**Arsenic absorption by *salvinia minima* L. in short and long ~~times in~~ contaminated waters and its possible use for bioremediation**

**ABSTRACT**

 Arsenic (As) is a ubiquitous metalloid in nature, which represents a serious environmental impact due to its accumulation and difficult removal. The objective of this study was to evaluate whether *Salvinia minima* L., with great vegetative reproduction, serves as a bioremediator. The plants were washed with a 10 mM EDTA solution and grown in batch hydroponics, using a modified Hoagland solution (190 μE·s⁻¹·m⁻²), 16/8h photoperiod (artificial light/darkness) and temperature of 25 +/- 2ºC, for 7 days of adaptation. The exposure to As was carried out with raw water and the addition of 0.2 ppm of Na2AsO4. Uptake was analyzed over short periods of time, for 42h, by extracting triplicate samples every 6,12,18,24,30,36,42h from contaminated water and plant material. Uptake was analyzed over long periods of time, extracting triplicate samples every 48,96,144,192,240,288,336 and 384 h from contaminated water and plant material.Leaf and root homogenates were subjected to HNO3 mineralization by wet microwave procedure using hermetic teflon reactors with a pressure valve. Water samples were extracted at the same times and acidified with 1% HNO3. [As] was determined by ICP-Mass. Statistical analyses were performed by ANOVA followed by the Tukey test, with \*\*\*p≤0.001. The variation of As over short periods of time showed a significant decrease in culture solution at 42h with p \*\*p≤0.01 and in S. minima a significant increase was observed at times 36 and 42h with \*\*\*\*p<0.0001. The variation of As over long periods in Salvinia showed a significant increase between 48 h and 240 h and 336 h with a \*\*\*p≤ 0.001 and between 48 h and 384 h a very significant increase with a \*\*\*\*p<0.0001. The fresh weight presented a very significant increase at 384 h of exposure to As with a \*\*\*p<0.0001 and in dry weight there were no significant differences with a \*p<0.05. S. minima L. is a promising species for water bioremediation.

**Keywords**: Arsenic, Bioremediation, pollution, *Salvinia minima* L.

 **INTRODUCTION**

Among the wide variety of pollutants present in the environment there are some compounds found in nature in small quantities called heavy metals, which are removed very slowly, so when they accumulate in the environment, due to their degree of toxicity, they cause serious environmental impact (1)

. Arsenic is the number one substance in the most recent ~~(ATSDR)~~ Comprehensive, Environmental, Response, Compensation and Liability Act (CERCLA) Priority List of Hazardous Substances published by the Agency for Toxic Substances and Disease Registry (ATSDR). This list is comprised of substances found at hazardous waste sites on the National Priorities List. The substances are ranked on frequency or occurrence, toxicity, and potential for human exposure." (2)

Many industries, the application of fertilizers and pesticides, the dumping of sludge and the generation of municipal waste have been identified as the main sources of heavy metal contamination (3) Currently, heavy metal contamination of soils and groundwater, which flow into sources of water for human consumption, are a great concern. Contamination by these compounds produces deleterious effects on microorganisms and plants, representing a potential risk for animals and humans through different exposure routes, such as direct or indirect ingestion (4 ,5).

Arsenic may occur in the environment in four oxidation states: +V (arsenate), +III (arsenite), 0 (arsenic), and -III (arsine). In natural waters, it is mostly found in the inorganic form (iAs) and as oxyanions of pentavalent arsenate (AsV) and of trivalent arsenite (AsIII) (6,7,8). Arsenite is so easily oxidized to arsenate especially by microorganisms, since they enhance this process at least seven orders of magnitude faster than the abiotic rate (9) that a lack of an immediate and appropriate method to preserve species could result in questionable speciation data (10). Concentrations and relative proportions of arsenic species vary according to changes in arsenic source, redox conditions, and biological activity (11). Usually, arsenate is the thermodynamically stable state in oxic waters, while arsenite is predominant in anoxic and reduced environments (12). Consequently, in oxic waters of lakes, rivers, and oceans, arsenate is generally the dominant species, whereas high relative proportions of arsenite have been found in groundwater, in the hypolimnion of lakes with anoxic bottom waters, in river stretches close to inputs of arsenite-dominated industrial effluents, and in waters with a component of geothermal water (12).

**Arsenic in plants: Metabolism and effects at the cellular level.**

Plants readily absorb arsenic species and the bioavailability of the element depends on each plant species. Absorption through the roots is the main route of entry of trace elements into plants, so that, in general, the composition of a plant reflects the elemental composition of the medium in which it has developed (13) . Plants will absorb arsenic through the roots and the leaf mass, and translocation phenomena have been observed in some species (hyperaccumulators). Generally, plants stop growing and developing after prolonged root exposure to large amounts of arsenic. Inorganic As(III) penetrates the plant cuticle to a greater extent than As(IV). The predominant forms of inorganic As in both terrestrial and aquatic plant tissues are As(V) and As(III), but their proportion is relative and varies between plant species . Large amounts of As(III) have been found in the foliage of As-hyperaccumulator ferns (14). Arsenite can form complexes with a variety of thiol compounds in plant tissues (15). As is present mainly as As(III) in *Brassica juncea* and *Arabidopsis thaliana* in compounds such as phytochelatins (FQ) and glutathione (GSH) (16). Plants obtain carbon/oxygen/nitrogen, minerals, water, and energy to sustain themselves. Heavy metals (HMs) are naturally occurring elements and some of them are essential in minimal quantities in plants to maintain regular functions and include Co, Cu, Mn, Mo, Fe, Mg, Se, Ni, Se, and Zn, but above physiological levels tolerance, they become toxic and induce negative influences on the plants' growth (17). However, HMs such as As, Cr, Cd, Pb, Hg, and Ag exhibit deleterious effects even in lesser quantities (18). Aquaporins, aquaglyceroporins, phosphate, and sulfate transporters in plant systems facilitate arsenic absorption. Since As(V)’s oxyanion chemical structure is physically similar to that of Pi, it rapidly enters plant roots via phosphate (Pi) transporters and is the predominant As species in aerobic soils (19).

Phytoremediation is defined as the use of plants to remove contaminants from the environment and/or render them harmless. It has generated much attention in recent years as it is an effective and ~~environmentally friendly~~ technology (20, 21). The most well-known plants for phytoremediation are glycophytes, which can survive the combination of salt and heavy metal contamination (22). For this reason, they are an ideal alternative for phytoremediating toxic metals in contaminated waters. Five phytoremediation processes have been identified (23,24) Phytoextraction: plants remove metals from the soil and concentrate them in harvestable parts of plants (25) Phytodegradation: plants associated with microorganisms degrade contaminants (26) Rhizofiltration: plant roots absorb metals from stream waste (27) Phytostabilization: plants reduce the mobility and bioavailability of contaminants in the environment by immobilization or prevention of migration (28) Phytovolatilization: volatilization of contaminants into the environment by plants (29)

 **Species selected for the study**

*Salvinia minima*, ~~Family: Salviniaceae, genus~~ *~~Salvinia~~*~~, of the Salviniaceae family,~~ is composed of 12 known species (30). *Salvinia minima* Baker is a small, free-floating fern in freshwater, widely distributed in tropical and temperate regions of the world (31). The efficiency of pollutant removal during the phytoremediation process will depend mainly on the plant species used, the growth state of the plants, their seasonality and the metal species to be removed. Therefore, to achieve good results, the plants to be used must have the following characteristics: Be tolerant to high concentrations of metals, be metal accumulators, have a rapid growth rate and high productivity, be local species, representative of the natural community, and be easily harvested. This plant can be found floating near the edges of slow-moving streams and in nutrient-enriched ponds. *Salvinia* *spp* forms a "blanket" that covers very large portions of ~~the body of water~~ where it grows. It reproduces exponentially by vegetative fragments, with a leaf duplication time of 3.5 days (32,33) The unique characteristics of *Salvinia spp* make it an ideal candidate for phytoremediation (34,35,36), including: rapid and exponential growth, high capacity for acclimatization to a wide range of temperatures and tolerance to a large number of contaminants. The objective of this work was to analyze the bioextraction of arsenic (as arsenate) by *Salvinia minima* from water artificially contaminated with As(V) at short and long times of exposure.

**MATERIALES Y MÉTODOS**

**Plant material:** *Salvinia minima* plants were washed with distilled water and a 10mM EDTA solution, then placed in "batch" type hydroponic systems, using standard Hoagland solution with a pH 6.0-6.5, with artificial lighting (190μ E.S-1.m-1) using a programmable analog switch with a 16/8 photoperiod (light / dark) and controlled temperature (25 +/- 2ºC) for 7 days as an adaptation period (37)

**Experimental Model:** The collection of blanks was carried out before proceeding to exposure to As, 3 blanks were taken from plants not exposed to As. For exposure to As, 3 tanks of 50cm long x 30cm wide x 25cm high were filled with raw water (not treated), two of them were added Sodium Arsenate such that the concentration of As was 0.2 ppm and a third tank was left as a control. Each tank was filled with 45 g of *S. minima.*

**Sampling**: Once the plant samples were extracted, they were washed with distilled water and 10mM EDTA solution according to (38). The sampling frequency for the As uptake tests during the first 42 h was every 6 h (short times) and then every 48 h (long times) for a total period of 384 h. Samples of whole plants (root and leaves) and culture solution were extracted in triplicate. The plant samples were preserved in Petri dishes, tilled and stored at -20ºC until As quantification. 10 ml of culture solution samples were acidified with 1 drop of nitric acid and stored at -20ºC until As quantification.

**Sample treatment:** Ten drops of distilled water and 1 ml of concentrated nitric acid were added to each plant sample in Hatch tubes, and the tubes were covered and placed in a Zeltec® heating system with a temperature ramp from 50°C to 150°C for 2 h until the solution acquired a light yellow color. Once this was achieved, ten drops of 100 vol hydrogen peroxide were added to each tube and they were heated again using the same procedure. Once mineralization was complete, all samples were transferred to tared and labeled 20 ml polypropylene tubes and diluted to 15 mg (approximately 15 ml). The water samples were transferred to tared and labeled polypropylene tubes, then made up to 15 mg

**Arsenic quantification and chemo metric studies**: The determination of As concentration was performed on blanks, water and plant samples by ICP-Masses, with an ultrasonic nebulizer due to its high sensitivity and selectivity to determine Arsenic. An equipment was used: ELAN DRC-e, PERKIN ELMER SCIEX ICP - MS conditions: Monitored isotope: 75As.with RF power: 1050W, resolution: normal, scan mode: Peak Hop. Dwell time: 500 ms and gas flow rate: Plasma: 13 L / min, Auxiliary: 1.35 L / min, nebulizer: 0.75 L / min. Detection and quantification limits for 75As: o L / D = 1.025 ug / L. L / C = 1.853 ug / L. Methodological validation was performed using the standard aggregate method, where 1 drop (0.05 ml) of a solution containing 0.01 ppm of As was added to the As-free plants. The concentrations measured in the validation for these samples were: 10,240 ppm, 9,618 ppm, 9,602 ppm. The sample blanks received the same wet mineralization treatment as the methodological validation samples. All sample blanks yielded non-detectable arsenic (N/D).

**STATISTICAL ANALYSIS:**

Time series were calculated using one-way ANOVA, followed by Tukey's post-hoc test for specific comparisons; p ≤ \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, and \*\*\*\*p≤0.0001. Significance was considered when the means indicated in the figures were compared with the corresponding maximum values ​​within each group.

**RESULTS**

1. **Arsenic in culture solution and plant samples in short periods**



**Figure 1:** Temporal patterns of ppm As in culture solution over short periods of time. The bars represent As values ​​at different times. Each point represents the mean ± SEM of n = 3 culture solution samples. Statistical analysis was performed using one-way ANOVA followed by Tukey's test, with \*\*p < 0.01; means were compared with the corresponding maximum values.

The variation in As in S. minima culture solution samples over short periods of time showed a statistically significant decrease at 42 h, with \*\*p ≤ 0.01, compared to 6 h.



**Figure 2:** Temporal patterns of ppm As in S. minima over short periods of time.

The bars represent the As values ​​at different times; each point represents the mean ± SEM of n = 3 *S. minima* samples. Statistical analysis was performed using one-way ANOVA followed by Tukey's test, with a \*\*\*\*p < 0.0001; means were compared with the maximum value.

*S. minima* plants showed a highly significant increase in As levels at 36 and 42 h compared to 6 h, with a \*\*\*\*p ≤ 0.0001.

**Arsenic in plant samples and culture solution in long periods**



**Figure 3**: Temporal patterns of ppm As in the culture solution over long periods of time. The bars represent As values ​​at different times; each point represents the mean ± SEM of n = 3 culture solution samples. Statistical analysis was performed using one-way ANOVA followed by Tukey's test, with \*p < 0.05; means were compared with the maximum value.

The variation in As over long periods in the culture solution did not show a significant difference in the time series analyzed.



**Figure 4:** Temporal patterns of ppm As in S. minima over long periods. The bars represent the As values ​​at different times; each point represents the mean ± SEM of n = 3 S. minima samples. Statistical analysis was performed using one-way ANOVA followed by Tukey's test; with \*\*\*p < 0.001, and with \*\*\*\*p < 0.0001, the means were compared with the maximum value.

The variation in As over long periods in S. minima showed a significant increase between 48 h and 240 h and 336 h with \*\*\*p ≤ 0.001, and a highly significant increase between 48 h and 384 h with \*\*\*\*p < 0.0001.

**Evolution of fresh and dry weight of *S. minima alo largo del tiempo***



**Figure 5:** Evolution of fresh weight in S. minima plants. The bars represent the values ​​in g at different times; each point on the curve represents the mean ± SEM of n = 3 samples. Statistical analysis was performed using one-way ANOVA followed by Tukey's test, with \*\*\*p < 0.0001; Plant fresh weight was compared at 48 h of cultivation with respect to treatment times.

Fresh weight showed a very significant increase after 384 hours of exposure to As, with \*\*\*p < 0.0001.



**Figure 6:** Dry weight of S. minima plants over cultivation. The bars represent the weights in g of S. minima at different times; each point represents the mean ± SEM of n = 3 plant samples. Statistical analyses were performed using one-way ANOVA followed by Tukey's test, with \*p < 0.05. Plant dry weight was compared at 48 h of cultivation with respect to treatment times.

No significant differences were observed in dry weight between the treatments studied, with \*p < 0.05.

**DISCUSION**

Arsenic (As) is an environmental pollutant, and its concentrations have increased in freshwater sources around the world, through both geological processes and anthropogenic activities such as mining, burning fossil fuels, and agricultural use of fertilizers, pesticides, and herbicides containing As (39). The most common inorganic As forms in the aquatic ~~environmen~~ are arsenate (AsV) and arsenite (AsIII). Is considered more toxic and also more present in groundwater sources, in many regions of the world, water intake with high As concentrations represent a serious threat to the health of human populations because of the pollutant toxicity and carcinogenic potential.

Our findings demonstrate that *Salvinia minima* possesses the capacity to phytoremediate arsenic-contaminated water bodies through prolonged As uptake without exhibiting signs of phytotoxicity. Previous studies support its metal tolerance; for instance, Hoffman et al. (2004) reported that toxic effects in *S. minima* occurred upon exposure to 20 μM Pb(II) and 100 μM AsO₄³⁻ under conditions of 30 leaves and 15 pseudo-roots per 150 mL of Hoagland medium. Furthermore, (32,33) highlighted the species’ potential to remediate Cu(II) at concentrations up to 100-fold higher than those typically observed in natural waters. Nonetheless, their results also indicated a sensitivity to Cu(II), with a gradual decline in growth observed after 7 days of exposure at concentrations up to 3.0 mg/L, and a marked reduction in CO₂ assimilation and photosynthetic pigment production after 14 days. In contrast, in our experiment, *S. minima* maintained sustained growth throughout 16 days of arsenic exposure. No reduction in dry biomass was detected, and a significant increase in fresh weight was observed.

Other studies on this genus and its various species have also demonstrated their capacity to remove heavy metals from aqueous media. For instance, (30) reported that *Salvinia natans* was capable of removing up to 90% of Hg(II) and Cu(II) from solutions with initial concentrations below 5 mg/L and up to 50 mg/L, respectively. Similarly, in *Salvinia molesta*, (31) observed growth inhibition at sublethal concentrations of Cd(II) (0.05 to 0.075 mg/L), using 42 leaves per experimental unit. (40) investigated the potential of *S. herzogii* to remove Cr(III) from water at concentrations ranging from 1 to 6 mg/L. The accumulation rate was directly proportional to the initial metal concentration in the solution. Notably, Cr(III) accumulation in the roots was rapid, reaching 6.2 mg/g dry weight within the first 24 hours. However, translocation to aerial parts was minimal, as Cr content in fronds was much lower (0.44 mg/g dry weight), likely due to direct contact between the leaves and the solution rather than active transport.

These findings, along with our results, reinforce the suitability of *Salvinia* species for phytoremediation applications. However, interspecific variability in metal tolerance and accumulation patterns highlights the importance of species-specific evaluation when designing remediation systems. In our case, the sustained growth and biomass increase of *S. minima* under arsenic exposure further supports its robustness and potential as a phytoremediator, particularly in As-contaminated waters.

Our experiments demonstrated that during the first 48 hours of arsenic exposure, *S. minima* significantly increased its internal As accumulation, which coincided with a marked decrease in arsenic concentration in the growth solution. This initial uptake phase suggests a highly efficient removal mechanism. However, arsenic dynamics within the plant may also involve efflux processes. It has been reported that As(III) can be transported out of the plant cells into the external environment via efflux mechanisms (19,41). Aquaporins, a class of intrinsic membrane proteins, have been identified as bidirectional transporters in some plant species and are capable of facilitating As(III) efflux from the symplast as a defense response (42).

The combination of arsenic efflux and translocation to aerial tissues may help explain why pollutant accumulation is predominantly observed in the floating leaves of *S. minima*, rather than in root tissues or in direct correlation with increasing As(III) concentrations in the external solution (43). These physiological mechanisms likely play a key role in the species' tolerance and detoxification strategy under arsenic stress.

(35 y 36) investigated the ability of *S. minima* to bioaccumulate Pb(II), Cd(II), and Cr(VI), evaluating the influence of pH and light intensity in batch systems. These authors were the first to report that *S. minima* functions as a Cd hyperaccumulator, as the bioconcentration factor (BCF) exceeded 2000 in all tested conditions, and Pb content in biomass reached 1.1%. However, the removal of Cr(VI) was very limited. Later studies examined the influence of environmental factors and nutrient availability on the removal mechanisms and Pb(II) distribution across different compartments (biomass, water column, and sediments) in lagoon microcosms containing *S. minima*. In the absence of EDTA and phosphates, adsorption onto the biomass surface was identified as the predominant removal mechanism, even at Pb(II) concentrations as high as 13 mg/L. Under these conditions, a BCF of 2065 ± 35 and a Pb(II) content of 2.7% in the biomass were reported, further confirming that *S. minima* is a Pb hyperaccumulator.

In such batch-operated lagoon microcosms, the mechanisms of Pb(II) removal and its compartmentalization were found to primarily depend on the presence of specific nutrients and ligands, with environmental conditions playing a secondary role. Based on these references, and considering the high As accumulation observed in our own experiments, it is reasonable to propose that *S. minima* may also be categorized as a hyperaccumulator of arsenic under certain conditions.

In our long-term experiments, with exposure times reaching up to 384 hours, *S. minima* continued to grow, as indicated by the increase in fresh weight and the stable dry weight throughout the experiment. Moreover, a highly significant accumulation of arsenic in plant tissues was observed at 240, 336, and 384 hours, reinforcing its classification as an arsenic-concentrating species according to the bioconcentration factor (BCF) values. These results are consistent with the findings of (38) and further support the remarkable metal uptake capabilities of this species.

Therefore, based on our results, *S. minima* can be considered a promising candidate for the phytoremediation of arsenic-contaminated environments. Its ability to grow under prolonged exposure, combined with its high accumulation capacity, highlights its potential role in the recovery and/or restoration of degraded water bodies and soils, ultimately contributing to the improvement of environmental quality.

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