# ARSENIC UPTAKE IN LETTUCE: ITS IMPACT ON CROP QUALITY AND METABOLIC STRESS

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**Abstract.** Currently, the presence of arsenic (As) in groundwater is a global concern to agricultural irrigation. It can produce a stressful environment in plants, causing both physiological and metabolic changes. The objective of this study was to evaluate the physiological and metabolic effects derived from As absorption in a hydroponic lettuce crop. A hydroponic system with different concentrations of As (0-16 µM) was used. The physiological development and quality of the crop were evaluated. The absorption of As in vegetal structures was evaluated to obtain the translocation and bioconcentration factors. The lettuce absorbed As in a range of 0.22-2.18 and 1.34-68.23 mg As/kg in leaves and roots, respectively. Arsenic caused a decrease in plant growth (>1 µM As) and biomass (<2 µM As). At 16 µM As, the observed changes were in color and Chlorophyll content of leaves. Phenolic compounds presented activation and inactivation at different As concentrations. The bioaccumulation factor shows that lettuce can efficiently absorb As; however, as it has low translocation factor, it is considered that As absorbed by the plant was not translocated to the aerial part. The study demonstrates the ability of lettuce to withstand As toxicity and still grow to a size suitable for commercial purposes, even under high As concentrations. It highlights the role of hydroponic system in facilitating As absorption, particularly in roots. Even though As accumulates within lettuce structures, it has limited movement within the plant. Notably, the study reports a high bioconcentration factor, suggesting that lettuce could serve as an effective As accumulator. Therefore, lettuce might be a viable option for As remediation due to its ability to absorb substantial amounts.

**Keywords:** *plant stress, arsenomic, arsenic pollution, arsenic bioaccumulation, hydroponic lettuce*

## Introduction

Nowadays, arsenic (As) contamination in water supplies still represents a serious problem, threatening the health of billions people around the globe. Especially aquifers are gravely affected by the presence of this metalloid (Alam et al., 2019), since many of them exceed the maximum As levels of 10 µg/L, permitted by the World Health Organization (WHO, 2001). The presence of As in water is related to both natural and anthropogenic causes (Shakoor et al., 2015). The latter involve coal combustion, mining, smelting, textile industry, and the use of arsenical pesticides, all contributing to the pervasive presence of As in the environment (Yañez et al., 2019). The Agency for

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Toxic Substances and Disease Registry (ATSDR, 2007) in the USA have concerns about As presence in groundwater for agricultural irrigation through the use of organic As in some chemical products (pesticides), which mainly contain compounds such as cacodylic acid, disodium methyl arsenate (DSMA), and monosodium methyl arsenate (MSMA), among others. However, there are sites where the main source of As contamination is due to natural sources, i.e., volcanic activity, rock erosion, and minerals (Arreguín et al., 2012; Martínez-Toledo et al., 2017). These, together with anthropogenic activities, increase the risk of As presence in aquifers.

A great number of studies have reported that human exposition to As is related to the development of several diseases, including respiratory affectations, neurological disorders, and several types of cancer (Rasheed et al., 2018, 2016; Sodhi et al., 2019). Hence, As has been declared a member of the carcinogenic group 1 by the International Agency for Research on Cancer (IARC) (IARC, 2012). Recently, As contamination in the water-soil-plant system has received special attention due to its ability to enter the food chain seriously affecting human health (Molin et al., 2015; Sarkar et al., 2016).

The phytotoxicity of As from irrigation water is variable, since plants have different capacity to absorb it (Saldaña-Robles et al., 2018). Plants might be either accumulative or sensitive towards As. Generally, As presence and absorption induce a stressful environment for plant growth (Kalita et al., 2018). Previous research reported that As stress in plants can cause (a) decrease in harvest yield (Rasheed et al., 2018; Sarkar et al., 2016), (b) root reduction and shoot growth (Singh et al., 2016), and (c) modifications of the primary and secondary metabolites (Martinez-Castillo et al., 2022). It also causes stress in plants due to the hyperproduction of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), which might provoke changes in the activity of some antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione synthetase (GSH), as well as the levels of endogenous antioxidants (Farooq et al., 2021, 2016). ROS and RNS hyperproduction leads to the peroxidation of membrane lipids, causing cell death (Farooq et al., 2016). In general, some toxic effects of As on plants include physiological and metabolic damages affecting their development and growth; however, with increased As concentrations, plant death may occur. In addition, As is distributed and bioaccumulated in different vegetable structures (root, stem, leaf, flower, and fruit).

There are crops of high commercial value that are exposed to As stress directly through irrigation. In lettuce, absorbed As accumulates in greater quantities in the root than in the aerial part (leaves) (Cadet et al., 2021; Sandil et al., 2019). The behavior of this plant commonly produces low translocation and high bioaccumulation of As (Yañez et al., 2019). The presence of As in lettuce affects it on the physiological level, since it causes a decrease in the growth and biomass of the plant (Yañez et al., 2019). At the metabolic level, As can produce the destruction of the chloroplast membrane and induce an abnormal metabolism of secondary metabolites (Wang et al., 2022).

There is still little information on how secondary metabolites act when lettuce activates its defense mechanism against stress due to the absorption of As. There are some reports that these metabolites can increase in stress situations, although a decrease has also been reported (Martinez-Castillo et al., 2022). In addition, the relationship that these metabolites have with other physiological variables (already reported in the lettuce crop) has not been discussed in detail. Therefore, the aim of this work is to evaluate the physiological and metabolic effects derived from As induced stress in a hydroponic lettuce crop and also to determine the absorption of As in vegetable structures of lettuce plants, including As bioaccumulation and transfer factors.

## Materials and methods

### *Lettuce plant culture*

Seedlings of Romaine type lettuce (*Lactuca sativa* L. var. longifolia) after 4 weeks of germination were employed. These seedlings were obtained from the Alfaro hotbed located in Hidalgo, Mexico. A disinfection protocol was established for the seedlings, which consisted in washing the root with purified water, followed by a wash with a 10 % hydrogen peroxide solution for 5 min (Manrique, 2011). Finally, the root was rinsed with purified water. Subsequently, the seedlings were transplanted to the hydroponic system to allow their acclimatization. The Douglas universal nutrient solution (Douglas, 1976) was added every 10 days.

### *Stress in lettuce by As exposure*

This experiment was carried out in a NFT (Nutrient Flow Technic) hydroponic system, located at the University of Guanajuato (20°74’43.2” N-101°33’65.2” W)., six independent treatments with five lettuce seedlings in each treatment were placed in the hydroponic system. The treatments were adapted with an individual recirculation system, using a nutrient solution with different As concentrations for each treatment (T1 = 0, T2 = 1, T3 = 2, T4 = 8, T5 = 12, T6 = 16 µM As). Arsenic was supplied in the form of arsenate ($AsO_3H_2$) by dissolving $Na_2HAsO_4*7H_2O$ in the Douglas universal nutrient solution (optimal nutrient range in ppm: N = 250, Ca = 200, Mg = 75, P = 80, K = 300, S = 400, Cu = 0.5, B = 5, Fe = 5, Mn = 2, Mo = 0.002, Zn = 0.5) (Douglas, 1976). The experiment was carried out by duplicates. After the acclimatization period, the As solution was added to stress the plants every 10 days. The nutritive solution contained the proper amount of As for each treatment. During the lettuce cultivation period, measurements (plant length and weight) were carried out to evaluate the effect of As on plant physiology and secondary metabolism. At harvest time, As absorbed in different plant structures was evaluated, including its bioaccumulation and transfer factors.

### *Physicochemical determinations*

#### *Length and weight*

Physical measurements were evaluated at the beginning and end of the harvest period. The measurements were made by using a vernier with a millimeter scale. The length of the root as well as the aerial part was measured (Rengifo and Avila, 2013). In addition, the weight of each plant structure (roots and leaves) was determined to obtain the total weight (biomass) by using an analytical balance (Rengifo and Avila, 2013).

#### *Color*

The color of lettuce leaves was determined from the color scale of the CIELab model (coordinates L*, a*, b*, c* and angle h°), using a ColorFlex Ez CFEZ 0483 colorimeter (HunterLab) (López-Valencia et al., 2018).

### *Determination of total chlorophyll (TC)*

The amount of TC in lettuce was measured in leaves. The sample was dehydrated at 30 °C, and an extract 1:200 (weight/volume) using acetone (80 %) as extraction solvent. An UV-Vis Genesys 10S spectrophotometer was employed to determine chlorophyll A at 664 nm and chlorophyll B at 647 nm. A blank with extraction solvent was used for each of the readings.

### *Determination of total phenolic compounds (TPC)*

For TPC determination, the Folin-Ciocalteu method proposed by Singleton et al. (1999) was employed with slight modifications. First, a hydroalcoholic extract (80 %) with lettuce plant (root, leaf) 1:10 (weight/volume) was obtained by the maceration technique. An aliquot of the obtained extract was reacted with 1 N of Folin reagent and 7.5 % sodium carbonate, then it is measured in a UV-Vis Genesys 10S spectrophotometer at a wavelength of 760 nm. The results was expressed as mg gallic acid equivalent (mg GAE/g of extract).

### *Determination of total As*

The total quantification of As in plant parts (root and leaves) was performed after acidic digestion on a Atomic Emission Spectroscopy (AES) coupled with a graphite furnace (Thermo, iCE 3500). The digestion of vegetable matrix (root, leaves) was carried out with HCl and $HNO_3$ for 1 h at 90°C (EPA, 2007a, b). A nitrous oxide/acetylene and a stoichiometric flame were used in a range of 2100 to 2600°C of atomic emission. Measurements were carried out to an emission wavelength of 235 nm and a fuel flow rate of 4.1 to 4.6 L/min. The calibration curve was constructed in the range of 1-100 μg/mL.

### *Determination of bioconcentration and translocation factors*

The As mobilization in lettuce structures was evaluated by calculating the bioconcentration (BCF) and translocation (TF) factors. The BCF is the ratio of total As concentration in the root of vegetable dry weight basis with respect to the total As concentration in the soil/substrate (*Eq. 1*).

$$\mathrm{BCF} = \frac{\text{As root}}{\text{As hydroponic system}} \tag{Eq.1}$$

The TF in the lettuce plant was evaluated according to *Equation 2*, where TF is the ratio of As concentration in leaves with respect to the As concentration in the root.

$$\mathrm{TF} = \frac{\text{As aerial}}{\text{As root}} \tag{Eq.2}$$

### *Statistical analysis*

The statistic analysis were performed with the software STATGRAPHICS Centurion XVI.I. All results are expressed as mean values (5 samples per treatment) with standard deviation (means ± SD). Significant differences were determined using a simple analysis of variance (ANOVA) with the Tukey comparisons test at $p < 0.05$ significance level. In addition, a correlation matrix of the physicochemical and metabolic variables was carried out for the different As concentrations absorbed in the lettuce plant (roots and leaves).

## Results and discussion

### *Effect of As on the growth and development of lettuce plants*

The harvest of lettuce plants was carried out after 50 days of cultivation. *Table 1* shows the results of growth and development of lettuce plants. The weight of the whole plant was affected by As stress, this weight was used to determine the Total Biomass (TB) of lettuce plants. TB was determined based on the total dry weight of the plant and is a critical parameter for assessing the impact of As stress on the plant growth (Yañez et al., 2019). All treatments of TB presented statistically significant differences ($p \leq 0.05$) with respect to the control treatment (T1). It has been reported that a decrease in TB is one of the main effects of As stress on several plants (Singh et al., 2016), as it occurs in this work from T3 to T6. In lettuce plants, Yañez et al. (2019) reported a TB decrease ranging from 30 % to 40 % when the plant was exposed to As stress through the soil and irrigation water. Similar results were reported by Gusman et al. (2013), where the authors reported a reduction in TB by 39 % and 29 % for stress by As(V) and As(III), respectively, in lettuce crop plants under hydroponic conditions.

As observed in *Table 1,* the length of the whole plant was also affected by As stress. The treatments T1 and T2 (0 and 1 μM As) show the largest length of lettuce plant with 45.30 ± 6.81 and 47.67 ± 6.43 cm respectively. This largest length for T1 (0 μM As) was due to the absence of a stressing agent that limits the development of the plant, while the length of the plant in T2 (1 μM As) was possibly stimulated by the response before stress in plants, this is according to the reported by Lichtenthaler (1996). The greatest negative effect of As on lettuce length is observed in T3, T5, and T6 (~30 cm), in which no significant statistical difference ($p \leq 0.05$) was observed among treatments, and this is due to an increase of As concentration in treatments. It is hypothesized that the decrease in the length of plant as a whole could be related to the damage suffered by the root, since this is the first absorption filter that removes As from water, and by affecting the radicular part. Consequently, the aerial part is affected as well. There are reports of damage mainly in the root area of a lettuce plant, with a decrease in root growth for As concentrations of 5-12 mg/kg (60-160 μM) (Kim et al., 2018) and 10-25 mg/kg (133-333 μM) (Cadet et al., 2021) in lettuce crops grown in soil.

***Table 1.*** *Growth and development parameters in lettuce plants*

| Parameter | | T1 | T2 | T3 | T4 | T5 | T6 |
|---|---|---|---|---|---|---|---|
| Length plant (cm) | Root | $15.0\pm3.61^{b}$ | $8.83\pm0.29^{a}$ | $8.17\pm1.44^{a}$ | $9.17\pm0.29^{a}$ | $9.50\pm1.32^{a}$ | $8.50\pm0.87^{a}$ |
| | Leaf | $32.2\pm1.61^{c}$ | $38.83\pm6.25^{d}$ | $20.0\pm2.50^{a}$ | $29.33\pm1.76^{bc}$ | $23.83\pm2.47^{ab}$ | $25.17\pm0.29^{ab}$ |
| | Whole plant | $45.30\pm6.81^{c}$ | $47.67\pm6.43^{c}$ | $28.17\pm1.44^{a}$ | $37.50\pm1.50^{b}$ | $33.33\pm3.06^{ab}$ | $33.67\pm1.15^{ab}$ |
| Weight plant (g) | Root | $2.73\pm0.57^{b}$ | $2.76\pm0.15^{b}$ | $2.39\pm0.47^{a}$ | $1.66\pm0.22^{a}$ | $1.52\pm0.45^{a}$ | $1.57\pm0.11^{a}$ |
| | Leaf | $9.37\pm3.43^{b}$ | $4.61\pm0.93^{a}$ | $3.38\pm0.30^{a}$ | $2.03\pm0.18^{a}$ | $1.50\pm0.49^{a}$ | $2.85\pm0.42^{a}$ |
| | Whole plant | $18.22\pm2.39^{c}$ | $10.91\pm0.69^{b}$ | $9.40\pm0.20^{a}$ | $7.01\pm0.21^{a}$ | $4.47\pm0.13^{a}$ | $6.11\pm0.46^{a}$ |
| Number of leaves | | 14±3 | 15±2 | 10±2 | 13±1 | 12±2 | 14±1 |

The results were obtained after 50 days of experimentation. Lowercase letters between columns represent statistically significant differences ($p \leq 0.05$). As concentrations: T1 = 0 μM (control), T2 = 1 μM, T3 = 2 μM, T4 = 8 μM, T5 = 12, and T6 = 16 μM As

The number of leaves in lettuce plants appears to be more affected in T3. The As did not directly affect the number of leaves in a proportional manner as the As concentration was increased in the treatments. However, there is a relationship between TB and plant length in T2, which has the highest number of leaves. Probably, the As concentration in T3 (2 µM As) caused a negative stress that does not allow the growth of plants and affects their physiological parameters, while As concentrations in other treatments (T2 and T4) produce positive stress or stimulation (Martinez-Castillo et al., 2022).

### *Effect of As on color parameters of lettuce plants*

The color changes of lettuce leaves is reported in *Table 2* by CIELab system. The coordinate a* shows a trend towards greenish tones (negative values), which is characteristic of lettuce, with such a trend noticeable in all treatments, showing that this parameter was not affected by As stress. The coordinate b* shows a significant statistical difference ($p \leq 0.05$) in treatment T6 (b* = 24.79 ± 1.87) with respect to all treatments, indicating a trend to yellowish tones, which is due to the highest As concentration in this treatment. The luminosity (L*) was also significantly affected in T6 (51.06 ± 2.75), since this is the treatment exposed to the highest As concentration. Both b* and L* parameters can produce a color change in lettuce leaves; however, here it is not appreciated by the human eye. The changes found in the color parameters (b*and L*) can be related to the interference of As with the photochemical and biochemical stages of photosynthesis (Gusman et al., 2013).

***Table 2.*** *CIELab parameters of lettuce plants*

| Parameter | T1 | T2 | T3 | T4 | T5 | T6 |
|---|---|---|---|---|---|---|
| L* | $36.44\pm2.09^a$ | $39.02\pm2.22^a$ | $36.38\pm4.11^a$ | $36.91\pm1.63^a$ | $39.08\pm3.56^a$ | $51.06\pm2.75^b$ |
| a* | $-7.75\pm0.78^{ab}$ | $-7.80\pm0.43^{ab}$ | $-6.73\pm0.92^b$ | $-7.46\pm0.91^b$ | $-6.54\pm0.59^b$ | $-8.86\pm0.50^a$ |
| b* | $15.23\pm2.10^a$ | $15.49\pm2.50^a$ | $12.81\pm3.66^a$ | $13.58\pm1.99^a$ | $12.60\pm1.24^a$ | $24.79\pm1.87^b$ |
| C* (croma) | $17.10\pm2.10^a$ | $17.36\pm2.42^a$ | $14.49\pm3.66^a$ | $15.50\pm2.18^a$ | $14.20\pm1.27^a$ | $26.34\pm1.85^b$ |
| h° (hue) | $195.77\pm6.98^a$ | $196.20\pm5.86^a$ | $192.34\pm10.28^a$ | $191.31\pm2.72^a$ | $194.88\pm5.52^a$ | $213.22\pm2.64^b$ |
| ΔE | ND | 2.6 | 2.62 | 1.73 | 3.92 | 17.51 |

The results are determined after 50 days of experimentation. Lowercase letters between columns represent statistically significant differences ($p < 0.05$). As concentrations: T1 = 0 µM (control), T2 = 1 µM, T3 = 2 µM, T4 = 8 µM, T5 = 12, and T6 = 16 µM

### *Effect of As on total chlorophyll (TC) of lettuce plants*

*Table 3* shows the metabolic and pigment parameters obtained for lettuce plants. The highest As concentration affects the TC production, therefore T6 shows the lowest value of TC (2.72 mg of chlorophyll/g sample). On the other hand, the maximum values of TC are observed for T4 and T5 with 9.70 and 9.03 mg of chlorophyll/g sample-of-leaves respectively, because these As concentrations were employed to stimulate the production of such pigment in the treatments. TC has a negative effect on the physical color (L* of CIELab system) in the T6, and the relation between both TC and L* observed with As absorbed in the plant can be related to the energy dissipated by the plant as heat or fluorescence (Caporale et al., 2013; Iriel et al., 2015).

The behavior of TC was evaluated during the growth period of the lettuce (*Fig. 1*). It is observed that the maximum values of TC were obtained after 20 and 50 days of sampling. The latter value occurs in the phenological stage of the plant, which is closest to harvest, and thus the lettuce leaves tend to get stronger and take the color that characterizes the plants, that is, color with green tones. Initially, there is no statistically significant difference for TC, while the statistical difference between treatments for CT increased over time. On day 20, it is observed that TC begins to differ for all treatments. However, this effect of different CT concentrations is very clear in the measurement made on day 50, where T2 showed a higher percentage of TC, while T6 and T4 showed the lowest percentage of TC. This decrease is in agreement with Duman et al. (2010), who reported that TC tends to decrease at high concentrations of As (in a range from 1 to 64 µM in water). The results found in the present work indicate that TC decreases when the As concentrations were near 8 and 16 µM.

***Table 3.*** *Metabolic and pigments parameters of lettuce plants*

| **Treatment** | **Chlorophyll *a*** | **Chlorophyll *b*** | **Total chlorophyll** | **TPC** | **TPC** |
|---|---|---|---|---|---|
| | **mg/g** | **mg/g** | **mg/g** | **mgGAE/g (roots)** | **mgGAE/g (leaves)** |
| T1 | 4.04±0.42$^{b}$ | 2.33±0.24$^{b}$ | 6.37±0.64$^{b}$ | 10.72±0.83$^{b}$ | 16.05±0.65$^{abc}$ |
| T2 | 4.45±0.58$^{bc}$ | 2.51±0.18$^{bc}$ | 6.95±0.75$^{bc}$ | 8.42±1.76$^{a}$ | 17.46±1.64$^{c}$ |
| T3 | 4.52±0.45$^{c}$ | 2.09±0.24$^{b}$ | 6.61±0.69$^{b}$ | 7.06±1.25$^{a}$ | 12.02±1.22$^{ab}$ |
| T4 | 6.23±0.91$^{c}$ | 3.47±0.60$^{c}$ | 9.70±1.50$^{c}$ | 7.75±1.09$^{a}$ | 16.22±0.94$^{bc}$ |
| T5 | 5.75±0.54$^{bc}$ | 3.27±1.42$^{bc}$ | 9.03±3.68$^{bc}$ | 8.28±0.62$^{a}$ | 11.19±2.70$^{a}$ |
| T6 | 1.75±0.54$^{a}$ | 0.98±0.28$^{a}$ | 2.72±0.82$^{a}$ | 7.76±1.09$^{a}$ | 12.13±2.46$^{ab}$ |

The results were obtained after 50 days of experimentation. Lowercase letters between columns represent statistically significant differences ($p \leq 0.05$). TPC = Total Phenolic Compounds, GAE = Gallic Acid Equivalents. As concentrations: T1 = 0 µM (control), T2 = 1 µM, T3 = 2 µM, T4 = 8 µM, T5 = 12, and T6 = 16 µM of As

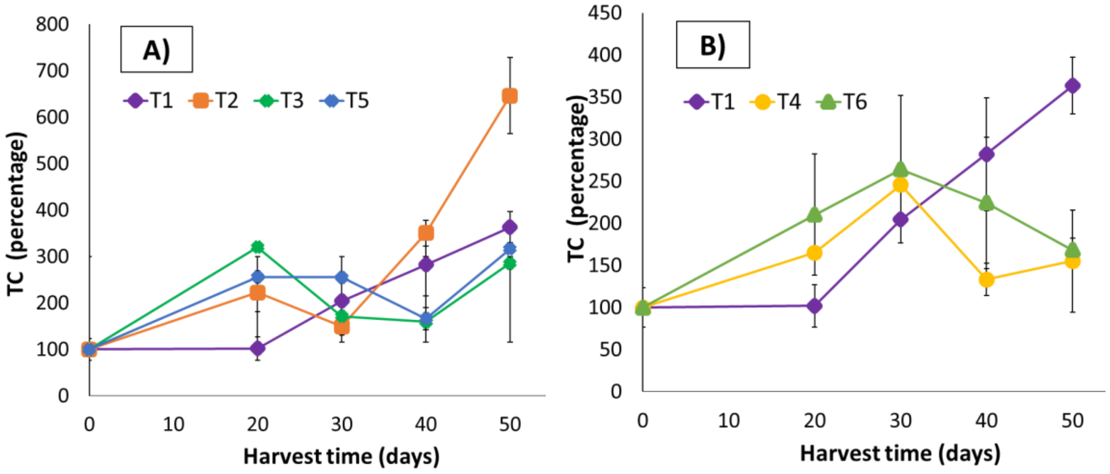

***Figure 1.*** *Chlorophyll behavior in lettuce plants. 0 (initial), 20, 30, 40, and 50 (final) for each treatment, the results are expressed in percent. (A) TC in T2, T3 and T5. (B) TC in T4 and T6*

### *Effect of As on total phenolic compounds (TPC) of lettuce plants*

The behavior of TPC was evaluated in the leaves of the lettuce plant (*Fig. 2*). Initially, no differences were observed in the content of TPC in the treatments, the differences observed from 10 days and on day 20 are shown in *Figure 2*. A possible response to the behavior of TPC that occurred on day 30 was attributed to environmental factors (climate, temperature, and rainfall) since T1 (control) displayed the same behavior. However, at day 50, the behavior of TPC in T1 is attributed to the effect of the phenological stage of the plant, which probably, like TC, is due to the harvest stage in which the plant maintains homeostasis between the values of TPC and other derived compounds of the metabolism. The behavior of TPC in T5 and T6 (highest As concentration) with respect to T1 (control) is shown in *Figure 2B*, where it is observed that the TPC tends to decrease over time. However, the T3 and T4 respond to the situation of As stress in the same manner as in TC (stimulant), where a decrease is observed in TPC on day 30, followed by a stimulus on day 40, and finally, the TPC tends to decrease, which is probably a defense mechanism that is activated in lettuce plant by As stress.

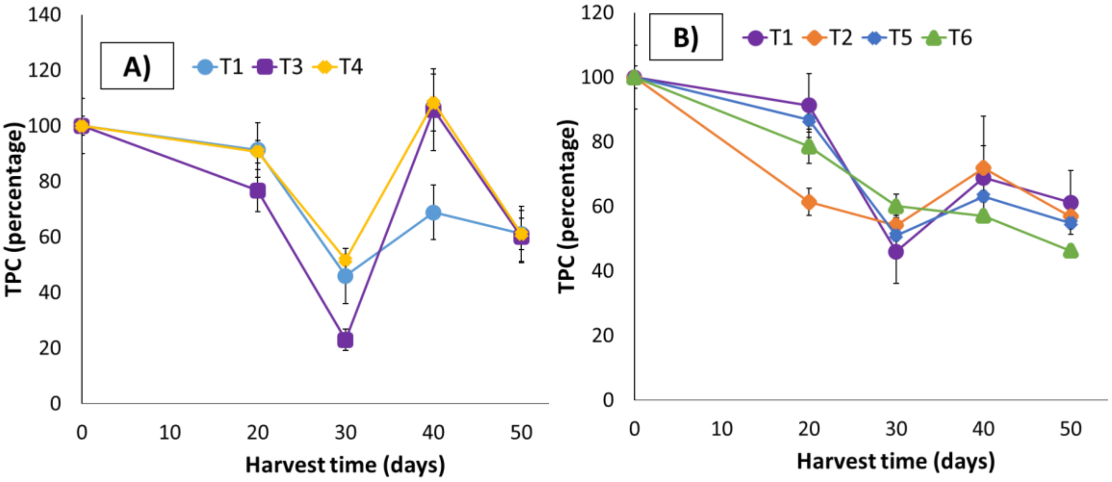

***Figure 2.*** *The behavior of TPC in lettuce plants. 0 (initial), 20, 30, 40 and 50 (final) for each treatment, the results are expressed in percent. (A) TPC in T3 and T4. (B) TPC in T2, T5 and T6*

### *Arsenic uptake in lettuce plants*

The concentration of accumulated As increased on the leaves and roots of lettuce as the concentration of As increased, in the hydroponic medium. This is in agreement with the results reported by Castillo et al. (2013), where it was shown that the concentration of As in vegetables increases as As concentration contents in irrigation water increases.

In the lettuce root, the increase in absorbed As showed a statistically significant result ($p < 0.05$) on treatment from T3 to T6 with respect T1 (control), and increased until reaching an amount of 68.23 ± 2.10 mg of As/kg root DW (dry weight) in the T6 (16 μM As). This amount in T6 increased 15 times more with respect to the amount of the lowest treatment of As (T2). Similar results have been found in the literature on the root structure of lettuce, i.e. 29.33-88.73 mg As/kg plant exposed to As-contaminated soil (Wang et al., 2022), while Cadet et al. (2021) reported that the entire lettuce plant can absorb between 78.7-109.0 mg As/kg-plant in contaminated soils.

In lettuce leaves, *Figure 3* shows that there are no significant statistical differences ($p < 0.05$) between T3 and T4. However, the T5 and T6 increased 6 and 9 times with respect to the amount of the lowest treatment of As (T2). Values of As absorption of lettuce plant have been reported, showing that lettuce plant accumulates concentration of As of 0.08–0.22 mg As/kg (Wang et al., 2022) and 8.76 ± 1.33 mg As/kg (Yañez et al., 2019) in leaves in experiments with As contaminated soil. In contrast in this study, the maximum amount absorbed in lettuce leaves found is 2.48 ± 0.56 mg of As/kg left DW in T6.

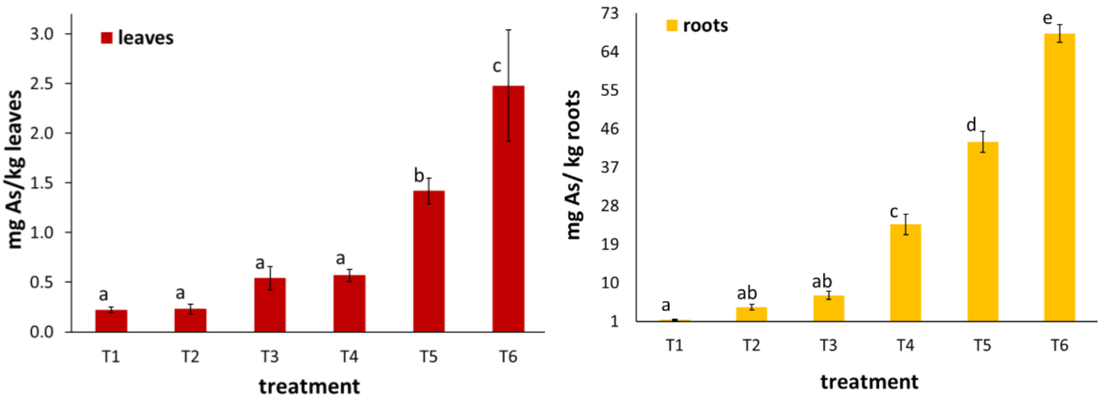

***Figure 3.*** *Absorption As in lettuce plants. T1 = 0 µM (control), T2 = 1 µM, T3 = 2 µM, T4 = 8 µM, T5 = 12, and T6 = 16 µM of As. Lowercase letters between bars indicate statistically significant difference by Tukey test*

Importantly, the highest concentration of As was absorbed by the lettuce root, where the average As concentration in roots was approximately 27 times higher than in the leaves, which indicates a limited translocation rate of the toxic systemic level (Smith, 2009). In this sense, the values of BCF and TF are shown in *Table 4*.

***Table 4.*** *Arsenic bioaccumulation and translocation factor of lettuce structures*

| Structure | T1 | T2 | T3 | T4 | T5 | T6 |
|---|---|---|---|---|---|---|
| TF | 0.152±0.03$^{b}$ | 0.068±0.02$^{a}$ | 0.070±0.00$^{a}$ | 0.025±0.00$^{a}$ | 0.043±0.02$^{a}$ | 0.045±0.01$^{a}$ |
| BCF | ND | 57.82±8.99$^{c}$ | 23.55±3.17$^{a}$ | 39.83±3.99$^{b}$ | 50.07±0.04$^{bc}$ | 56.90±1.75$^{c}$ |

The results are expressed in µM of As. Different lowercase letters between columns represent statistically significant differences ($p \leq 0.05$). BCF = bioconcentration factor, TF = translocation factor. ND = Not determinate. As concentrations: T1 = 0 µM (control), T2 = 1 µM, T3 = 2 µM, T4 = 8 µM, T5 = 12, and T6 = 16 µM of As

The value of TF indicates the capacity that the plants have to mobilize a toxic agent from the root part to the aerial part (Yañez et al., 2019). It is observed that the control treatment (T1) has a higher TF (TF = 0.152 ± 0.03 in T1), than the treatments that were exposed to As in the hydroponic system, so the treatments with the highest exposure to As (T3, T4, T5) implement a mechanism that prevents the mobilization of As towards the leaves. Huang et al. (2005) mentioned that, at a higher concentration of As, plants avoid As intoxication by expelling it (Huang et al., 2005), and this is due exists the excluding plants (Sinha et al., 2007). However, in the present work this behavior was not observed, namely, that at higher As concentrations, plants do not expel As, but instead they tend to have a greater accumulation of it in the root, and thus not mobilizing it to other plant structures. This is a characteristic behavior of a hyperaccumulating plant, as has been reported in lettuce (Yañez et al., 2019).

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On the other hand, BCF is a quantifiable indicator of plant contamination that has been commonly used to estimate the transfer of contaminants from soil to plant (Chang et al., 2014). For BCF $\leq 1$, the plant cannot only absorb, nor accumulate heavy metals and as the value of BCF > 1, the plant is able to accumulate metals. In the present work, BCF values varied from 23.55-57.82, and thus, the lettuce acts like an arsenic accumulator plant. These results are supported by the greater absorption of As in roots, where a reduction of As(V) supplied in the hydroponic system to As(III) probably occurred, which could form As-PC complexes for its sequestration in the vacuoles (Kofronova et al., 2019). This mechanism can occur mainly in the roots (Liu et al., 2010). In this way, the aerial part of the lettuces achieved their physiological development.

### *Correlation of As absorption with the development of lettuce*

Statistical correlations were reported by Spearman rank between each pair of variables, i.e., As in root, As in leaf, As water, total chlorophyll (determined in leaves), TPC roots, TPC leaves, length in the plant (root + leaf) and plant weight (root + leaf). No correlation were found between As in the physiological parts of the plant and metabolic variables. However, there was a statistically significant negative correlation between the As concentration in water and the whole plant weight ($r^2 = -0.826$, $p = 0.042$), due to the physiological affectations such as the length and number of leaves described in the last section. In addition, there was a positive correlation between TPC in leaves and plant length ($r^2 = 0.872$ and $p = 0.023$), due to the fact that metabolites such as TPC are important to some functions in the development of the lettuce plant.

## Conclusions

This work shows that the exposure to As and its absorption produce negative effects at metabolic level. When As concentrations in water were higher than 8 µM, the weight and length of the lettuce plant decreased. Compounds such as TPC and TC were stimulated at 2 µM As (T3); however, a decrease in these compounds was observed when As concentration in water increased (>8 µM). These effects did not translate into the physical parameters of lettuce, since the commercial harvest size was achieved. Therefore, lettuce growth can be achieved without visible post-harvest effects of As.

Importantly, the hydroponic lettuce cultivation system plays a fundamental role in As absorption, since our results differ from studies of lettuce cultivation in soil contaminated with As. A greater absorption of As occurs while the concentration of As increases in the hydroponic system, where the root accumulates the highest level of total As compared to the other organs of the plant. In addition, this study provides useful information for a better understanding of the effects that the absorption and translocation of As have on the different structures of lettuce, where it is shown that the absorbed As is not effectively mobilized in the structures of the plant. Therefore, the high BCF reported in this work points to a possibility and opportunity for lettuce as an As accumulator plant.

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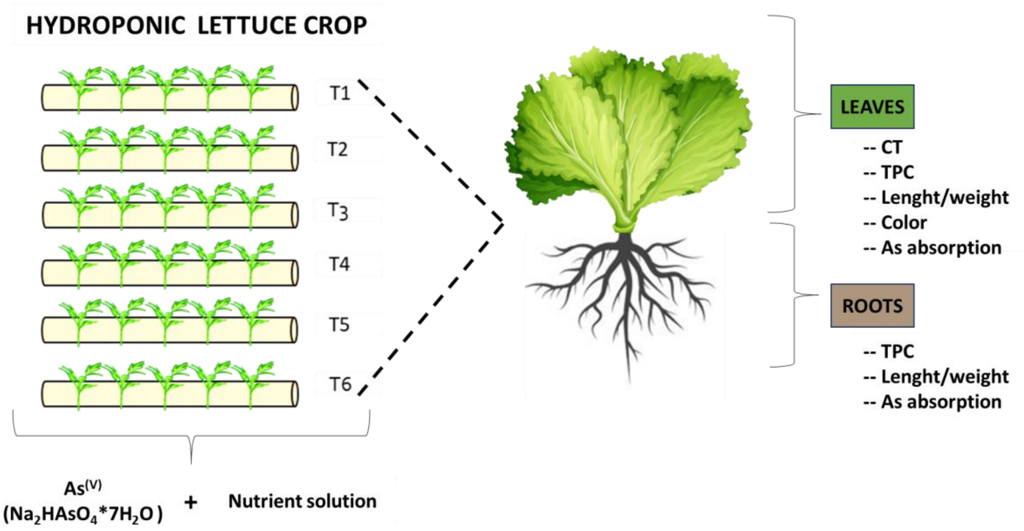
# APPENDIX



*Graphical abstract*