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Bioremediation of highly arsenic-contaminated soils: A case study in the Central Andes of Ecuador

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ABSTRACT

In this research, the capacity of three phytoremedial species in an agricultural soil highly contaminated with Arsenic (As) of the Tiliche San José Irrigation Board, Cotopaxi-Ecuador, was evaluated. A Full Randomized Design was used with three treatments (*Canna indica* L., *Medicago sativa* L. and *Cortaderia nitida* L.) and three replications; the comparison of means was performed with Tukey's test at 5% significance. The reduction of arsenic in the agricultural soil, transfer factor (TF), bioaccumulation (BF) and development of the species at 90 and 210 after transplantation were determined. The results revealed that the bioremediation of As with *Medicago sativa* L. is highly significant ($p < 0.01$) achieving a reduction of 85±1.5%, but decreased with *Canna indica* L. (70±2%) and *Cortaderia nitida* (45±0.7%) at the end of the experiment. However, the treatments did not achieve even 1/3 of the height of their species under normal conditions, evidencing the complex relationship between the accumulation of As and plant development. The phytoremediation of As was superior with *Medicago sativa* L. due to its greater transfer capacity and bioaccumulation, so it absorbs As in its tissues, while reducing the concentration of it in highly contaminated soil. But, *Cortaderia nitida*, a plant from the area with slow growth and reduction of Ace, could be a long-term alternative.

Keywords: arsenic, *Cortaderia nitida*, *Canna indica* L., *Medicago sativa* L.

INTRODUCTION

Soil contamination by heavy metals (HMs) has become one of the main environmental problems, caused both by human activities such as mining, industry and fertilizer use, as well as by natural processes such as erosion and volcanic activity (Guzmán et al., 2019; Shehata et al., 2019). Although heavy metals are natural components of soil, the problem arises when their concentrations exceed safe levels, causing toxicity, bioaccumulation in the food chain and adverse effects on human health, such as kidney and neurological diseases, as well as on biodiversity (Awa & Hadibarata, 2020). Similarly, river ecosystems are affected, as rivers act as dispersal vectors, transporting polluted sediments and retaining heavy metals in their beds, amplifying environmental impact (Al-Amrani & Onaizi, 2024; Guzmán et al., 2019). Soil and water are fundamental resources for the development of irrigated agriculture, but they are also means through which plants and humans can be affected by contaminants such as arsenic (As).

(Polechońska et al., 2022; Velázquez et al., 2022). The agricultural sector relies heavily on river water for crop irrigation; however, the use of contaminated water such as As, directly affects the quality of soil and vegetation, since all these factors make soils arid and inefficient for agricultural activities (Aguilar & Cubas, 2021; Calcina-Benique et al., 2022). Recent studies have shown that irrigation with contaminated water increases the accumulation of heavy metals in the soil, which are absorbed by plants and subsequently enter the food chain, affecting human health (Jomova et al., 2025; Kayode et al., 2021; Mahurpawar, 2015).

In regions where irrigation water contains high levels of As, significant accumulation of this metal has been observed in crops such as rice and vegetables, posing a risk to consumers (Genchi et al., 2022; Rahaman et al., 2021; Shaji et al., 2021). In Ecuador, real cases of As contamination have been reported in provinces such as Manabí and Esmeraldas, where levels of As have been detected in soils that exceed 20 mg/kg due to the use of contaminated water for irrigation (Jiménez et al., 2023).

Currently, various technologies have been developed for the removal of As from the soil, such as chemical leaching, electro-remediation and phytoremediation. Among these methods, phytoremediation stands out for being a sustainable, economical and ecologically viable technique (Sevak & Pushkar, 2024). And addition, phytoremediation not only removes contaminants, but also improves the physical, chemical, and biological quality of the soil, making it a preferred choice for the recovery of contaminated soils (Yao & Zhou, 2024). Unlike other methods, phytoremediation uses plants to absorb, accumulate, or degrade pollutants, minimizing environmental impact and reducing costs (Mendarte-Alquisira et al., 2021). One of the species that stands out in recent years within phytoremediation is *Medicago sativa* L. because it is capable of absorbing up to 4 mg/kg of As in its stem, leaves (aerial part) and small amount in the root (Puente-Valenzuela et al., 2019; Helaoui et al., 2023). Another important species is *Canna indica* L., studies reveal the removal of heavy metals, since this plant concentrates mainly on the roots (accumulated organ), followed by the aerial parts, where it mainly affects with the decrease of plant biomass (de Meyer et al., 2017; Sandil et al., 2021). Water and soil contamination, specifically arsenic, poses a significant risk to human health in irrigated agricultural areas. Therefore, in this research the removal of high concentrations of As was evaluated in a case study of the Andean region of Ecuador, using two phytoremdiating species (*Medicago sativa* L. and *Canna indica* L.) and a local plant known as Sigse (*Cortaderia nitida*).

MATERIAL AND METHODS

Study location

The research was carried out in the area of the Tiliche San José Irrigation Board, Tanicuchí parish (3115 meters above sea level), Cotopaxi province, in the central Andes of Ecuador (Figure 1). The area is characterized by an average temperature ranging between 13 and 14 °C and a bimodal precipitation regime (662 mm/year) (Ilbay-Yupa et al., 2021). The waters with which the Board irrigates correspond to a tributary of the Pumacunchi

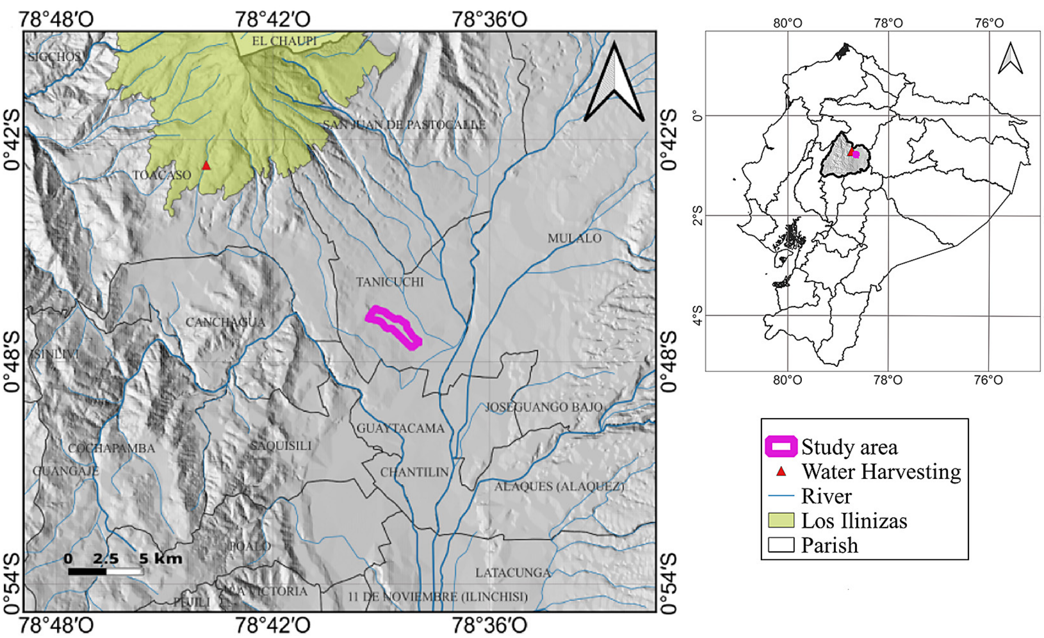

Figure 1. Map of location, altitude of the study area

River with arsenic concentration levels of 0.91 mg/L (Pazmiño et al., 2022), which is born from the Los Ilinizas Ecological Reserve. In other words, the board has been using polluting water since 2018 with values that exceed the regulations for irrigation use (0.01 mg of As/L) (TULSMA, 2015), the same that have accumulated in the soil.

Vegetative material

The present study used 15- and 30-day-old seedlings of alfalfa (3 branches of 0.10 m) and achira (2 true leaves) respectively; In the case of the *Cortaderia nitida*, a cutting with 2 canes (0.15 m) and roots (≈ 0.05 m) was used.

Alfalfa (*Medicago sativa* L.) is a legume widely used in agriculture due to its ability to fix atmospheric nitrogen, which contributes to soil enrichment. Due to its deep root system, it improves soil structure and increases water retention capacity (Baragaño et al., 2022). It is a plant with a great capacity to accumulate HM, recent studies have shown that alfalfa can harbor high concentrations of these elements in its tissues (Helaoui et al., 2023), these characteristics make it a promising species for use in phytoremediation strategies (Raklami et al., 2021). In Mexico, alfalfa demonstrated an absorption capacity of up to 25.25 ± 3.55 mg of As/kg of soil when exposed to a concentration of 10 mg/L in irrigation water (Puente-Valenzuela et al., 2019)

Achira (*Canna indica* L.) is a species that grows in poor soils, with a high production of leaf mass, which improves soil structure and increases organic matter content (Di Luca et al., 2024). Widely used in phytoremediation due to its adsorption of HM in its roots, and subsequently transported to parts such as leaves and stems (Chen et al., 2022.; Sharma et al., 2014). In turn, this plant produces phytochelins that bind to metals such as As and reduce their toxicity, so it can survive in highly contaminated soils (Martínez-González et al., 2017).

Sigse (*Cortaderia nitida* L.) belongs to the native grasses of the Andes, used for soil conservation and erosion prevention in the highlands, due to its dense root system (Paredes-Páliz et al., 2024). Regarding phytoremediation, (Bech et al., 2017), established that Sigse is a plant with an accumulation potential in its leaves unlike roots, this because grasses are not efficient in root accumulation.

Initial state of the soil

The determination of the concentration of As was carried out in 9 experimental units, each with an area of 6.05 m². To take soil samples, the zigzag method was used, recognized for its effectiveness in heavy metal studies (Ojeda, 2021; Unda et al., 2021). This technique ensured a representative collection of contaminated soil samples, which were subsequently analyzed to assess arsenic levels using inductively coupled plasma atomic emission spectrometry.

Experimental design

Three phytoremediation plants (Treatments) were evaluated such as Achira (*Canna indica* L.), Alfalfa (*Medicago sativa* L.), and Sigse (*Cortaderia nitida*) with three replications in an area of 73.62 m^2; each experimental unit consisted of 2.46 m in width and length and a road separation of 0.3 m; the separation of 0.2 m plants for a total of 144 plants per experimental unit. A complete randomized design (CRD) was used, which is used for the comparison of three or more treatments, in all experimental executions were performed randomly. This to determine the existence of a significant difference between the treatments, when comparing the Alpha value of the Fisher statistic calculated with the Fisher tabulated at 0.05 and 0.01, which determines that if F calculated is greater than 0.05 there is a significant difference and if the difference is 0.01 it is highly significant; otherwise, the treatments would not differ between them.

The mathematical model for a CRD is as follows:

$$\gamma_{ij} = \mu + \tau_i + \varepsilon_{ij} \quad (1)$$

where: γ_{ij} – corresponds to the response variable of the *j-th* observation to the *i-th* phytoremediation plant, which correspond to the treatments; τ_i – corresponds to the effect of each of the treatments, i.e., the effect of each of the arsenic phytoremediation plants, related to the deviation from the overall mean of the treatments; ε_{ij} – corresponds to the general error, generated by the random effect of each of the *j-th* observations at the *i-th* treatment, whose random value is independent and is normally distributed. The residues generated by the experiment, corresponding to ε_{ij} and follow a normal distribution, according to the test carried out by Kolmogorov-Smirnov, as follows:

$$F_n(x) = \frac{1}{n}\sum_{i=1}^{n} 1\,(X_i \leq x) \tag{2}$$

where: $F_n(x)$ is an empirical function that allows the cumulative distribution of a sample of size n, where X_i corresponds to each of the observations of the ordered sample. The indicator function takes the value 1 if $X_i \leq x$, otherwise it is zero. On the other hand, the theoretical function given by

$$F(x) = P(X \leq x) \tag{3}$$

In our case, it corresponds to a normal distribution with mean and variance $N(\mu, \sigma^2)$. The probability P of the random variable X takes a value less than or equal to x. When calculating the maximum absolute distance (D_n) between $F_n(x)$ and $F(x)$ and comparing it with the critical value obtained in tables, it was possible to answer that the null hypothesis (H0) is accepted, i.e., that the residues follow a normal distribution in all the treatments and variables studied.

For the homoscedasticity test, Bartlett's test (B) was performed, which follows a chi-square distribution (χ^2) with k – 1 degrees of freedom, as follows:

$$B \sim \chi^2_{(k-1)} \tag{4}$$

Associated with a significance level of α equal to 0.05, where, if p is greater than or equal to α, the null hypothesis is accepted, which indicates that the existence of homoscedasticity of the variances is assumed, as was given in the present research in all variables.

Tukey´s test

The statistical significance, with α = 0.05 and α = 0.01, determines that there is a difference between the treatments, that is, if $p < 0.05$ or $p < 0.01$, the null hypothesis is rejected, which establishes that the means of the treatments are different. This result allowed Tukey's test to be applied at 5% significance, to compare the means of the treatments by pairs. The equal letters establish a similarity between, with the most relevant to the least relevant being those determined as a, then b and so on. The confidence interval between two means is calculated as follows:

$$Tukey = D \pm q \times \sqrt{\frac{MSE}{n}} \tag{5}$$

D corresponds to the difference between the means, q is Tukey's critical value, MSE represents the mean square of the error obtained from ANOVA and n is the sample size.

This test can be used when trying to compare the effect of contamination, measured in germination and biomass, in phytotoxicity tests (Zawierucha et al., 2022), it has also been applied to identify differences in hydrocarbon degradation, metal removal, bacterial proliferation and toxicity reduction, therefore, this test, it is considered feasible to compare the effect of *Canna indica* L., *Medicago sativa* L., *Cortaderia nitida* for arsenic phytoremediation.

Experiment management

Seedlings and cuttings were planted on April 20, 2024; after 8 days, a transplant was performed, mainly of *Cortaderia nitida,* to replace the plants that failed to adapt due to their initial slow growth. Subsequently, a biostimulant enriched with phosphorus and potassium was applied to promote root development and improve plant nutrition. Irrigation was carried out by a sprinkler system, using water for human consumption with a pH of 7.5, and was applied every 8 days to maintain an adequate water supply. In addition, hilling work was carried out 30, 60 and 90 days after transplanting, which allowed for better soil aeration, strengthening of the root system and weed elimination, thus reducing competition for resources.

Measuring plant growth

Plant height was measured from the base of the root neck of 10 randomly selected plants in each experimental unit every 30 days. This procedure was essential to evaluate the growth and development of plants, since it allows quantifying changes in their vertical structure over time (Di Benedetto & Tognetti, 2016).

Percentage reduction of arsenic in soil

The percentage of reduction of an As in soil is calculated based on the initial concentration of HM in contaminated soil (mg/kg) and the concentration after the treatment process is completed (Estrada, 2024) as detailed below:

$$PR = \frac{Initial\ concentration\ of\ As - Concentration\ final\ of\ As}{Initial\ concentration\ of\ As} \tag{6}$$

Determination of transfer factor (TF) and bioaccumulation (BF)

The determination of these factors is a key indicator to evaluate how HMs are distributed in plant tissues and their impact on plant growth and survival (Shukla et al., 2024). TF is defined as the ratio of As concentration in the aerial part of the plant (usable part) to the concentration of As in the roots. This parameter reflects the efficiency with which HM is translocated from the roots to the upper tissues of the plant (Loredo-Tovías et al., 2022). On the other hand, BF refers to the ratio between the total concentration of As in the plant (or in a specific part of it) and the concentration of the metal in the soil. This indicator measures the plant's ability to absorb and accumulate metal from the soil (Lesmeister et al., 2021). Arsenic levels in plant roots and aerial parts were determined by inductively coupled plasma atomic emission spectrometry.

$$TF = \frac{Concentration\ of\ As\ in\ the\ usable\ part}{Concentration\ at\ the\ root} \quad (7)$$

$$BF = \frac{Total\ Ace\ concentration\ in\ the\ usable\ part}{Concentration\ in\ the\ soil} \quad (8)$$

RESULTS AND DISCUSSION

Arsenic extraction

The ANOVA analysis shows that there are highly significant differences in the percentage of As reduction in the soil at 90 and 210 days ($p < 0.01$) due to the effect of the three phytoremediation species. The As at the beginning of the experiment was 14.62 mg/kg of soil, a value that exceeds the Ecuadorian norm (5 mg As/kg) (TULSMA, 2015). At 90 days after sowing, the three species caused a reduction in As of more than 43% and at 210 days the values increased, reaching a reduction of 85±1.5% in *Medicago sativa* L, 70±2% in *Canna indica* L. and 45±0.7% in *Cortaderia nitida* (Figure 2). The greater capacity of the first species is due to adsorption in its roots, accumulation in all its organs, rapid growth and biomass production (Raklami et al., 2021).

Arsenic concentration in soil, cauline system and root system

The results of the analysis of variance showed highly significant differences ($p < 0.01$); this indicates that As concentration varied significantly depending on the treatments applied. The soil under the treatment of *Cortaderia nitida* L. presented the highest concentration of arsenic both at 90 days (8.20 mg/kg) and at 210 days (8.01 mg/kg). In contrast to *Medicago sativa* L. which showed the lowest concentration of As in the two periods (3.68 and 2.17 mg of As per kg of soil). In the case of the root system, *Canna indica* L. and *Medicago sativa* L. shows a greater accumulation of As; but at 210 days *Cortaderia nitida* led the contraction (2.51 mg As per kg of root). In the cauline system, *Medicago sativa* L. generated the highest accumulation of As at 90 and 210 days (8.62 and 10.75 mg/kg); followed by *Canna indica* L. (9.63 mg/kg) and finally (4.1 mg/kg) for the last period (Figure 3). The results show that the accumulation of As in plant organs not only varies between species, but also as a function of time and plant tissue evaluated. Phytoremediation studies report that

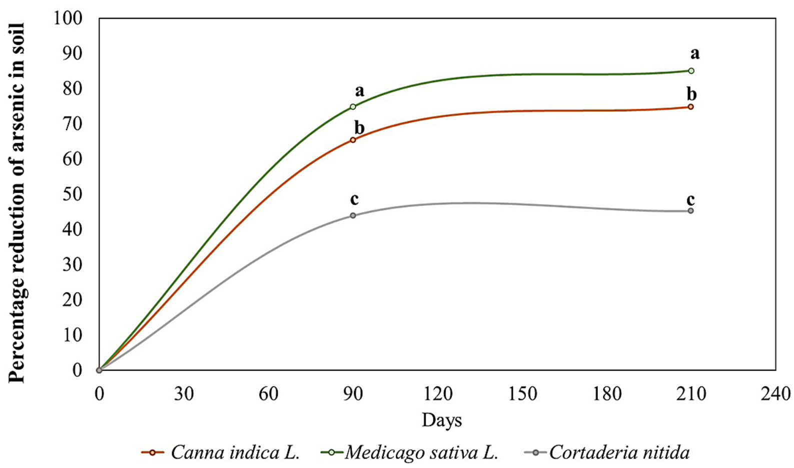

Figure 2. Arsenic reduction curve in agricultural soils

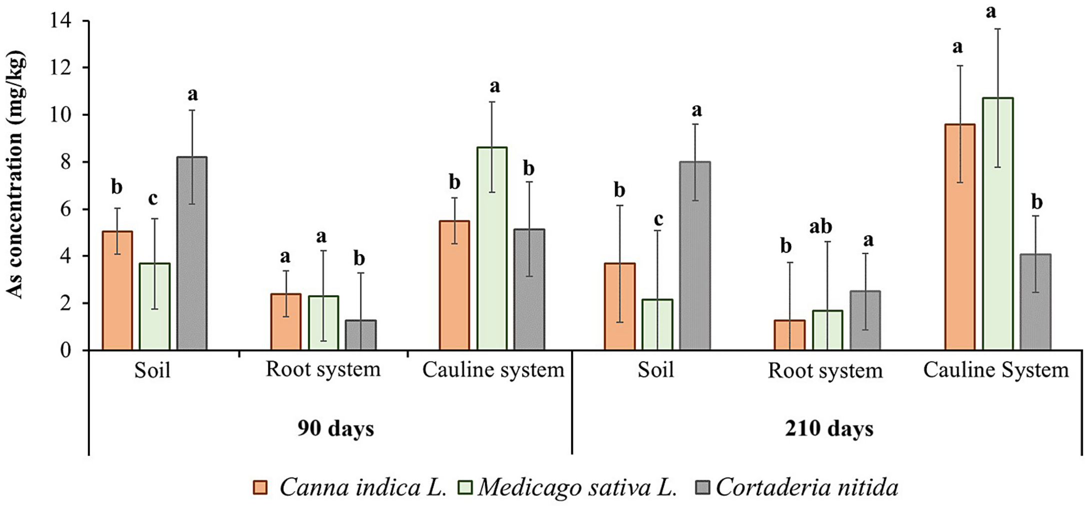

Figure 3. Arsenic concentration in soil, cauline and root system at 90 and 210 days

Medicago sativa L. obtains a higher percentage of As extraction compared to *Canna indica* L. (Chen et al., 2022; Puente-Valenzuela et al., 2019) however, *Cortaderia nitida* could have slower mechanisms of absorption in the long term.

Transfer factor and bioaccumulation factor

The analysis of variance for the three species of phytoremedial plants shows that there are highly significant differences in the FC and BF of As in agricultural soil at 90 and 210 days ($p < 0.01$). The TF for *Medicago sativa* L. at 90 and 210 days presented values of 3.85 and 6.39, respectively, unlike the other species that did not exceed the value of 2.07 at 210 days (Figure 4a). In other words, the study evidences the ability of plants to absorb and transfer As from the soil to its different parts (Loredo-Tovías et al., 2022). Therefore, the higher values recorded in the study reveal a greater transfer of As by *Medicago sativa* L. from the root to the different parts of this plant, due to the fact that the plant has a fairly strong root system so they can absorb and distribute PTEs (Chen et al., 2022).

In the case of BF, *Medicago sativa* L., presented the highest result at 90 and 210 days with 2.35 and 5.00, respectively, these values are similar to that of the study presented by (Castro-González et al., 2018). *Cortaderia nítida* reached a value of up to 0.63; thus, as *Canna indica* L., 0.16 in the 90 days (Figure 4b). In the same way, the stems and leaves are the organs where As accumulates compared to the concentration in the soil.

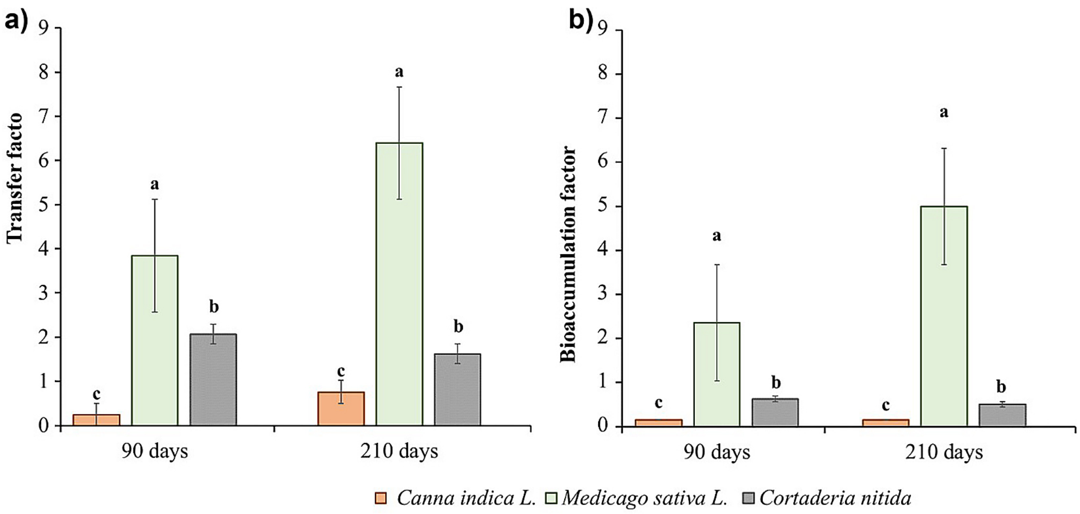

Figure 4. Transfer factor and bioaccumulation factor of the tree species

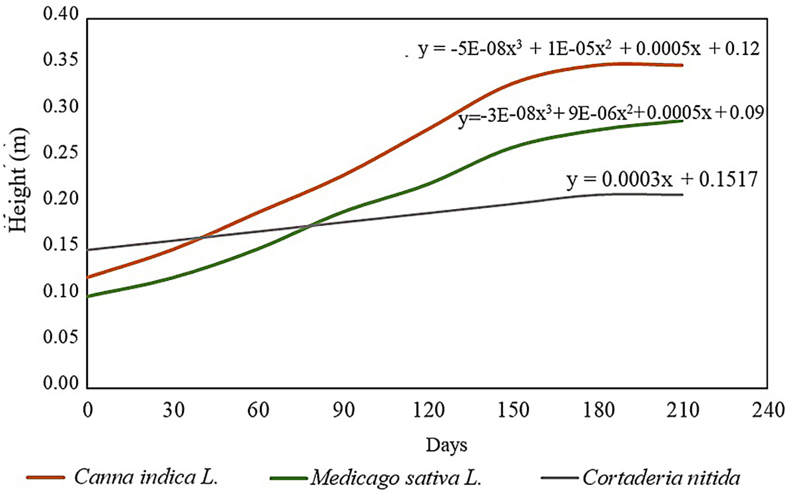

Figure 5. Growth curves of the three phytoremediation species

Effect of arsenic on plant development

The development of the three species was affected; thus, the height of *Canna indica* L. reached 0.35±0.04 m, with a polynomial growth of order 3 (0.033 m/month); in the same way, *Medicago sativa* L. presented a polynomial growth reaching 0.3±0.035 m (0.03 m per month). That is, these two species initially showed rapid growth, but from 150 their growth stabilized. For *Canna indica* L. the growth was different during the 210 years its height increased on average 0.01m per month, reaching only 0.21±0.02 m, when this plant can reach 3 m (Figure 5). The results suggest a complex relationship between the accumulation of heavy metals and their development, the toxicity caused by high concentrations of heavy metals results in chlorosis and slow growth (Ruiz-Huerta & Armienta-Hernández, 2012). *Canna indica* L. and *Medicago sativa* L. was able to adapt initially but not tolerate stress due to the accumulation of high levels of As. On the other hand, *Cortaderia nitida* had a slower growth, which can be said that high concentrations of As affected its reproductive processes (Rodríguez et al., 2016).

CONCLUSIONS

The three species evaluated in this research show significant differences in their ability to accumulate and transfer arsenic *Medicago sativa* L. emerges as the most effective species for arsenic phytoremediation, due to its high biomass, ability to accumulate arsenic in its tissues, particularly in the cauline system and physiological adaptability, achieving a reduction of 85±1.5%. These values were higher than *Canna indica* L. and *Cortaderia nitida*, which obtained 70±2% and 45±0.7% respectively. The main difference between the three species lies in the balance between growth, stress tolerance and translocation efficiency, even after the plants failed to develop even 1/3 of what they normally should grow.

Phytoremediation with *Medicago sativa* L. in a case study from the Ecuadorian Andes allowed a drastic reduction of arsenic in highly contaminated agricultural soils, confirming its suitability for active phytoextraction strategies. In contrast, *Canna indica* L. and *Cortaderia nitida* showed significantly lower bioaccumulation ($p < 0.01$) which limited their efficacy. However, *Cortaderia nitida*, which grows and reduces slowly, could be a long-term alternative.

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