Effects of cadmium on the physiology of *Solanum lycopersicum* L. grown in alternative hydroponic media

Efectos del cadmio en la fisiología de *Solanum lycopersicum* L. cultivados en medios hidropónicos alternativos

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ABSTRACT

Cadmium (Cd) is one of the most toxic metals for the physiology of plants. Proper nutrient management through wastewater reuse can be an efficient strategy to mitigate its effects. In this research, the effects of cadmium were evaluated in the hydroponic cultivation of *Solanum lycopersicum* L. We conducted two experiments: One using mining wastewater with concentrations of 0, 5, 10, and 15 mg L^{-1} of Cd^{2+} (Experiment 1) and another using deionized water with concentrations of 0, 2.5, 5, 10, and 15 mg L^{-1} of Cd^{2+} (Experiment 2). Cadmium stress in plants reduced leaf area, chlorophyll content, and concentrations of potassium (K) and manganese (Mn), and increased concentrations of sulfur (S), phosphorus (P), iron (Fe), and copper (Cu). The employment of mining wastewater improved the plant's response to Cd stress by reducing the translocation of Cd and increasing the contents of P, S, calcium (Ca) and magnesium (Mg) in leaves. At the same time, the use of deionized water decreased the contents of Cu in leaves. These nutrition-related effects influenced leaf area and chlorophyll content, as both indicators showed less impairment in the experiment with wastewater. These results provide additional value to the reuse of wastewater in agriculture.

Key words: heavy metal, tomato, wastewater, hydroponics.

RESUMEN

El cadmio (Cd) es uno de los metales más tóxicos para los procesos fisiológicos de las plantas. El manejo adecuado de nutrientes a través de la reutilización de las aguas residuales puede ser una estrategia eficiente para minimizar sus efectos. En el presente estudio se evaluaron estos efectos en el cultivo hidropónico de *Solanum lycopersicum* L. Se realizaron dos experimentos: uno con aguas residuales mineras y concentraciones de 0, 5, 10 y 15 mg L^{-1} de Cd^{2+} (Experimento 1) y otro con agua desionizada y concentraciones de 0, 2.5, 5, 10 y 15 mg L^{-1} de Cd^{2+} (Experimento 2). El estrés por Cd en plantas redujo el área foliar, el contenido de clorofila y las concentraciones de potasio (K) y manganeso (Mn) y aumentó las concentraciones de azufre (S), fosforo (P), hierro (Fe) y cobre (Cu). El uso de aguas residuales mineras mejoró la respuesta de las plantas al estrés por Cd al reducir su translocación y aumentar los contenidos de P, S, calcio (Ca) y magnesio (Mg) en las hojas. Al mismo tiempo, el uso de agua desionizada disminuyó el contenido de Cu en las hojas. Estos efectos relacionados con la nutrición influyeron en el área foliar y el contenido de clorofila, ya que ambos indicadores mostraron un menor deterioro en el experimento con aguas residuales. Estos resultados proporcionan un valor adicional a la reutilización de aguas residuales en la agricultura.

Palabras clave: metal pesado, tomate, aguas residuales, hidroponía.

Introduction

Due to industrial and anthropogenic activities, concentrations of toxic metals in water, soil, sediments, and other ecosystems have significantly increased in recent decades. Among them, cadmium (Cd) has drawn the attention of soil and plant sciences due to its high toxicity, mobility, and bioaccumulation potential (Abdel-Satar *et al*., 2017; Bala Murugan *et al*., 2019; El Rasafi *et al*., 2022).

In the 1940s, Cd pollution in the Jinzu River and in rice cultivation became evident when over 100 people in Japan died from a disease named Itai-Itai (Ogawa *et al*., 2004). Additionally, scientific evidence of Cd contamination in water,

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sediments, and plant tissue has been reported in various regions worldwide. Examples include lakes in northeastern Wisconsin (USA), Central Ontario (Canada), northeastern Sweden, southeastern Norway (Spry & Wiener, 1991), the Huelva River (Spain) (Martorrell *et al*., 2009), and soils and vegetables in Ethiopia (Duressa *et al*., 2015) and India (Gimba *et al*., 2015).

This metal is recognized as one of the most toxic for plant physiological processes (Li *et al*., 2023). In many crops, including the tomato, Cd has been reported to inhibit growth, photosynthesis, transpiration, and the formation of photosynthetic pigments. It also causes chlorosis, nutritional imbalances, oxidative stress, and modifies the activity of enzymes involved in organic acid metabolism (Hédiji *et al*., 2015). To mitigate its effects, proper nutrient management is known to be an effective alternative (Samet *et al*., 2017) and can be achieved through the reuse of previously treated wastewater.

In addition to Cd pollution, another issue currently affecting agriculture is the high levels of water deficit reached in many regions of the world (Muller, 2017). As a solution, different countries have proposed the reuse of wastewater, recognized since ancient times as its high nutritional value (Ramírez *et al*., 2021; Samet *et al*., 2017).

Despite the above research, studies to thoroughly understand the effects of Cd on physiological and nutritional indicators of plants, as well as the role of nutrients in tolerance to this type of stress, are insufficient. Because of this and the need to reuse wastewater, the objective of the following research was to evaluate the effect of Cd on growth indicators, photosynthetic activity, and the nutrient composition of tomato plants grown in two different hydroponic media: wastewater and deionized water.

Materials and methods

Growth conditions of tomato plants

We conducted two experiments in a growth chamber under controlled light conditions (12/12 h light/darkness), temperature (18/25ºC light/darkness), and light intensity (800 µmol m^{-2} s^{-1}). In both experiments, the tomato seeds germinated and grew in vermiculite. After 7 d, we transplanted the plants into a hydroponic system in plastic trays measuring 30 x 60 x 80 cm. We maintained the system continuous aeration. The Hoagland and Arnon (1950) nutrient solution (Tab. 1) was used that had been modified at the Laboratory of Plant Physiology of the National Institute of Agricultural Sciences (Cuba). The pH was adjusted to 6.5 and the nutrient solution was renewed every 8 d.

The main difference between both experiments was the type of water used in the hydroponic solution. In experiment 1, water was collected from the Biajaca River, where residues from the Castellanos Mines in the province of Pinar del Río, Cuba, are discharged. In contrast, deionized water was used for experiment 2.

After 15 d of germination, treatments were applied following a completely randomized design with 20 plants per treatment. In experiment 1, the treatments were as follows: T1 - deionized water, T2 - wastewater, T3 - wastewater with 5 mg L^{-1} of Cd, T4 - wastewater with 10 mg L^{-1} of Cd, and T5 - wastewater with 15 mg L^{-1} of Cd. In experiment 2, the

TABLE 1. Composition of the modified Hoagland and Arnon (1950) solution.

Salt	Content (mM)	Element	Content (mg L^{-1})
$Ca(NO_3)_2$	2.5	Ca	103
KH_2PO_4	0.5	N	105
KNO_3	2.5	K	118
$MgSO_4$	1.0	S	33
$ZnSO_4$	0.00039	Mg	25
$MnSO_4$	0.0046	P	15
$CuSO_4$	0.00016	Fe	10
H_3BO_3	0.0234	B	0.25
H_2MoO_4	0.000051	Mn	0.25
Fe- EDTA	0.179	Zn	0.025
		Cu	0.01
		Mo	0.0052
		Cl	0.50

treatments were as follow: T1 - deionized water, T2 - deionized water with 2.5 mg·L^{-1} of Cd, T3 - deionized water with 5 mg L^{-1} of Cd, T4 - deionized water with 10 mg L^{-1} of Cd, and T5 - deionized water with 15 mg L^{-1} of Cd. Cadmium chloride ($CdCl_2$) was used as the metal carrier salt.

The wastewater used was classified as sulfated bicarbonate of chlorinated sodium calcium magnesium (SO_4 = HCO_3^- > Cl-Na = Ca > Mg). The electrical conductivity (270 µS cm^{-1}), alkalinity (1.03 meq L^{-1}) and the concentrations of N, K, Ca, Mg, Fe, Mn, Zn, B, Mo, Cu, Co, Ni, Na, Al, Cd, Pb, As, Cr, Li, Be, Sr, Sb, Se, V, Tl, Rb, Bi, Cl^-, NO_3^-, SO_4^{2-}, Br^- were below the permissible limits established by FAO (Hernandez-Baranda *et al.*, 2018). The initial pH was 4.0, which did not fall within the range established by the FAO. However, the water was neutralized to a pH of 7.0 before being used. The chemical composition of the water utilized is presented in Hernandez-Baranda *et al.* (2018). After 25 d of treatments, evaluations were performed at a rate of eight plants per treatment, as detailed below.

Leaf area

The leaf surface area was calculated through image analysis. Initially, all leaves of each plant were scanned using a Canon MF4800 scanner in jpg format. Subsequently, the images were processed in Adobe Photoshop SC5 image analysis software, and the area in cm^2 was determined.

Chlorotic leaf area

For the damaged leaf area, the same procedure was followed as above, but only the damaged surface or leaves with obvious symptoms of chlorosis were calculated.

Chlorophyll content

Six well-developed leaves of the upper third of the plants were measured with a Portable MINOLTA Chlorophyll Meter SPAD 502 Plus.

Plant chemical analysis

A destructive sampling was carried out, where the organs were separated into root, stem, and leaves. Subsequently, they were dried in an oven at 80°C until reaching a constant mass, and the samples were ground. The content of Cd, P, K, S, Ca, Mg, Cu, Mn, and Fe in each organ was determined by ICP-OES after microwave-assisted acid digestion. The procedure for this analysis was established at the Ionomics Laboratory of CEBAS-CSIC (Hernandez-Baranda *et al.*, 2018).

Statistical analysis

Results underwent one-way ANOVA (factor with five levels). Indicators with differences were further analyzed using Duncan's Multiple Range Comparison test ($P \leq 0.05\%$). Additionally, an independent sample T-test compared Cd concentrations in root, stem, and leaves (5, 10, and 15 mg L^{-1}) between the two experiments.

Results and discussion

Effects of Cd on leaves of tomato plants

The presence of Cd in the environment, even at its lowest concentrations, caused a significant reduction in leaf area and led to chlorosis-related damage, affecting over 40% of leaf tissue in both experiments (Fig. 1). These damages

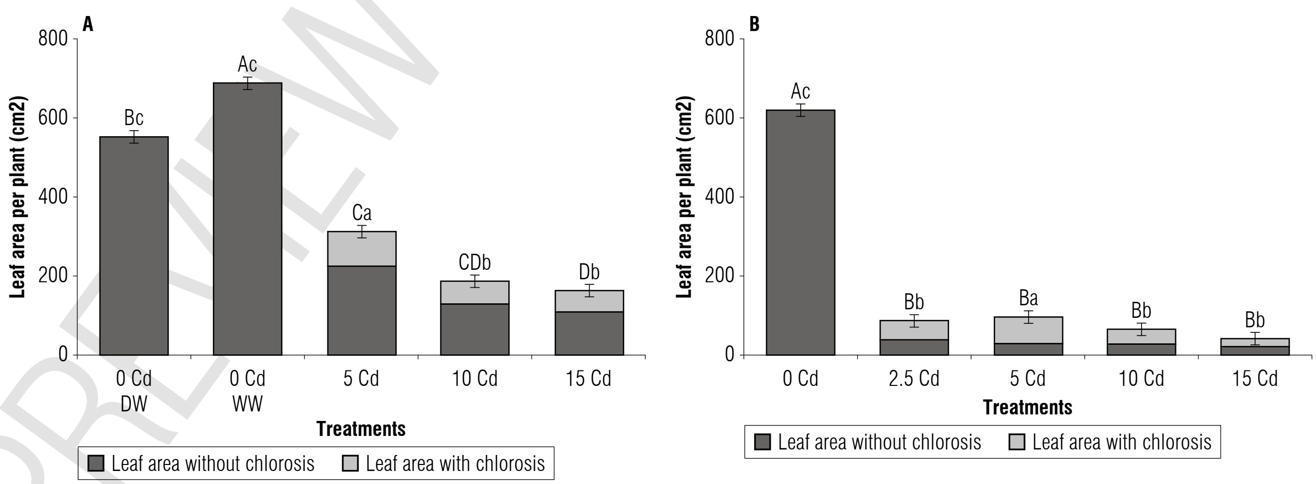

FIGURE 1. Leaf area of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10, and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Capital letters compare the total leaf area and the lowercase letters that leaf area with chlorosis according to the Duncan's multiple range comparison test, $P \leq 0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

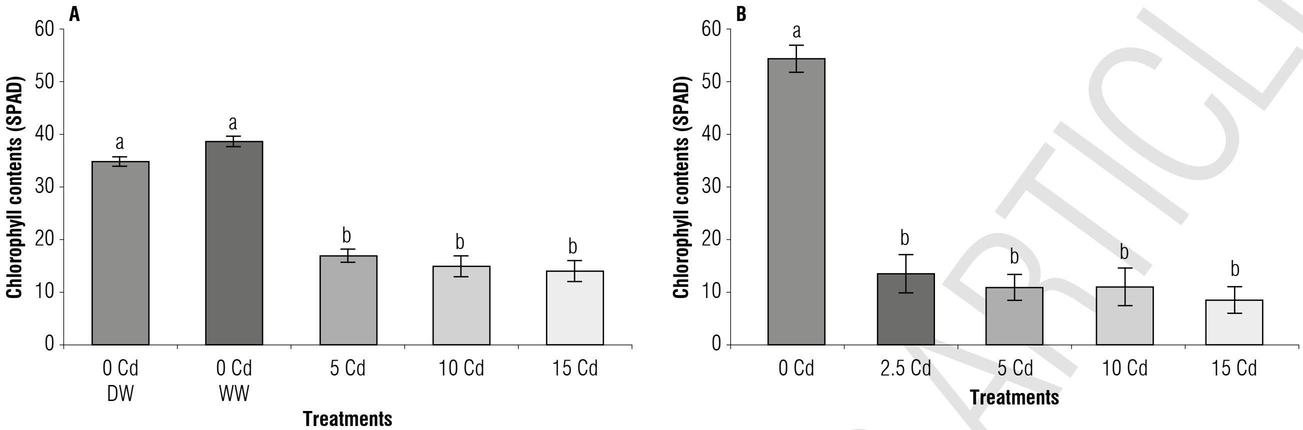

FIGURE 2. Chlorophyll content (SPAD units) in leaves of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10, and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Capital letters compare the total leaf area and the lowercase letters that leaf area with chlorosis according to the Duncan's multiple range comparison test, $P \leq 0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

were mainly evident in young leaves. Notably, reductions in leaf area were less severe in experiment 1 (67%, 81%, and 84% in T3, T4, and T5) compared to experiment 2 (85%, 89%, and 93% in T3, T4, and T5). Initial Cd doses also led to less chlorosis damage in experiment 1, suggesting wastewater cultivation positively mitigated Cd toxicity in this indicator.

Leaf tissue damage is evident in chlorophyll content estimates (Fig. 2). Cadmium toxicity caused reductions exceeding 50% compared to control treatments. Similar results were obtained in other crops exposed to high concentrations of Cd, such as peas (Agrawal & Mishra, 2009), soybeans (Xue *et al.*, 2014), lettuce (Monteiro *et al.*, 2009) and potatoes (Xu *et al.*, 2013). Notably, similar to leaf area, experiment 1 showed less pronounced reductions in chlorophyll content (around 57%) compared to experiment 2 (around 73%). This indicates that wastewater cultivation had a beneficial impact on mitigating Cd toxicity in this indicator.

Effects of Cd on the concentration of mineral nutrients in roots, stems, and leaves

Cd toxicity altered normal nutrient concentrations in the organs of tomato plants. Control treatment levels of nutrients closely matched standard nutrient concentrations in plant tissues (Azcón-Bieto & Talón, 2013).

Cadmium

Different authors have studied plant response mechanisms to Cd toxicity, including exclusion via root accumulation and detoxification in leaf organelles. Figure 3 indicates that roots accumulated the highest Cd levels, consistent with exclusion behavior (Seregin & Kozhevnikova, 2004). This doesn't imply a singular tolerance mechanism, rather it suggests a combination of mechanisms. Accumulation of Cd in roots is proposed as a significant contributor in this study. Similar results were reported by Hernández *et al.* (1998) in pea plants (*Pisum sativum* L.) exposed to concentrations of 10 mM and 50 mM of Cd in a hydroponic system. They described that the reason for this higher accumulation in roots is that the metal is primarily concentrated in the cell wall, in the soluble fraction, associated with molecules of molecular weight higher than 6-8 kDa, possibly corresponding to phytochelatins (Hernández *et al.*, 1998).

Nevertheless, in leaf tissue, plants from both experiments accumulated Cd concentrations similar to known hyperaccumulator plants of Cd, such as *Viola baoshanensis*, *Arabis paniculata*, *Potentilla griffithii* (Liu *et al.*, 2004; Qiu *et al.*, 2011; Zeng *et al.*, 2009). The Cd levels (4.7 mg g^{-1}) are comparable to hydroponically grown tomato varieties (Sagardoy Calderón, 2011), three orders of magnitude higher than soil-grown varieties identified as tolerant (4.3 mg kg^{-1} and 13.4 µg g^{-1}) (Andal, 2016; Sbartaï *et al.*, 2017). This highlights greater Cd availability and absorption in hydroponic systems.

Figure 3 shows that as Cd levels in the solution increased, its content in leaves and stems also increased. However, root behavior differs; in some cases, plants exposed to higher toxicity accumulated less Cd than in previous treatments. This suggests greater Cd accumulation in roots at lower toxicity levels, with consistent patterns at higher levels. Notably, at these higher levels, Cd translocation to aerial organs increased.

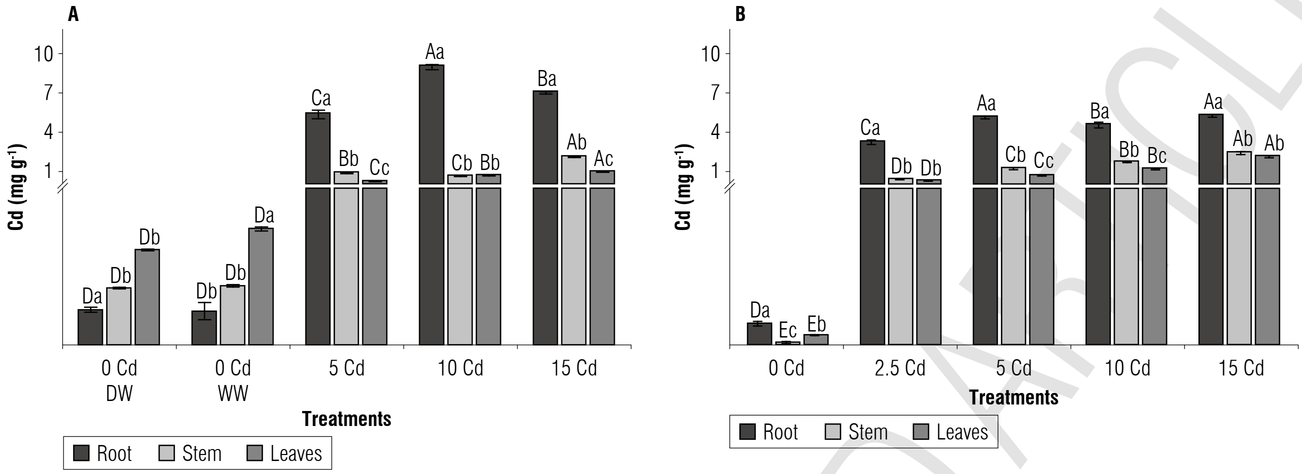

FIGURE 3. Concentration (mg g^{-1}) of Cd in root, stem, and leaves of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10, and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Capital letters compare the total leaf area and the lowercase letters that leaf area with chlorosis according to the Duncan's multiple range comparison test, $P \leq 0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

TABLE 2. Concentration (mg g^{-1}) of Cd in roots, stems, and leaves of tomato plants grown in different treatments of Cd (5, 10, and 15 mg L^{-1}). *P-value*<0.05 indicates statistical differences between the experiment with wastewater (1) and the experiment with deionized water (2).

Exp	Roots			Steams			Leaves		
	5 Cd	10 Cd	15 Cd	5 Cd	10 Cd	15 Cd	5 Cd	10 Cd	15 Cd
1	5.45	9.08	7.10	0.96	0.71	2.18	0.30	0.76	1.04
2	5.23	4.65	5.36	1.31	1.80	2.51	0.77	1.28	2.22
P	0.55	0.0	0.00	0.00	0.00	0.02	0.00	0.00	0.00

Exp: experiment, *P*: P-value.

Table 2 compares Cd concentrations in the most stressed treatments between the two experiments. Experiment 1 shows higher Cd concentration in roots, while experiment 2 exhibits higher Cd concentration in aerial tissues (stem and leaves). This suggests increased Cd translocation to aerial organs in experiment 2, where nutrient availability in the growth medium is lower.

Two genotypes of pea and wheat with different levels of Cd tolerance have been identified, and the authors attributed their higher tolerance to a reduction in Cd translocation to leaves (Ci *et al*., 2011; Rahman *et al*., 2017). Therefore, we suggest that, due to reduced translocation, plants cultivated with wastewater have developed more efficient Cd response mechanisms than those grown solely with nutrient solution.

One of the tolerance strategies of plants to Cd stress is the immobilization of the ion in the roots through chelation with sulfur-rich proteins (Nocito *et al*., 2011). This coincides with the fact that the higher concentration of sulfate in wastewater is one of the most pronounced differences compared to deionized water. Consequently, plants from experiment 1 grew with a higher available sulfur content. Both criteria suggest that sulfur may be responsible for the observed lower translocation of Cd in experiment 1 and its higher accumulation in the roots in both experiments.

Sulfur

Sulfur concentrations in the root (Fig. 4 A and B) corroborate the assumption of the relationship between the content of this element and the immobilization of Cd. In treatments 3 and 4 of experiment 1 and treatments 2 and 3 of experiment 2, the sulfur concentrations in the root were increased when the Cd content in this organ increased. Subsequent treatments also found a total correspondence between the behavior of these elements (S and Cd), decreasing both in treatment 5 of experiment 1 and in treatment 4 of experiment 2.

In leaves, which are the primary organ for sulfur accumulation, discordant results were observed between the experiments. Unlike experiment 1, in experiment 2, there was no consistent correspondence between Cd and S concentrations. Up to and including treatment 3, both elements increased, but in treatments 4 and 5, S concentration decreased, reaching levels similar to the control and lower than experiment 1. Conversely, Cd concentration increased to higher levels than in experiment 1. Thus, the greater Cd accumulation in experiment 2 leaves is unrelated to S concentration. However, in experiment 1, where sulfate concentration in the solution is higher, a relationship between them exists.

Plant exposure to Cd could increase sulfate assimilation (Nussbaum *et al*., 1988) and this coincides with the increase in S observed in the Cd concentration levels of both experiments (Fig. 4). Notably, the S increase in leaves across all stress treatments in experiment 1, and in treatments 2 and 3 of experiment 2, exceeded concentrations (0.1%) considered adequate for S in plant organs (Azcón-Bieto & Talón, 2013).

Various authors have linked sufficient S availability to the biosynthesis of Cd detoxifying agents, mitigating their effects (Anjum *et al*., 2012; Hassan *et al*., 2006). Given this evidence, the greater impact on leaf tissue in experiment 2 is attributed to lower S availability in the hydroponic medium, leading to reduced accumulation in the leaves.

Potassium and manganese

Analysis of K behavior in both experiments revealed progressive decreases in concentrations across all organs with increasing Cd in solution (Fig. 5). In the most toxic treatment of experiment 1, K concentrations decreased by 51%, 55%, and 48% in the root, stem, and leaves. In experiment 2, the decreases were 42%, 57%, and 60% in the same organs.

Regarding the decrease of the concentration of K, the leaf was the organ least affected in experiment 1 and, in turn, the most affected in experiment 2, due to the higher concentration of Cd found in the leaf tissues of this last experiment. The decrease caused was of such magnitude that, according to the sufficiency interval, 3-6% (Azcón-Bieto

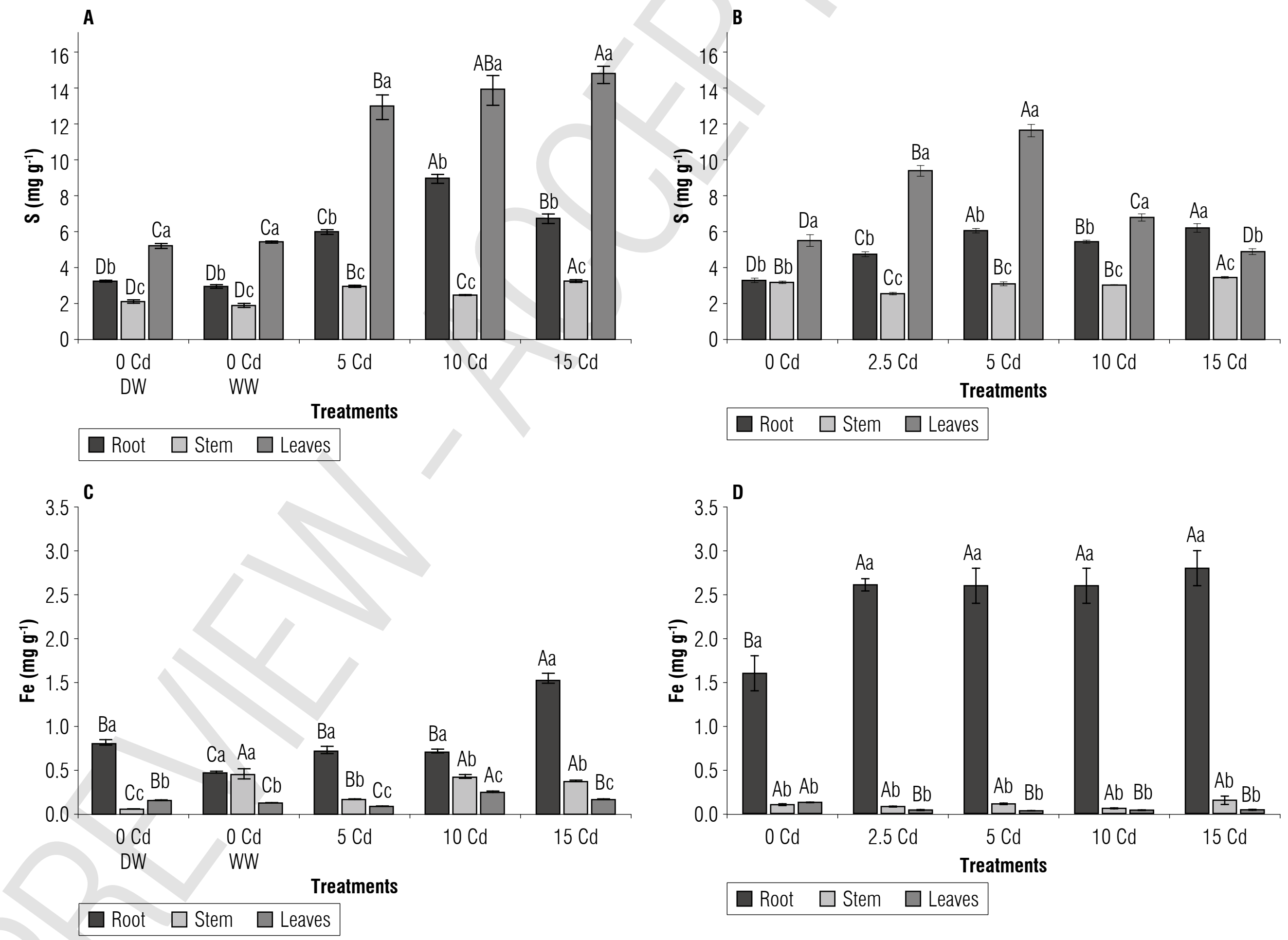

FIGURE 4. Concentration (mg g^{-1}) of S (A and B) and Fe (C and D) in root, stem, and leaves of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10 and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Capital letters compare the total leaf area and the lowercase letters that leaf area with chlorosis according to the Duncan's multiple range comparison test, $P \leq 0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

& Talón, 2013), the plants were grown under 15 mg L^{-1} of Cd in experiment 1 showed a deficiency of K. For experiment 2, the deficiency of K was marked from the treatment with 5 mg L^{-1} of Cd. Due to the vital role of K in plant cells, plants with a significant deficiency of this nutrient end up showing a reduction in growth, especially in the aerial part (Azcón-Bieto & Talón, 2013), similar to the changes observed in the present study.

Cd toxicity led to a decline in Mn concentration throughout the plants as well (Fig. 5 C and D). The root was the most affected organ in both experiments, showing a difference of over 30% compared to the stem and leaves. This impact is attributed to the chemical similarity between these elements, facilitating the entry and transport of Cd through Mn-specific transporters (Conn & Gilliham, 2010). In this scenario, Cd displaces Mn in the binding sites of the transporter protein, consequently reducing its uptake into the plants. There is an antagonistic relationship between Cd and Mn in root tissue in pea plants (*Pisum sativum* L.) grown in hydroponics (Hernández *et al.*,1998).

The concentration of Mn in the root exhibited reductions of 56%, 68%, and 71% (Experiment 1) and 88%, 90%, and 91% (Experiment 2) in the treatments with 5, 10, and 15 mg L^{-1} of Cd (Fig. 5 C and D). The most significant impacts occurred in experiment 2, where, unlike experiment 1, the root was not the organ with the highest accumulation of Mn.

Phosphorus and copper

Phosphorus and Cu, unlike K and Mn, exhibited a positive correlation with Cd (Fig. 6). Similar outcomes are reported by Nogueirol *et al.* (2016) in two tomato varieties exposed to varying Cd levels (0, 3, 6, and 12 mg kg^{-1}). They observe antagonistic associations with Mn and synergism with P. Positive correlations between Cd and Cu are also identified in various rice cultivars (Liu *et al.*, 2003).

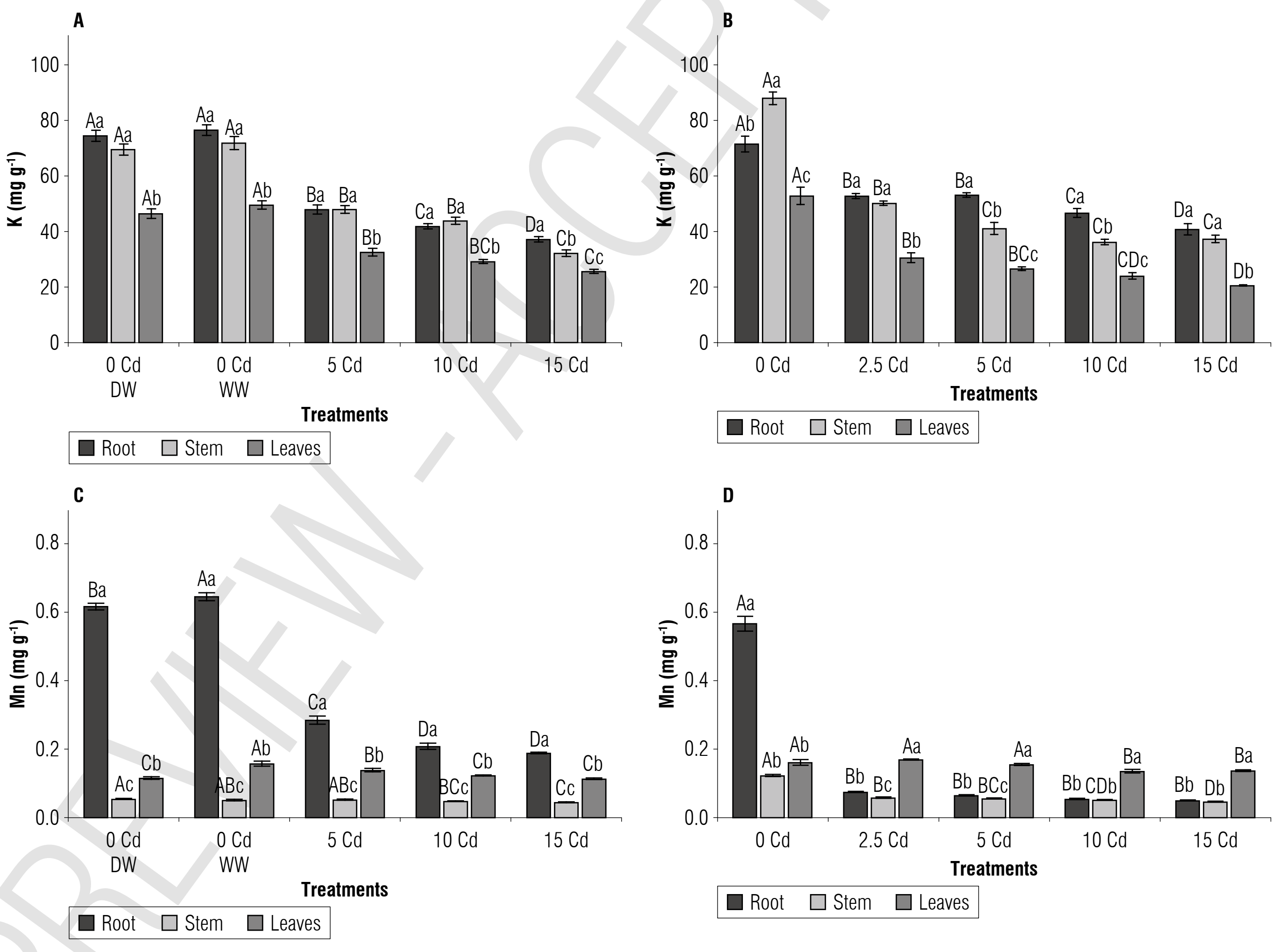

FIGURE 5. Concentration (mg g^{-1}) of K (A and B) and Mn (C and D) in root, stem, and leaves of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10 and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Capital letters compare the total leaf area and the lowercase letters that leaf area with chlorosis according to the Duncan's multiple range comparison test, $P \leq 0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

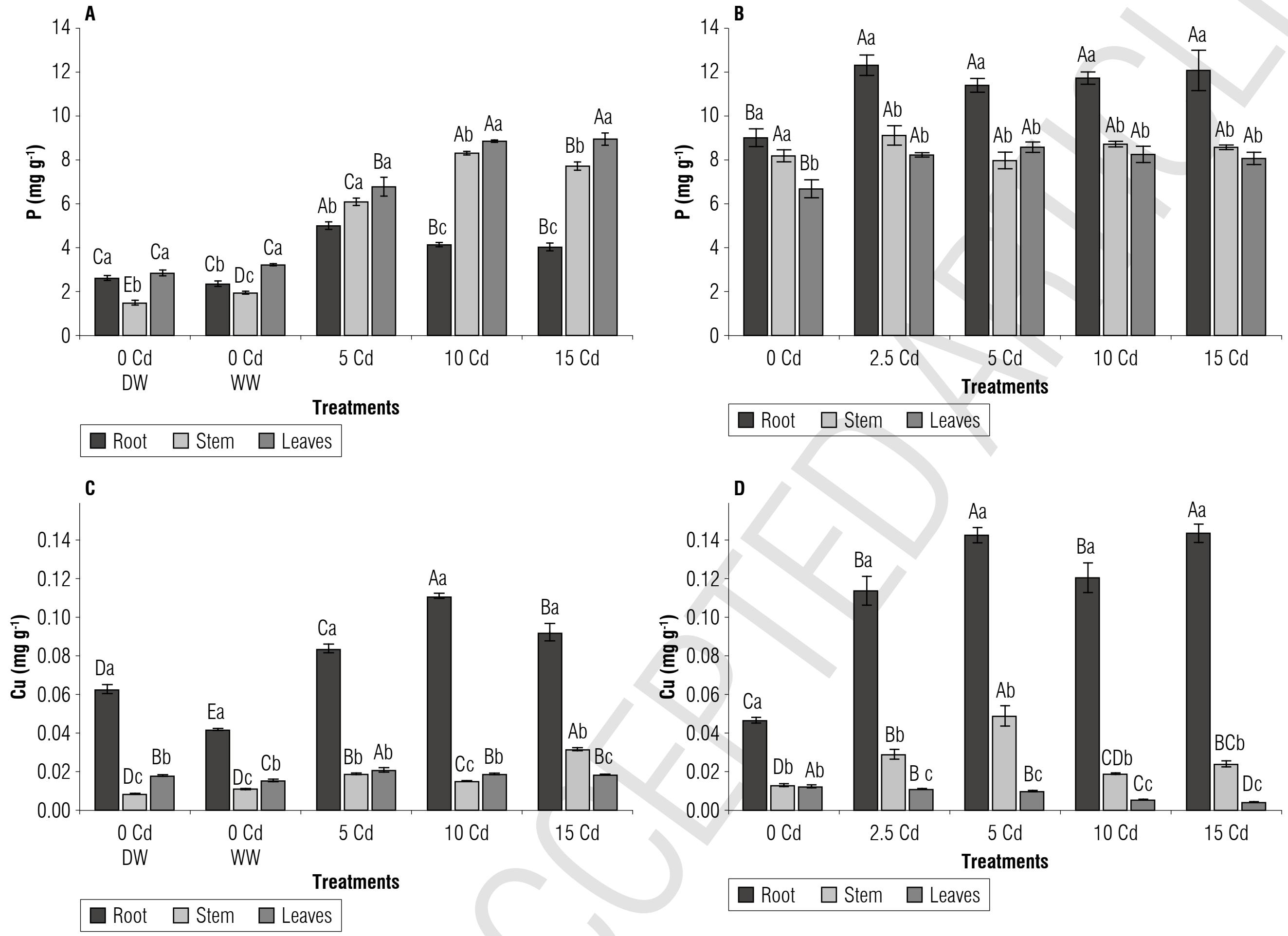

FIGURE 6. Concentrations (mg g^{-1}) of P (A and B) and Cu (C and D) in root, stem, and leaves of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10 and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Capital letters compare the total leaf area and the lowercase letters that leaf area with chlorosis according to the Duncan's multiple range comparison test, $P \leq 0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

As a common result between both experiments, the increase in toxicity caused an increase in P content in all organs, except in the stem of experiment 2. It's noteworthy that the increase in leaves in both experiments surpassed the sufficiency range (0.3-0.6%) typical for tomato plants at the same phenological stage as in this study (Azcón-Bieto & Talón, 2013). Some authors have noted the onset of nutritional stress in tomato plants when P levels exceed 1.0% (Benton Jones Jr, 1998) and 0.6% (Peñalosa *et al*., 1989), particularly in hydroponic crops.

P distribution varied between experiments; in experiment 1, leaf tissue had the highest P accumulation, while in experiment 2, it was the root that had the highest P accumulation. In both experiments, the organs with the highest accumulation were the most affected by Cd toxicity. Notably, prior to treatment application, experiment 2 exhibited higher P concentration in leaves compared to experiment 1, and after Cd exposure, experiment 1 showed a higher P concentration in leaves. This suggests that plants grown in wastewater displayed greater P translocation, intensified with increasing Cd doses, thus recognized as another response mechanism that contributed to mitigating the effects of Cd on the leaves of experiment 1.

Similar to manganese in experiment 1, the root was the organ with the highest copper accumulation (Fig. 6) and was the most affected by Cd toxicity. Cu levels in the roots were approximately twice as high in stressed treatments as in the control. In the leaves, the content of Cu only decreased in experiment 2 and that is attributed to greater leaf damage seen in this experiment that can affect the accumulation of this element in the leaf tissue.

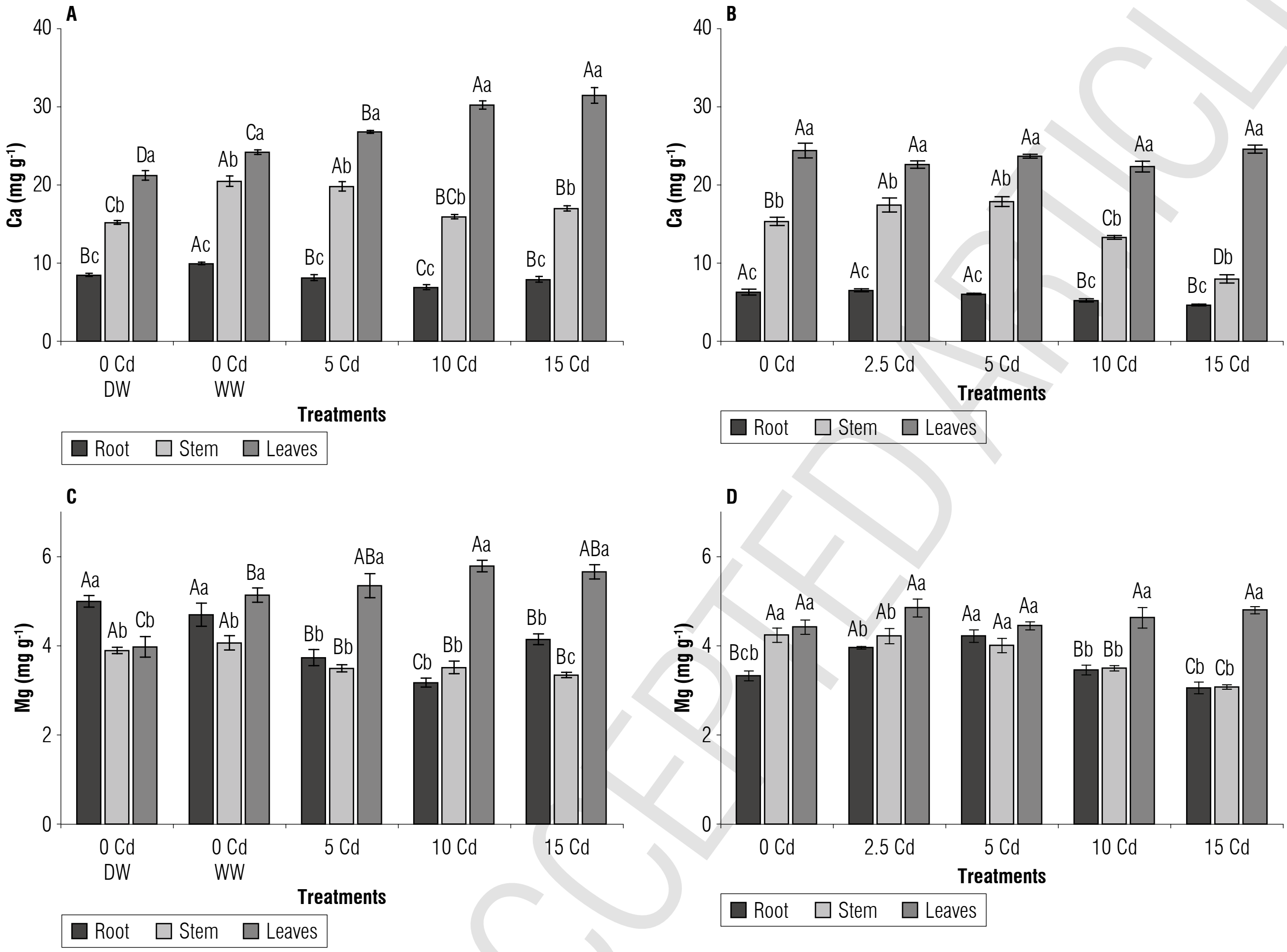

FIGURE 7. Concentration (mg g^{-1}) of Ca (A and B) and Mg (C and D) in root, stem, and leaves of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10 and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Capital letters compare the total leaf area and the lowercase letters that leaf area with chlorosis according to the Duncan's multiple range comparison test, $P \leq 0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

Calcium and magnesium

As Cd toxicity levels increased, the concentrations of Ca and Mg decreased in the root and stem in both experiments, while they only increased in the leaves of experiment 1 (Fig. 7).

Ca, like S, is another predominant element in wastewater that distinguishes the two hydroponic media. Previous studies suggest that adequate Ca availability inhibits Cd accumulation in plants like *Arabidopsis thaliana* and *Trifolium repens* (Suzuki *et al*., 2005; Wang *et al*., 2009). Therefore, the higher Ca content present in the leaves of experiment 1 could be another possible cause of the observed lower translocation of Cd in this experiment.

Previous research has also found a positive correlation between Cd and Mg in the leaves of rice plants (Liu *et al*., 2003). Additionally, Küpper *et al*. (2002) identified, in a Cd toxicity study, that mesophyll cells show higher concentrations of Mg than normal, interpreting it as a defense mechanism against the substitution of Mg by Cd in chlorophyll molecules. This mechanism is presumed to be related to the findings in experiment 1 as well.

Iron

In both experiments, Cd presence elevated Fe concentrations in the roots, making it the organ with the highest Fe accumulation (Fig. 4 C and D). Similar increases in root Fe content were noted in previous studies on tomatoes (Sagardoy Calderón, 2011) and rice (Liu *et al*., 2003) under Cd treatments.

Cd had different effects in the leaves. In experiment 1, Fe concentration increased in the treatment with 10 mg L^{-1} of Cd and then decreased in the high-stress treatment. In experiment 2, it decreased from the first level of Cd, persisting in subsequent treatments. It's noteworthy that the 5 mg L^{-1} Cd treatment in both experiments accumulated a lower Fe concentration in the leaves and simultaneously exhibited a larger leaf area with chlorosis (Fig. 1). A similar effect on chlorophyll content in sugar beet was observed in the lower stress Cd treatment, attributed to induced Fe deficiency at low Cd levels (Larbi *et al*., 2002).

Cd toxicity can occur due to the exchange of Fe for Cd (Kabata-Pendias *et al*., 2010). Based on the results presented and the type of damage observed in the leaves, it is considered that the observed chlorosis is not only a result of Cd toxicity but also characteristic of Fe deficiency. However, in experiment 1, the relationship between Fe content and chlorosis seems to contradict this, as the treatments with higher stress showed 49% leaf damage and the Fe contents in the leaves are slightly higher than the controls (Fig. 4 C and D).

The iron chlorosis paradox, where Fe-deficient chlorotic leaves often exhibit higher Fe concentrations than green leaves, suggests that accumulated Fe might be in an unavailable form for the plant (Römheld, 2000). Phosphates and a high pH of the apoplast might cause the precipitation of Fe outside the cell, preventing its utilization (Römheld, 2000). This aligns with elevated P levels in leaves, indicating that Fe precipitation as phosphates could contribute to the apparent contradiction in experiment 1.

In experiment 2, Fe levels in the leaves of stressed treatments were below concentrations considered adequate in plants (100 mg kg^{-1}) (Azcón-Bieto & Talón, 2013). Given the crucial role of Fe in chlorophyll biosynthesis and photosynthetic electron transport, its deficiency in leaves could, along with other factors, contribute to the observed decline in chlorophyll content.

Conclusion

The stress caused by Cd, even at low doses (2.5 and 5 mg L^{-1}), resulted in a significant reduction in chlorophyll content and leaf area, and caused chlorosis damage affecting more than 40% of leaf tissue in both experiments. The presence of Cd was observed to increase the concentrations of P, Fe, Cu, and S, while decreasing the concentrations of K and Mn. Roots and leaves were the organs most affected for K, with roots being the most affected organ for Mn, P, and Cu. The greater availability and concentration of essential nutrients such as S, Ca, and Mg in the hydroponic medium from the Biajaca river enhanced the response of tomato plants to Cd stress by reducing the translocation of this element to the leaves and minimizing the impact on leaf area and chlorophyll content. Additionally, in the experiment with wastewater, there was greater translocation to the leaves of S, P, Ca, Mg, and Fe, with evidence of lesser reductions in the concentrations of K and Mn. These results provided information on the effects of Cd on leaf area and nutrient homeostasis in tomato plants, highlighting the potential of using mining wastewater as a strategy to mitigate these effects. However, it is crucial to know the composition of such waters beforehand to ensure that they receive appropriate treatment.

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Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

YHB contributed to the research process in the experiment conception and design; data acquisition; analysis and interpretation of results, article writing and review. ZNCP, YMH, OCR participated in the data acquisition, analysis, interpretation of results, and article review. MPI, JLMO, IEM, and MME participated in the analysis, interpretation of results, and article writing and review. PRH contributed to the research process in experiment conception and design; data acquisition; analysis, interpretation of results, and article writing and review. Al authors reviewed the final version of the manuscript.

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