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Senecio bonariensis Hook. & Arn. promising arsenic phytoextractor from water in laboratory and field trials

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ABSTRACT

The use of plant species to cleanse groundwater with excessive concentrations of arsenic (As) derived from contact with weathered materials has become a valuable option to treat it. The aim of this work was to analyze the bioaccumulation capacity of As of *Senecio bonariensis* (Asteraceae) through controlled laboratory tests and uncontrolled trials in the field in order to generate a low cost method applicable in rural areas that do not have systems of water treatments. Plants collected from the natural environment were arranged in hydroponic crops under controlled and uncontrolled conditions, in the first case with increasing concentrations of As for 45 days, and in the second, with a constant concentration of As for a period in a range between 45 and 90 days. The plants were processed and dried for the measurement of As. In both tests, in all the samples there was a noticeable accumulation of As, generally greater in roots than in leaves. Under controlled conditions the plants accumulated more As in relation to greater concentration of this element in the water. In all the trials a high bioaccumulation of As was found, which turns the plant into a hyperaccumulator. Due to the ability of *S. bonariensis* to accumulate As, and even more because of the great biomass produced by this species, it becomes an excellent one to be used for the remediation of arsenical waters.

1. Introduction

Water, soils and atmosphere all around the world are contaminated with various agents of natural origin, typical of the earth's crust, or human activities such as mining, industrial processes, agriculture, etc. [1]. Although pollutants of anthropogenic origin often exceed those from natural sources, but both must be evaluated.

There are several regions in the world with excessive concentrations of toxic elements in water derived from contact with weathered materials [2]. The contamination of water by arsenic (As), fluorine (F), vanadium (V), heavy metals, nutrients and organic constituents is of particular concern [3]. Specially As, ranks as the 20th most occurring trace element in earth's crust, 14th in seawater, and 12th in human body [4]. In both developed and developing countries, the economic implications of getting water to have an acceptable concentration of arsenic have opened an important debate about the level to be set [5–7].

The most affected areas in the world by high concentrations of As in groundwater are the South-East, South-West and North-East of USA, Mongolia (China), coastal regions of Taiwan, Sonora (Mexico), Pampa plain (Argentina), West Bengal (India), Northern Chile and Bangladesh (Singh et al., 2015). In these regions As concentrations vary between 0.5 and 5000 mg l^{-1} when the recommended limit of arsenic in drinking water is 0.01 mg l^{-1} [8]. For example, in Argentina, in a large part of the rural areas in the Pampa plain, where groundwater is widely used for human consumption, the magnitudes of As are greater than 0.1 mg l^{-1}, reaching in up to

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$0.24\ mg\ l^{-1}$ [9].

The adverse effects of arsenic can be direct (irreversible diseases) and indirect (general contamination of the environment), in both cases they significantly affect the quality of animal and human life [10–13]. Chronic exposure to arsenic is manifested through multiple health problems, known as arsenicosis or chronic endemic regional hydroarsenicism [6,14]. Due to the harmful effects of arsenic, currently water treatment plants include water removal systems, however, rural people from areas with low population density or those who do not have access to water are left unprotected [6,15]. People that rely on an income level of less than $4 per day are particularly vulnerable [16]. Recently, an effective small-scale remediation method has been developed, although its costs and feasibility of implementation are still under study [17]. In response to this need, alternative techniques for the elimination of arsenic from water become increasingly important, with special attention paid those that involve the use of macrophytes in hydroponics or in artificial wetlands [4,6,18–21]. These mechanisms can be applied in the long term, although they are limited by the tolerance, uptake and immobilization of arsenic by the species used as biofilters [22]. These techniques, called together as phytoremediation, are a method of removal of various pollutants used successfully since several years ago in the world [23]. These technology is also seen as an indirect As remediation method [24].

The term phytoremediation, used for the first time in 1991 [25], is a technique that employ plants to degrade, contain, extract or immobilize soil or water pollutants, widely studied due to its good cost-effectiveness ratio and absence of adverse implications for the environment [26,27]. The knowledge for its proper implementation and success have been increasing, now it is known that the selection of plant species to be used must be made according to the abundance in the area where the system is to be installed, to the adaptability of the same to the local climate, to the oxygen transport capacity to the rhizosphere, to the tolerance to high concentrations of contaminants as well as to the capacity for their assimilation and ease of later collection of the plant [28].

There are numerous species used in bioremediation, such as species belonging to the genera *Cyperus*, *Glyceria*, *Phalaris*, *Phragmites*, *Pontederia*, *Scirpus* and *Typha* [29–32]. Especially as hyperaccumulating species of arsenic are listed: *Agrostis castellana* and *A. delicatula* [33], *Eichornia crassipes* and *Lemma minor* [34], *Pteris cretita* [35], *Pteris vittata* [18] and woody species of the genus *Populus* and *Salix* [36].

Different studies support the use of plants of the Asteraceae family for the remediation of different areas. The mechanisms by which these plants remove contaminants include extraction, transformation and stabilization, as well as degradation in the rhizosphere, where plants promote the growth of bacteria that transform contaminants [37]. *Senecio bonariensis*, a representative of the Asteraceae family, was studied by López et al. [38] and Siniscalchi [39], who concluded that it is a good assimilator of nitrogen and phosphorus. Taking into account that the mobilization of arsenic is carried out through phosphate channels and, due to its morphological and physiological characteristics, it is considered as a good candidate as biofilter for arsenic.

The aim of this study, is to evaluate the capacity of arsenic bioaccumulation of *Senecio bonariensis* and to determine in which plant organs is observed the highest accumulation of this element, through controlled laboratory experiments and uncontrolled ones, in the field. The field experiments were designed in order to generate a low cost method applicable to rural areas that do not have specialized water treatment systems.

2. Material and methods

Specimens of *S. bonariensis* were obtained from the natural environment in Bahía Blanca city (Prov. Buenos Aires, Argentina) and nearby places (Fig. 1 A), to perform the trials of arsenic removal under controlled and uncontrolled conditions (referring exclusively to atmosphere conditions). For each experiment, control specimens were kept in order to compare the arsenic base values present in plants of the natural environment with those resulting from the different treatments.

2.1. Experimental design

2.1.1. Test under controlled conditions

The experiments were performed in a culture room for 45 days. Before starting arsenic treatments, medium plants (the length of the lamina was between of 15–30 cm) were transplanted into PEP containers covered with aluminum with 1.75 l Arnon and Hoagland solution (Table 1) for adaptation for 7 days. The solution was constantly aerated and the pH was adjusted between 5.5 and 6.5 with $0.1\ mol\ l^{-1}$ solution of NaOH or HCl. This pH range is the one suggested in order that the plants can have all the nutrients. The solution was renewed weekly or at the time the plant used up the water in the container.

After the adaptation period, arsenic was added to the nutrient solution. Arsenic was supplied as $Na_2HAsO_4 \cdot 7H_2O$ at six concentrations (0, 0.05, 0.1, 0.25, 0.5 and $5\ mg\ l^{-1}$) in three replicates arranged in a completely randomized experimental design, totaling 18 containers containing one plant each one. Concentrations up to $0.05\ mg\ l^{-1}$ were used to simulate uncontaminated water while concentrations of $0.1–5\ mg\ l^{-1}$ constituted those referred to waters contaminated with arsenic. Treatments in increasing concentrations were named A, B, C, D, E and F.

2.1.2. Test under uncontrolled conditions

Two experiments were carried out, the first was in autumn-winter (A) while the second was in spring-summer (B). For both, 36 plants were randomly selected and categorized according to the length of their lamina in: 12 big (>30 cm long), 12 medium (15–30 cm) and 12 small (<15 cm). In the case of trial B, large plants were collected with incipient development of the floral scape (Fig. 1 J), in this way it was possible to evaluate how the flowering and fruiting phase affects the phytoextracting activity of the plant. The 12 plants of each group were suspended in 500 l PVC containers (Fig. 1 F) in a hydroponic culture system (container 1: big plants, container 2: medium plants, container 3: small plants) (Fig. 1 G). In these tests plants of different sizes and in different phenological stages were used in order to check if these plant conditions interfered in the plant's remediation activity.

For these experiments, underground water (20 m depth) was used (extracted by a perforation), with a concentration of arsenic in a range between 0.23 and $0.25\ mg\ l^{-1}$. Because this concentration of arsenic could produce severe stress in the plants, it was decided to dilute the underground water resource with free arsenic water in a tank of 500 l, decreasing the arsenic content to the half ($0.12\ mg\ l^{-1}$).

The water coming from the tank recharges the three PVC containers automatically through a system of individual floatings which independently regulate the load of each container. At the opposite end of the water intake was connected a half inch hose provided with a self-compensating dropper that releases a flow of $1\ l\ h^{-1}$ and generates a continuous stream of liquid in each PVC container. Despite differences in the hydraulic load of each self-compensating dropper, the flow rates of the same remained constant in the gauging carried out during the test. Under these

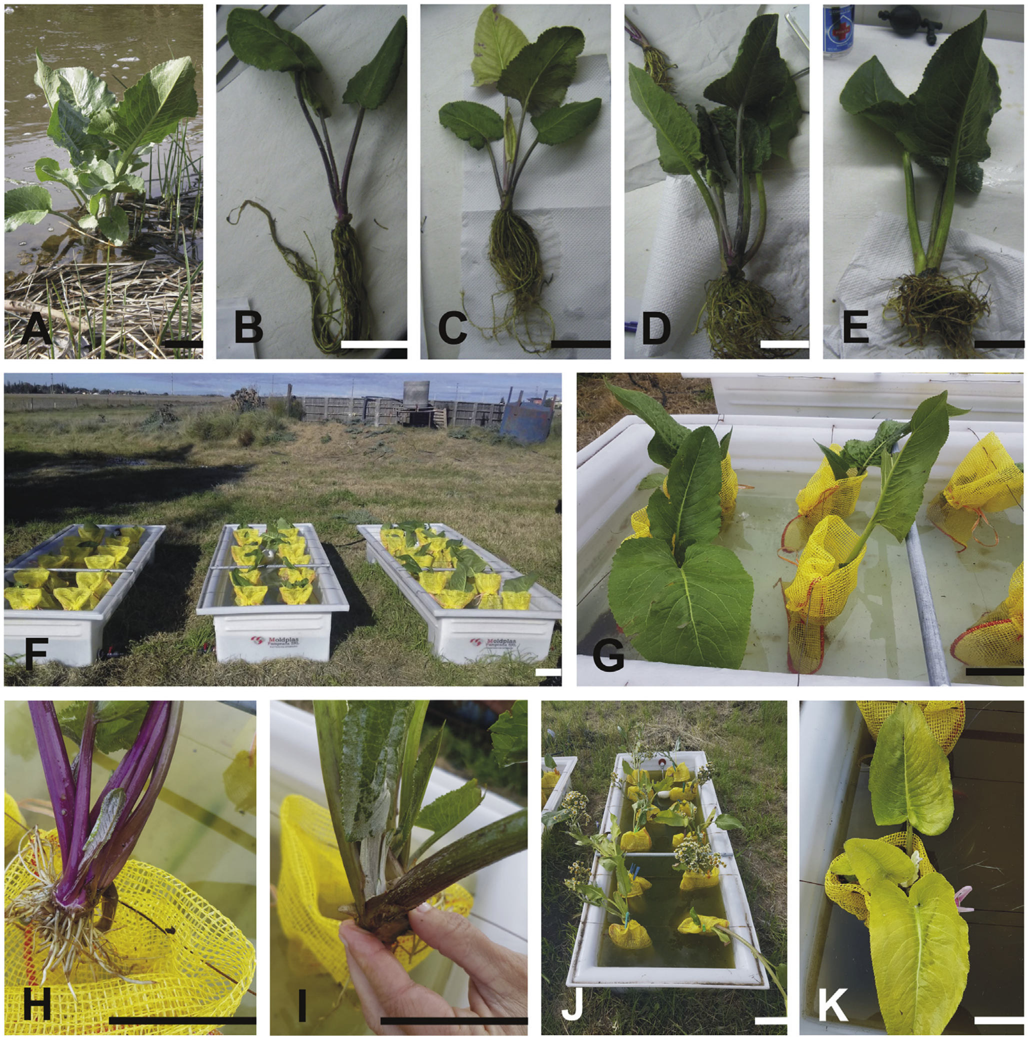

Fig. 1. *Senecio bonariensis* a phytoremediator species. A, Plant in its natural environment. B-E, Experiment under controlled conditions. F-K, Experiment under uncontrolled conditions. B, Control exemplary (A). C, Plant exposed to 0.1 mg l^{-1} of As. D, Plant exposed to 0.5 mg l^{-1} of As. E, Plant exposed to 5 mg l^{-1} of As. F, Trial A under uncontrolled conditions. G, Specimens of *S. bonariensis* growing in hydroponic culture in a 0.12 mg l^{-1} concentration of As. H, Detail of the development of roots. I, Detail of the development of new shoots. J, Plants in bloom. K, Chlorotic leaves. Bars: 10 cm.

Table 1
Solution of Arnold and Hoagland (macro and micronutrients).

Solution of Houagland-Arnon macronutrients	
	Culture solution $cm^3.l^{-1}$
KNO_3	6
$Ca(NO_3)_2 \cdot 4H_2O$	4
$NH_4H_2PO_4$	2
$MgSO_4 \cdot 7H_2O$	1
Solution of Houagland-Arnon micronutrients	
	Culture solution $mg.l^{-1}$
H_3BO_3	2.86
$MnCl_2 \cdot 2H_2O$	1.81
$CuSO_4 \cdot 5H_2O$	0.08
$ZnSO_4 \cdot 7H_2O$	0.22
$H_2MoO_4 \cdot 2H_2O$	0.09

conditions, the treated water effluent is 24 $l.day^{-1}$ for each container of PVC and the volume of water corresponding to 0.5 m^3, has a hydraulic retention time of 20.8 days.

2.2. Removal, accumulation and tolerance analysis of arsenic

In the experiment under controlled conditions after 45 days the plants were removed from the solution. In the experiments under uncontrolled conditions were removed after 90 days in case A, and 45 days for B. In all cases the plants were processed by separating the different organs and/or parts (roots, rhizomes, leaves and floral escapes) to record their fresh weight. The samples were washed and placed in a drying stove at a temperature of 60 °C for 72 h (constant dry weight), then their dry weight was recorded.

The samples from the experiment under controlled conditions were digested following the EPA 3050 standard adapted for plant tissue, while those from the uncontrolled conditions were pulverized and sieved.

The quantification of arsenic concentration in plant tissues was carried out with an Inductive Coupling Plasma Atomic Emission Spectrometer (ICP-AES), Shimadzu Sequential 1000 mod III in line with a Volatile Hydride Generator, according to EPA Standard 200.7.

The ability of plants to extract arsenic from water was evaluated using two parameters, the bioaccumulation factor (FB = [As] tissue/[As] solution) and the translocation factor (FT = [As] leaves/[As] roots).

2.3. Statistic analysis

To the results of the experiment under controlled conditions Pearson correlation coefficient between content of P and As in leaves and root tissues of *S. bonariensis* was calculated to determine significant relationships between concentration of As and P in order to detect possible interference in the assimilation of these two elements. The data was transformed to a logarithmic scale to reduce the difference between ranges of As concentrations used in the experiment. Also, the mean values of biomass obtained were compared with Tukey test. Infostat Software version 2011 was used.

3. Results

3.1. Tests under controlled conditions

Only in treatments B, D and E did we obtain a final biomass that exceeds the initial biomass (Table 2). In treatment C, the final biomass did not differ from the initial one, while in treatments A and F, the final biomass was lower than the initial biomass (Table 2). The highest average final biomass was recorded in treatments D and E, with the lowest recorded in treatment C (Table 2). The highest Relative Growth Rate (RGR) was obtained in treatment B (Table 2). Although, when comparing statistically the mean values could be affirmed that treatments D and E had a higher final biomass than the rest of the treatments and that the means between them were not significantly different (Table 3).

The average concentrations of arsenic in roots and leaves at the start of each treatment were 0.6 mg and 0.1 As.kg PS^{-1} respectively. Table 4 shows the increase in arsenic concentration in plant tissues in all treatments (B-F) with respect to control (A). The highest concentrations of arsenic in the tissues of *S. bonariensis* were observed in the D-F treatments that correspond to the highest concentrations of arsenic in the culture medium (Table 4). The highest concentration of arsenic in both roots and leaves was found in treatment E, followed by F and D (Table 4). The concentration of arsenic in roots and leaves of the plants subjected to treatment F (5 mg $As.l^{-1}$) were lower than those in treatment E, although they exceed those found in the remaining B-D treatments (Table 4).

The translocation factors found varied between 0.02 and 0.23 and showed low transfer of arsenic from the root to the leaves (Fig. 2 A). On the other hand, the bioaccumulation factors calculated for the different treatments varied between 9 and 17 (Fig. 2C). The highest translocation and bioaccumulation factors were observed in the plants subjected to treatments C and D (Fig. 2 A, C).

The efficiency of removal of arsenic from the culture medium ranged between 25 and 65%, with the highest in treatments B and F, the lowest in treatment E (Table 5).

To detect possible interferences in the assimilation of P and As, there were taken P concentrations for each of the As concentrations. The efficiency of removal of P ranged between 44 and 70% (Table 5), with the highest efficiency corresponding to As concentrations of 5 mg l^{-1} (treatment F) (Table 5). Performing the analysis of correlation estimating the Pearson coefficient it was detected that there was not correlation between the concentration of As in the medium and the assimilation of P by part of *S. bonariensis* since the correlations were not significant for leaves (-0.02^{NS}) as for roots (0.12^{NS}).

Plants that grew at different concentrations of arsenic did not present morphological anomalies as symptoms of toxicity at any of the concentrations studied (0.05–5 mg $As.l^{-1}$). The specimens that were exposed to arsenic concentrations of 0–0.1 mg $As.l^{-1}$ had less robust plants with lower biomass (Fig. 1B and C). Specimens that were exposed in a range of 0.25–5 mg $As.l^{-1}$ of arsenic in the culture medium had robust plants and higher biomass (Fig. 1D and E).

3.2. Tests under uncontrolled conditions

3.2.1. Experiment A (autumn - winter)

In all organs, a marked accumulation of arsenic was recorded, from 30% to 170% more with respect to the control (Table 6). The roots were the organs that registered the highest accumulation of arsenic, reaching at least double the value of the control (Table 6). The large plants were the ones with the highest accumulation in roots, with the medium and small ones remaining in nearby values, although the latter recorded the lowest level (Table 6). In the case of the rhizomes, something similar happened in the roots, the largest ones having the highest value, followed by the medium and small ones (Table 6). In the leaves, the greatest accumulation was observed in the small plants while the medium and large ones were in similar values (Table 6).

During the 90 days that the trial A lasted, 6000 l of water with a total of 720 mg of arsenic passed through the PVC containers of the hydroponic system. The plants managed to extract a total of 23.9 mg of arsenic with a vegetal biomass of 189.81 g of dry weight, which represents 3.3% of arsenic extraction from the culture medium.

At 45 days, small plants began to show symptoms of suffering: yellowing of leaves (Fig. 1 K), death of roots and general decay. Medium and large plants did not show these signs, showing the development of new roots and buds (Fig. 1H and I). It is highlighted that the climatic conditions during the period in which this trial was developed were extreme. Severe frosts (where a layer of up to 3–4 cm of ice formed), sleet and hail fall, however the plants managed to survive these conditions, even the roots that were trapped in the ice water showed no adverse effects.

3.2.2. Experiment B (spring - summer)

In trial B, as in trial A, in all organs more As was detected, an increase of 23% and 130% more with respect to the control samples (Table 7). In the roots, lower concentrations were observed than those recorded in experiment A; the large plants (in flowering) having the highest value, followed by medium and smaller plants (Table 7). In the case of rhizomes, unlike experience A, the arsenic content was slightly higher in medium plants, followed by large and small plants (Table 7). In the case of the leaves, the medium plants showed the highest value, while the large and small plants presented low values, even close to that of the control (Table 7).

Table 2
Biomass and relative growth rate (RGR) of *S. bonariensis* for each of the treatments (A-F). Biomass values expressed as mean ± SD in grams of fresh weight (FW) and RGR expressed as $gFW.d^{-1}$.

	A	B	C	D	E	F
Initial biomass	27.8 ± 15	28.3 ± 9	16.2 ± 3.6	45.4 ± 38	49.5 ± 22.5	45.5 ± 29
Final biomass	25.5 ± 11	36 ± 14	16.2 ± 5.5	53.4 ± 8	52.5 ± 6.5	38.5 ± 25
RGR	0	0.02	0	0.01	0.004	0

Table 3
Comparison of mean final biomass obtained during the different treatments with As. In the Tuckey test the letters indicate between which means significant differences were obtained ($\alpha = 0.05$).

Treatments	A	B	C	D	E	F
Means	25.6	36.1	16.1	53.4	52.5	38.6
Order of treatments according to increasing means	C	A	B	F	E	D
Tukey test result	a	a	a	a	b	b

Table 4
Arsenic concentration in the tissues of *S. bonariensis* of the experiment under controlled conditions expressed in $mgAs.kgPS^{-1}$. The average values are shown with their respective standard deviations.

	A	B	C	D	E	F
Roots	0.8 ± 0.22	7.6 ± 1.7	7.9 ± 2.3	17.1 ± 5.5	45.3 ± 3.5	24.3 ± 11.6
Leaves	0.22 ± 0.09	4.25 ± 2.5	8.89 ± 7.8	27.3 ± 6.6	40.8 ± 15.8	35.1 ± 18.3

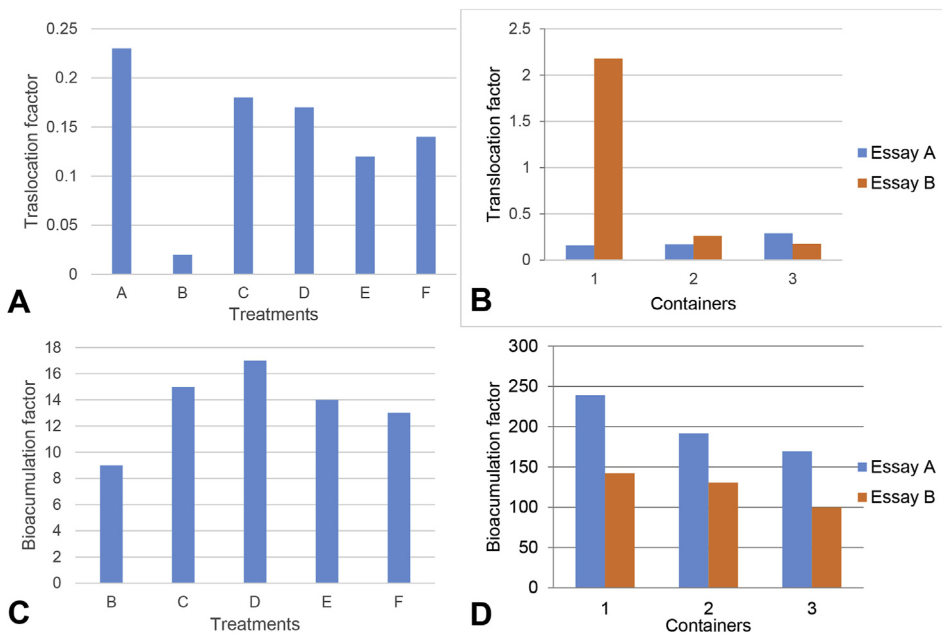

Fig. 2. Translocation factor (TF) and bioaccumulation factor (BF) of arsenic in experiments under controlled and uncontrolled conditions. A, C, Experiments under controlled conditions. B, D, Experiments under uncontrolled conditions.

Table 5
Removal efficiency (RE) of arsenic (As) and phosphorus (P) for each of the treatments under controlled conditions. The data are shown in percentage with its corresponding standard deviations.

Treatment	RE As	RE P
A	0	44 ± 7
B	55 ± 7	48 ± 1
C	36 ± 18	57 ± 6
D	46 ± 2	57 ± 5
E	25 ± 5	52 ± 6
F	65 ± 1	70 ± 3

In the arsenic dosage of floral escapes, the highest value was obtained with respect to all the organs studied (Table 7). Although the plants came to fruition, the dosing of As in fruits was not carried out because, due to its small size and low amount collected, the minimum sample for that study was not completed.

In the 45 days that this trial lasted the amount of water used was 3000 l with a total of 360 mg of arsenic. The plants managed to extract a total of 109.6 mg of arsenic in a biomass of 715.56 g of dry weight, which represents 30% of arsenic extraction from the medium. If in this last test we do not take into account the arsenic extracted by the floral scape, the quantity extracted is reduced to 59.78 mg in 630.66 g dry weight of vegetable biomass, which represents an extraction of 16.6%.

It was recorded that the water temperature of the containers was very high during the hours of high solar exposure, reaching 26–30 °C. Due to this, a noticeable deterioration in the roots was observed after 45 days of hydroponic culture.

In both field trials, the translocation values detected were less than one in all cases (Fig. 2 B) except for that recorded for container one in trial B (Fig. 2 B). For all plant organs analyzed, the bioaccumulation factor was high or very high, which places the plant as a hyperaccumulatory species (Fig. 2 D). It should be noted that the values found in trial A (Fig. 2 D) are higher than those in B (Fig. 2 D), where the plants were half the time in A.

4. Discussion

Phytoremediation is a set of technologies applied to eliminate, or diminish, harmful components for organisms through the use of

Table 6
Uncontrolled conditions Experiment A fresh (FW) and dry weights (DW) and arsenic concentration in the tissues of *S. bonariensis* expressed in mgAs.kgPS^{-1}. The values of the control specimens are also included.

Plant organ	Sample	FW (g)	DW (g)	As (ppm)	As mg.kg^{-1} PS	As mg.kg^{-1} PF
Roots	Control plants	21.9	5.3	10.61	10.61	1.11
	Container 1	312.1	26.06	28.66	28.66	2.39
	Container 2	219.7	19.14	23	23	2
	Container 3	90	4.9	20.32	20.32	2.24
Rhizome	Control plants	19.7	4.9	4.28	4.28	0.41
	Container 1	517.4	64.41	13.59	13.59	1.7
	Container 2	215.4	30.3	10.17	10.17	1.43
	Container 3	106.7	9.44	9.04	9.04	0.8
Leaves	Control plants	337.5	30.11	4.48	4.48	0.4
	Container 1	221.2	19.81	6.83	6.83	0.61
	Container 2	94.6	11.94	5.71	5.71	0.72
	Container 3	37.5	3.81	8.42	8.42	0.86

Table 7
Uncontrolled conditions Experiment B fresh (FW) and dry weights (DW) and arsenic concentration in the tissues of *S. bonariensis* expressed in mgAs.kgPS^{-1}. The values of the control specimens are also included.

Plant organ	Muestra	FW (g)	DW (g)	As (ppm)	As mg.kg^{-1} PS	As mg.kg^{-1} PF
Roots	Control plants	15.7	4.2	7.81	7.81	1.09
	Container 1	346.7	35.85	17.08	17.08	1.77
	Container 2	935.2	150.44	15.68	15.68	2.52
	Container 3	308.1	27.43	11.96	11.96	1.06
Rhizomes	Control plants	14.1	4.2	5.26	5.26	0.82
	Container 1	431.8	55	11.63	11.63	1.48
	Container 2	752.1	130.1	12.1	12.1	2.09
	Container 3	282	43.72	10.81	10.81	1.68
Leaves	Control plants	117.6	11.7	3.24	3.24	0.32
	Container 1	240	26.82	4.2	4.2	0.47
	Container 2	1034.2	120.75	7.33	7.33	0.86
	Container 3	346.6	40.61	4	4	0.47
Floral scape	Container 1	694.5	84.84	58.41	58.41	7.14

plants that have specific capacities to absorb contaminants.

When the remedial plants manage to establish themselves in large areas, they help to return utilitarian and/or economic potential to the environment, in addition to modifying the aesthetics of the landscape of the contaminated sites [40]. It is important to bear in mind that because contaminants can be phytotoxic and inhibit plant growth, preliminary studies to identify potentially remediating plant species are of extreme importance [40]. For example, recent studies in communities of ruderal plants that live in soils with a high concentration of arsenic determined that these species (99 in total) were adapted to this condition without having been previously classified as metaphytic or hyperaccumulating [41].

Pteris vittata was the first hyperaccumulating species of arsenic (more than 1000 mg As.kg^{-1} in dry weight of leaves) discovered by Ma et al. [18] while Wang et al. [42] recorded that this same species is able to accumulate more than 27,000 mg kg^{-1} of arsenic in dry weight growing in hydroponic media. Another important species is *Populus nigra* that reaches up to 200 mg As.kg^{-1} in the tissues of its roots [43]. According to some studies the accumulation of arsenic is strongly influenced by the habit of the plant, being higher in submerged plants than in emergent and/or terrestrial ones, one of the probable causes would be that the submerged leaves or, parts of them, could absorb arsenic complementing the roots [44].

The results obtained in this study revealed a great capacity of *S. bonariensis* for the bioaccumulation of arsenic. In the experiment under controlled conditions, *S. bonariensis* specimens treated with high concentrations of arsenic in the culture medium had higher biomass values than those subjected to low concentrations of the metal. In a similar way to that described for *Arundo donax* [45], *S. bonariensis* did not present great differences in the amount of final biomass produced by the control (A) and the treatments with a contamination of between 0.05 and 0.1 mg l^{-1} (B-C) and the treatment that included the maximum value of arsenic in the medium, 5 mg l^{-1} (F), while there were important differences between them and the final biomass of the treatments with concentrations of 0.25 and 0.5 mg l^{-1} (D-E). In this test, under controlled conditions, a direct relationship was found between the concentrations of arsenic in the medium and in the organs studied, so that an increase of arsenic concentrations in the medium reflected the accumulated concentration in leaves and roots, as observed in *Poa annua* [46] and in *Pteris vitatta* [47]. In the latter species a large accumulation of arsenic was found in the fronds as the concentration of arsenic in the soil increased, always giving bioaccumulation factors greater than 1, except for the places where the concentration of arsenic in the environment was extremely high [47]. The latter is similar to what happens with *S. bonariensis*, where bioaccumulation rates are always high, although they are slightly declining in treatments that have very high arsenic concentrations (E-F). The bioaccumulation factors shown by *S. bonariensis* during the laboratory test are comparable with those of *P. vittata* and *P. cretica*, both species considered hyperaccumulators of arsenic [35]. In these laboratory tests, the bioaccumulation values found in *S. bonariensis* varied between 9 and 17, these values are somewhat less variable and higher than those found in *P. cretica* (1.34–6.6) or *P. vittata* (0.06–7.43) [35]. Probably this difference in values could be due to the fact that the studies in *Pteris* were developed by growing the plants on soil, where there could have been interactions with different elements that have hindered the bioaccumulation process, while the trials with *S. bonariensis*, as they developed in a controlled environment, they allowed the species to express its maximum capacity.

In general, for the final arsenic dosages, the plants were divided into underground and aerial parts, without further discrimination in case the plant has other underground organs besides the roots. *S. bonariensis* presents its underground part formed by rhizomes and roots, so in the field trial (where a good development of rhizomes was achieved) the arsenic content was analyzed separately in both underground organs. This discrimination is very important since it provides a more specific data of the arsenic content in the plant, being important at the time of making management decisions after the phytoremediation action of the plants. In this sense, although the rhizomes presented higher levels of arsenic accumulation than the leaves, as in many other studied species, the highest accumulation of arsenic occurs in roots, which was verified in the experiments under controlled and uncontrolled conditions.

In both trials, under controlled and uncontrolled conditions, *S. bonariensis* showed values of arsenic concentration in leaves similar to *Ricinus communis* cv *Guarany* [48] and other species, such as *Echhornia crassipes* and *Lemma minor*, considered hyperaccumulators [34]. However, these concentrations found in the leaves of *S. bonariensis* are far from those reported for *Pteris cretita* or for *P. vittata* [35].

Comparing the results of arsenic accumulation of *S. bonariensis* in the field experiment with respect to the laboratory test developed in this study, it was observed that the accumulation in the roots of the plants of the field, Experiment A, presented similar values to those registered in the laboratory with a concentration of arsenic in the medium of 5 $mg\,l^{-1}$ while in the field, Experiment B, the values were similar to those of the treatment with 0.25 $mg\,l^{-1}$ of arsenic in the corresponding to the laboratory. Comparing the results in leaves, it was recorded that the accumulation in the field experiments was similar to that recorded in the laboratory tests with arsenic concentrations in the medium of 0.05 and 0.1 $mg\,l^{-1}$. This shows that in uncontrolled conditions the capacity of *S. bonariensis* to act as a phytoextractor is significant, reaching to extract large quantities of arsenic from the environment even when the concentration of this element is moderate.

In the field experiment it was possible to evaluate how the size of the plant and phenological status affects the bioaccumulation of arsenic. Large and medium plants have a great accumulation of arsenic in the roots regardless of phenological status, therefore both sizes can be considered ideal for a long-term phytoremediation experience. Small plants presented somewhat different values in terms of their accumulation of arsenic, and were also more susceptible to environmental stress factors, which is why they are not especially recommended for long-term phytoremediation processes.

S. bonariensis showed, under controlled and uncontrolled conditions, higher translocation values than those of *Ricinus communis* cv *Guarany* [48], showing that the highest concentration of arsenic is retained in underground parts (rhizomes and roots), unlike for example what happens in *Pteris vittata* [47] where the leaves have the highest concentration of arsenic in the tissues. For *S. bonariensis*, only in the case of Container 1 of Experiment B under uncontrolled conditions, the translocation factor was 2.18; in this case the aerial part included the floral stalk that possessed a very high arsenic content denoting a high translocation rate towards the reproductive parts of the plant. In this sense it is important to confirm the presence of arsenic in the fruits of *S. bonariensis* (which act as propagules in this species), since for example in *Pityrogramma calomelanos* (fern) there was a very high concentration of arsenic in their spores [13]. Although the spores and/or fruits represent a lower biomass, it is important to carry out appropriate management tasks to avoid re-entering the environment of at least part of the arsenic that was extracted.

The test under uncontrolled conditions proved an extraction of at least 30% of the As of the medium, this value is a promising one taking into account that represent the action of only 36 plants in the total system. Therefore, by increasing the number of plants per container, higher extraction values could be achieved, at which point work will continue in the following years until an optimum number of plants is defined to obtain water with acceptable parameters for human consumption (maximum of 0.01 $mg\,l^{-1}$).

In the test under controlled conditions, no specimen of *S. bonariensis* exposed to arsenic treatments showed brown spots or necrosis of leaf blade margins and margins, symptoms considered typical of toxicity [49], as were detected in other species previously studied as *Thelypteris palustris* [50]. In the experiments under uncontrolled conditions, different symptoms were observed in small plants, related both to stress to environmental conditions and also to arsenic accumulation in relation to plant biomass.

The design of the hydroponic culture used in the trial under uncontrolled conditions is promoted as a good option for its implementation for the extraction of arsenic in water on a small scale. Its simple design, low cost materials and easy maintenance aim to solve a serious problem that many people in rural or sparsely populated areas with scarce economic resources must face.

Finding phytoremediation plants is usually not an easy task, since although there are several species with the capacity to accumulate different metals, many tend to grow only under certain conditions or produce little biomass [51]. In the case of *S. bonariensis* multiple characteristics are combined, firstly its capacity as a phytoremediator as detailed above, and secondly, it has characteristics cited as fundamental by Visoottiviseth et al. [13] in its evaluation study of phytoremediation species: wide distribution in the environment, large amount of aerial biomass, high bioaccumulation values, short life cycle and high propagation rates. Added to this, the robustness and adaptability to different growing conditions and the needs of temperature, humidity and solar exposure of *S. bonariensis* make it an excellent candidate for its controlled use as a remediator in small or large scale artificial wetlands.

5. Conclusions

It is concluded that *S. bonariensis* possesses a great capacity of bioaccumulation of arsenic, registering the highest concentration of this element in rhizomes and roots. This plant species manages to extract arsenic even when the concentration of it in the medium is moderate. In this study it was found that the environmental variables do not interfere in the arsenic extraction capacity of *S. bonariensis*. This plant species is an effective arsenic extractor even in short periods of time. For all these characteristics *S. bonariensis* becomes a potential biofilter to treat arsenic water of any origin.

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