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Thallium Differentially Affects Macronutrients Concentration and Stoichiometric Ratios with Nitrogen in the Leaves of Chili Pepper Varieties

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Abstract This study aimed to assess the effects of thallium (Tl) on the leaf concentration of macronutrients (N, P, K, Ca, Mg, and S) and the stoichiometric relationships of P, K, Ca, Mg, and S with N in three varieties of chili pepper (*Capsicum annuum*): Jalapeño, Poblano, and Serrano. Sixty-day-old seedlings of the three varieties were treated with Tl, in doses of 0, 5.5, and 11 nM in the nutrient solution. After 80 days of exposure to Tl treatments, the nutrient concentration in leaf tissue was determined. With the data obtained, an analysis of variance and comparison of means with Tukey's test ($\alpha = 0.05$) were carried out, and through a meta-analysis, the size and direction of the effect of the evaluated Tl doses were determined, in the leaf concentrations of macronutrients. The 5.5 and 11 nM Tl doses increased the leaf concentration of P in Serrano and that of N in Poblano, respectively. Applying 5.5 nM Tl significantly reduced the leaf concentration of K in Jalapeño and Serrano, that of Ca in Poblano, and that of Mg in Serrano. In Jalapeño, both Tl doses tested reduced the leaf Ca concentration. Low Tl doses (5.5 nM) caused significant and positive effects on the leaf K concentration in all three varieties. High Tl doses (11 nM) caused significant negative effects on the leaf concentration of Mg. In the three varieties evaluated, the addition of Tl increased the leaf N:K ratio, that of N:Mg in Poblano and Serrano, the N:Mg ratio in Jalapeño, the P:K ratio in Serrano, and the N:Ca ratio in Jalapeño and Poblano. There was no effect of Tl on shoot dry biomass in any variety evaluated. In Tl-treated plants, foliar concentration of this element varied from12.0 to 26.6 mg kg^{-1} on a dry basis. The sum of principal component 1 and principal component 2 represented 80.8, 72.3, and 79.6% of the total variance of macronutrient concentration in leaves of the Jalapeño, Poblano, and Serrano varieties, respectively. We conclude that Tl had differential effects on the nutrient status among varieties of chili pepper, with Jalapeño being the most affected and Poblano the least.

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1 Introduction

Thallium (Tl) is a metal widely distributed on the planet, and no specific biological functions have been found for this element in any living being analyzed. On the contrary, this element is on the list of environmental pollutants of the Environmental Protection Agency (EPA) of the USA due to its toxic effects in various biological systems, including humans, animals, and plants (Galván-Arzate & Santamaría, 1998; USEPA, 2021).

The release of Tl to the environment can occur naturally and through anthropogenic activities, mainly involving the chemical industry and mining. This has caused its concentration to increase in recent decades, and it is estimated that each year, 5000 t of Tl are expelled into the atmosphere (Léonard & Gerber, 1997; Yu & Tsunoda, 2016). In the lithosphere, Tl concentrations range from 0.3 to 0.6 mg kg^{-1}; in igneous rocks, from 0.05 to 1.7 mg kg^{-1}; and in soils, their concentrations are associated with their origin and the minerals present (Frattini, 2005; USEPA, 2021). This element can also be present in bodies of water. For example, in Poland, 5 to 17 ng Tl L^{-1} (0.0245 to 0.0832 nM) can be found in rivers and 10 to 15 ng Tl L^{-1} (0.0489 to 0.0734 nM) in seawater. In Lake Ocrida, located between the Macedonian and Albanian border, Tl concentrations are 0.5 µg L^{-1} (24.4638 nM); the Huron and Raisin rivers in the USA contain 21 and 2621 ng Tl L^{-1} (01,027 to 12.8240 nM), respectively. It is recommended that water for human consumption does not exceed 2 µg Tl L^{-1} (Health Council of the Netherlands, 2002).

Tl is incorporated into the soil through the wastewater used for irrigation in farmland. Being available to plants in the soil solution in its ionic form Tl(I), this element is absorbed and accumulated in plant tissue, which represents a problem for humans if it is incorporated into the food chain, since the consumption of vegetables contaminated with this metal could represent a high health risk (Karbowska, 2016; Rodríguez-Mercado & Altamirano-Lozano, 2013). In these plants, the presence of contaminating compounds that contain metals can disrupt homeostasis and cause serious toxic effects, including nutritional imbalances (Rezaeian et al., 2019).

Nutrient imbalance in higher plants can trigger biochemical processes that have negative consequences on crop growth, yield, and quality (Cvjetko et al., 2010). Nutritional diagnosis is an extremely important tool to know the effect of Tl on plant nutrition, since it allows identifying nutritional deficiencies or toxicities in different phenological stages of the plant and learning its effects (Alcántar et al., 2016).

In white mustard (*Sinapsis alba* L.), Tl^{+} toxicity reduces photosynthesis, which alters ionic homeostasis and causes water imbalance, oxidative stress, and chlorosis (Mazur et al., 2016).

There are few studies that have quantified specific concentrations of Tl in plant tissues, although it is estimated that these may be less than 0.1 mg kg^{-1} on a dry basis (Kazantzis, 2000). In *Biscutella laevigata*, a pseudometallophyta species that often grows near mining areas, a marked ability to bioaccumulate trace metals, especially Tl, was found. The concentrations of Tl found in plant tissues ranged from 10 to 1000 mg kg^{-1} of plant biomass on a dry basis (Pavoni et al., 2016). In mining sites nearby Kütahya, Turkey, Tl mean concentrations in the soil, roots, and shoots of the studied plants were 170, 318, and 315 mg kg^{-1}, respectively. Good Tl bioaccumulators were *Cynoglossum officinale*, *Isatis* sp., *Silene compacta*, and *Verbascum thapsus*, which can be useful for remediation or phytoremediation of soils polluted by Tl (Sasmaz et al., 2016). With the exception of maize (*Zea mays*), other crops pose a significant risk to human health because of their abilities to accumulate Tl, which represent a dare need for Tl-focused management of soil/plant interaction (Jiang et al., 2020). Importantly, the effects that Tl has on the nutritional status in higher plants have not been deeply investigated. Therefore, the main objective of this study was to evaluate the effect of Tl on the leaf concentration of macronutrients in the Jalapeño, Poblano, and Serrano varieties of chili pepper (*Capsicum annuum*), grown hydroponically under greenhouse conditions. The tested doses of Tl were determined considering the maximum concentration of this element allowed in water for human consumption (2 µg Tl L^{-1}, which corresponds to 9.7855 nM Tl), as well as the levels reported in rivers and bodies of water that reach values of 12.824 nM Tl (Health Council of The Netherlands, 2002; Queirolo et al., 2009). As aforementioned, the experiment was carried out in hydroponics, since this system provides conditions that allow real measurement of the interactions between essential and non-essential elements (such as Tl) in the nutrient solution and may help explain how these interactions modify the availability of nutrients. In soil systems, nutrient bioavailability changes throughout the soil matrix as nutrients bind to soil particles creating micro-environments within the soil. This heterogeneity could add an extra level of complexity in experiments needing a precise control on the external concentration of nutrients or other molecules (Nguyen et al., 2016). Consequently, we decided to carry out the experiments in hydroponics under greenhouse conditions, using an inert substrate and a nutrient solution.

Within the genus *Capsicum*, *Capsicum annuum* is one of the five domesticated species, which shows great variation in fruit size, shape, and color (Kazantzis, 2000; Madejón et al., 2007). This vegetable is of great economic importance in Asia and the Americas, with China and Mexico being the leading world producers and distributors, respectively (FAO, 2022; Fernández, 2022).

2 Materials and Methods

2.1 Experimental Conditions and Plant Material

The study was carried out under greenhouse conditions with average day and night temperatures of 32 °C and 15 °C, respectively; relative humidity of 31% during the day and 87% at night; light intensity of 137 μmol m^{-2} s^{-1} with a photoperiod of 11.5 h.

Seedlings were produced from hybrid seeds of three varieties of *Capsicum annuum* L.: Jalapeño "Emperador" NUN 70,030, Poblano "Capulín," and Serrano "Coloso."

2.2 Treatment Design and Experimental Design

To establish the experiment, healthy and vigorous 60-day-old seedlings were transplanted into black bags with tezontle as substrate. The study was established in a completely randomized experimental design (CRD) with six replicates per treatment, irrigated with Steiner's universal nutrient solution (Steiner, 1984), which has the following chemical composition of macronutrients (in mol_c m^{-3}): 12 NO_3^-, 1 $H_2PO_4^-$, 7 SO_4^{2-}, 7 K^+, 9 Ca^{2+}, and 4 Mg^{2+}. The micronutrient concentrations used in mg L^{-1} were 5.000 Fe, 2.328 Mn, 0.466 Zn, 0.186 Cu, 0.432 B, and 0.173 Mo. The nutrient solution was formulated with analytical grade reagents (J. T. Baker; Radnor, PA, USA). The electrical conductivity of the nutrient solution was 2 dS m^{-1} and the pH was adjusted to 5.5. Irrigation was automated through a drip system.

Three levels of Tl were added to Steiner's nutrient solution: 0, 5.5, and 11 nM Tl from Tl $(OOCCH_3)$ (Sigma-Aldrich; Burlington, MA, USA), and the treatments were supplied for 80 days.

2.3 Evaluated Variables

On day 80 after beginning the application of the treatments, the plants were harvested and the leaves were separated from the aerial part to later be dried in a forced air oven (Riossa, model HCF-125; Guadalajara, Jalisco, Mexico) at 70 °C for 48 h. Once dry and at constant weight, the leaf samples were ground (particle size 2 mm) for macronutrient and Tl analysis. Foliar diagnosis is a direct evaluation method that utilizes nutrient concentrations in plant tissues as an indicator of nutritional status. In general, recent matured and physiologically active leaves are the plant organs, which better reflect the nutritional status. They respond more readily to variations in nutrient supply and are, thus, better qualified as samples (De Mello & Caione, 2012).

The leaf N concentration was evaluated in the extract resulting from the acid digestion of dry leaf tissue with a solution of $C_7H_6O_3$ in H_2SO_4 at a concentration of 3.3%, as described in the micro-Kjeldahl method (Alcántar & Sandoval, 1999). For the determinations of P, K, Ca, Mg, S, and Tl, the samples were subjected to acid digestion with HNO_3:$HClO_4$ (2:1; v:v) and the extracts were read in an inductively coupled plasma optical emission spectrophotometer (ICP-OES 725-ES; Agilent; Santa Clara, CA, USA). With the concentrations obtained, the concentration ratios between N and the rest of the macronutrients evaluated in each dose of Tl supplied were estimated. Additionally, as a growth parameter, the shoots dry biomass weight was considered, which was determined by adding the weights obtained in leaves and stems.

2.4 Data Analysis

From the data obtained, analysis of variance and comparison of means tests were performed (Tukey, 0.05) (SAS, 2011). To identify the main patterns of macronutrient foliar concentrations, we performed a principal component analysis (PCA), an exploratory statistical method for graphical description of the information present in large datasets. This analysis was carried out using the FactoMineR library of the RStudio v.1.2.1335 software (R Core Team, 2018).

The sizes of the standardized effects of Tl on the leaf concentrations of macronutrients were estimated using the natural log of the response relationship (ln RR) between plants treated with Tl and untreated plants (Hedges et al., 1999) as indicated in the following formula:

$$\ln RR = \ln[X_{Tl}/X_C]$$

Values of ln *RR* equal to zero indicate that there is no difference between Tl-treated and control plants. Positive and negative values of ln *RR* indicate positive and negative effects respectively; X_{Tl} represents the leaf concentration of macronutrient *X* in plants treated with Tl, and X_C represents the leaf concentration of macronutrient *X* in plants of the control treatment.

3 Results and Discussion

3.1 Leaf Concentrations of Macronutrients

Nitrogen The leaf N sufficiency ranges for the Jalapeño, Poblano, and Serrano varieties are from 38 to 42 (Johnson & Decotearu, 1996), from 45 to 53 (Cruz-Crespo et al., 2014), and from 21 to 34 g kg^{-1} on a dry basis (Carballar-Hernández et al., 2018), respectively. The results obtained here for Jalapeño, Poblano, and Serrano are within, below, and above the ranges of sufficiency, respectively, independently of the Tl treatments (Fig. 1). Tl had a differential effect on the N concentration among varieties. The significant reduction of 2.8% in Serrano stands out with the 11 nM Tl treatment, compared to the control. In Poblano, increases of 3.6 and 8.5% are recorded with respect to the control, with 5.5 and 11 nM Tl, respectively (Fig. 1). Increased N concentration is a response mechanism to counteract metal toxicity within cells in plants (Kapusta & Godzik, 2013). In poplar (*Populus deltoides* × *P. nigra*) exposed to toxic doses of Cd, the adequate supply of N increased the chlorophyll content and improved the health status of the plant (Zhang et al., 2014).

Phosphorus The sufficiency ranges of P for Jalapeño and Poblano are from 2.2 to 7.0 g kg^{-1} dry matter (Mills & Jones, 1996), and the results of this study are within this range. In the case of Serrano, at all Tl levels evaluated, the values were below the sufficiency range, which is from 2.3 to 2.6 g kg^{-1} on a dry basis (Cruz-Crespo et al., 2014). In Jalapeño and Serrano, treatment with 5.5 nM Tl increased P concentration by 4.8 and 18.6%, compared to the control, respectively. The increase in P concentration with low Tl doses has not been documented. The exposure to Cd increases the concentration of macronutrients as a consequence of the alteration of the nutrient absorption and distribution rates within the plant, as a strategy to inhibit the accumulation of the metal or to increase tolerance to Cd; this effect has also been observed with beneficial elements such as selenium (Se) (Nazar et al., 2012). Contrarily, in Poblano, the mean Tl dose reduced the leaf concentration of P by 4.8%, with respect to the control (Fig. 1).

Potassium The leaf K concentrations were significantly lower than the control when 5.5 nM Tl was applied in the Jalapeño and Serrano varieties. The leaf K concentrations obtained in Serrano pepper are within the sufficiency ranges of 47.6 to 50.8 g kg^{-1} dry matter (Cruz-Crespo et al., 2014). In Poblano chili pepper, the concentrations obtained are above the sufficiency ranges, from 4.6 to 16.9 g kg^{-1} dry matter (Carballar-Hernández et al., 2018). In Jalapeño, these concentrations are also higher than the range reported by Mill and Jones (1996), which were from 35 to 45 g kg^{-1} dry matter (Fig. 1).

Tl did not affect the leaf K concentration in the Poblano variety. Conversely, in Jalapeño and Serrano, the 5.5 nM Tl treatment reduced the K concentration by 15 and 13%, respectively (Fig. 1). The exact mechanism of Tl toxicity is not clear, but it is believed to be related to the interference of K-dependent vital processes, since Tl ions imitate the biological action, movement, and intracellular accumulation of K ions, thus affecting enzyme production, amino acid synthesis, and transport mechanisms. Furthermore, Tl interferes in the activity of Na^+/K^+ ATPase, since it has been found that Tl and K ions are univalent with similar ionic charges and radii (Tl^+, 1.76 Å; K^+, 1.60 Å), which causes cell membranes not to have the ability to distinguish between these two ions (Rader et al.,

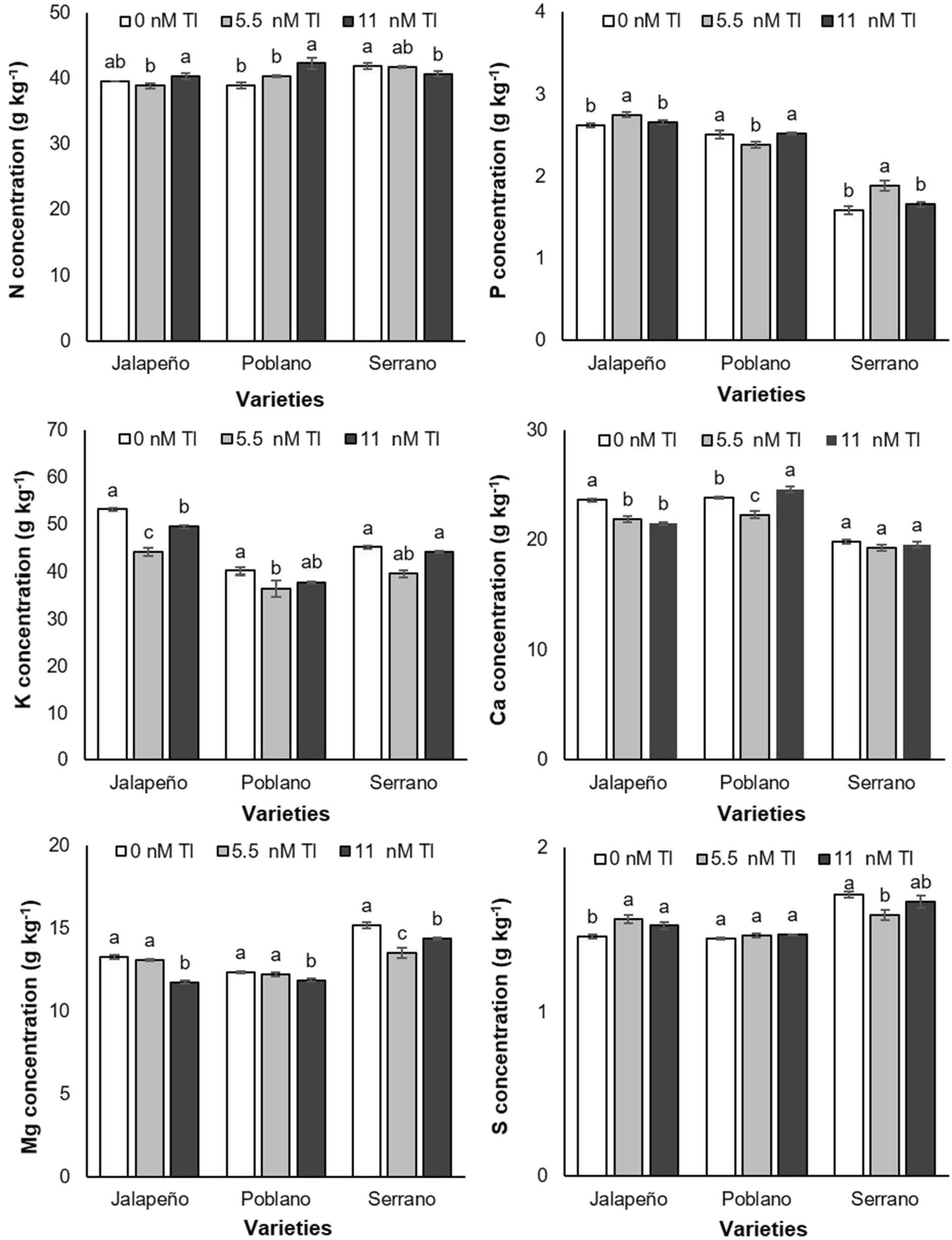


Fig. 1 Leaf concentration of N, P, K, Ca, Mg, and S in three chili pepper varieties treated with thallium ($n = 6$, $\alpha = 0.05$ by Tukey's multiple test)

2019; Viraraghavan & Srinivasan, 2011). Importantly, most of these studies have been carried out in animal systems.

Calcium Regarding the leaf Ca concentration, statistical differences were only observed among treatments in the Jalapeño and Poblano varieties. In Jalapeño, the 5.5 and 11 nM Tl doses reduced the Ca concentration by 8 and 10%, respectively. In Poblano, there was only a decrease with the 5.5 nM Tl dose compared to the 11 nM Tl dose. In Serrano, decreases in Ca concentrations were also observed with respect to the control, although differences were not significant for any of the treatments (Fig. 1).

In the Poblano and Jalapeño varieties, the leaf concentrations obtained for Ca were within the sufficiency ranges reported by Uchida (2000), which are from 10 to 25 g kg^{-1}, while those recorded in Serrano leaves are below the ones reported by Cruz-Crespo et al. (2014), ranging from 24.1 to 29.9 g kg^{-1} dry matter. Although the Ca concentrations in the three varieties are within the sufficiency ranges, significant reductions in this variable are observed in the Jalapeño variety when the plants were treated with Tl (Fig. 1). Conversely, plants exposed to metals such as Cd and Pb show increases in the Ca concentration as a defense mechanism to neutralize the toxicity of these elements given the function of Ca as a second messenger (Thévenod, 2009). In tomato (*Solanum lycopersicum*) and eggplant (*Solanum melongena*), the application of low concentrations of Cd (1.5 mg L^{-1}) and Pb (75 mg L^{-1}) had stimulating effects on plants, including an increase in Ca concentrations, although higher levels of these metals (i.e., 150 mg L^{-1} Pb and 3.0 mg Cd L^{-1}) caused phytotoxic effects, with the Ca concentration being inversely proportional to the Pb and Cd concentrations applied (Khan & Khan, 1983).

Metals such as Cd and Pb enter the cell through the same transport mechanisms as Ca, that is, calcium channels in the plasma membrane. The replacement of Ca^{2+} by these metals represents a serious imbalance in the cell, given the crucial role of Ca^{2+} as a second messenger in higher plants. As a pivotal second messenger, Ca interacts with proteins such as calmodulin and calcineurin that decode spatiotemporal patterns of intracellular concentration of this element, which in turn regulate a wide range of signaling pathways including responses to abiotic stress (Marchetti, 2013).

Magnesium Mg concentrations in the Poblano variety were not affected by Tl treatments. In Jalapeño and Serrano, Mg concentrations decreased when applying 11 and 5.5 nM Tl, respectively, in comparison to the control (Fig. 1).

The leaf Mg concentrations in the Jalapeño and Poblano varieties are within the sufficiency ranges reported for higher plants. Concentrations greater than 15 g kg^{-1} can be harmful to plants, limiting growth and inhibiting photosynthesis (Marschner, 2012). The critical level of Mg in leaf tissue for early identification of a deficiency is 2.1 g kg^{-1} dry matter (Hauer-Jákli & Tränkner, 2019; Riga & Anza, 2003). The leaf Mg concentration in Serrano is within the range reported by Cruz-Crespo et al. (2014), from 11 to 12.8 g kg^{-1} on a dry basis (Fig. 1). To date, there are no reports of leaf Mg concentrations in plants treated with Tl. Experiments evaluating the effects of Mg deficiency in plants show that visible symptoms appear at the leaf level below the sufficiency ranges: yellow to reddish leaves, interveinal necrosis, and senescence in mature leaves (Tanoi & Kobayashi, 2015). The presence of metals such as Al and Cd in plants causes damage to cell membranes, alters the absorption of nutrients, and decreases photosynthesis and the biosynthesis of many metabolites (Rengel et al., 2015). Furthermore, Mg, as a structural component of the chlorophyll molecule, is associated with numerous enzymes, including ATPases, RNA polymerases, protein kinases, phosphatases, glutathione synthase, and carboxylases. Mg is essential for membrane stabilization, and, consequently, when Mg is deficient, antioxidant protection mechanisms and cell homeostasis in the plant are hindered (Hermans et al., 2011).

Sulfur Thallium did not affect leaf S concentrations in any of the three pepper varieties evaluated (Fig. 1).

In Jalapeño chili pepper, leaf S concentrations have been reported higher than 0.39 g kg^{-1} (Azofeifa & Moreira, 2008). In Poblano, S concentrations of S were within the sufficiency ranges (1 to 5 g kg^{-1} S on a dry basis) reported for higher plants (Marschner, 2012). Finally, the concentrations in Serrano are below those reported by Barker and Pilbeam (2007), which range from 3 to 7 g kg^{-1} S on a dry basis. Maintaining S sufficiency concentrations is essential to the optimal development of plants. Since S-containing compounds such as the amino acids cysteine and methionine are crucial components in plant metabolism, S deficiencies may drastically hamper diverse biological processes, including hydraulic conductivity, stomatal opening, net photosynthesis, leaf area, chlorophyll concentration in leaves, and protein synthesis (Wawrzynska et al., 2015).

The macronutrient concentration results obtained in here indicate that Tl has differential effects among varieties. Although the effect of Tl on nutritional status in higher plants has not been extensively documented, it is well known that high levels of other metals such as Cu, Ni, and Cd affect the absorption and therefore the concentration of nutrients in plants (Arif et al., 2016; DalCorso et al., 2014).

Principal component analysis (PCA) revealed that the sum of principal component 1 and principal component 2 (PC1 + PC2) represented 80.8, 72.3, and 79.6% of the total variance of macronutrient concentration in leaves of the Jalapeño, Poblano, and Serrano varieties, respectively (Fig. 2), with PC1 being the dominant one. Likewise, in the Jalapeño variety, a clear separation of the nutritional concentrations was observed in the three clusters corresponding to the Tl levels. On the contrary, in the Poblano variety, there is less differentiation in the concentrations of macronutrients based on the levels of Tl. The following section presents the results of the size and direction of the effects of Tl, generated using the natural log of the response relationship (ln *RR*), an ad hoc meta-analysis approach for ecological studies related to stress factors, in this case presence of Tl, a non-essential element in higher plants.

3.2 Size and Direction of the Effects of Tl on Leaf Concentrations of Macronutrients

The size and direction of the effect caused by the 5.5 nM Tl dose on the leaf K concentration was significant and negative, respectively, in the three varieties of chili pepper. The same trend was observed in the leaf Ca concentrations of Jalapeño and Poblano with the 5.5 nM Tl dose. In hydroponic systems, Tl^+ can use the absorption routes of monovalent cations, mainly K^+, due to the similarity in ionic radii ($K^+ = 1.60$ Å; $Tl^+ = 1.76$ Å). Furthermore, Tl^+ tends to mimic the biochemistry of K^+ and has a higher affinity for organic ligands (Holubík et al., 2021; Kwan & Smith, 1991). Likewise, the size and direction of the effect of the 11 nM Tl treatment was significant and negative on the leaf Mg concentration. The reduction in the concentration of K^+ can affect the transport of water and the translocation of nutrients to the aerial part, among them Ca^{2+} and Mg^{2+} (Hasanuzzaman et al., 2018), as observed in this study.

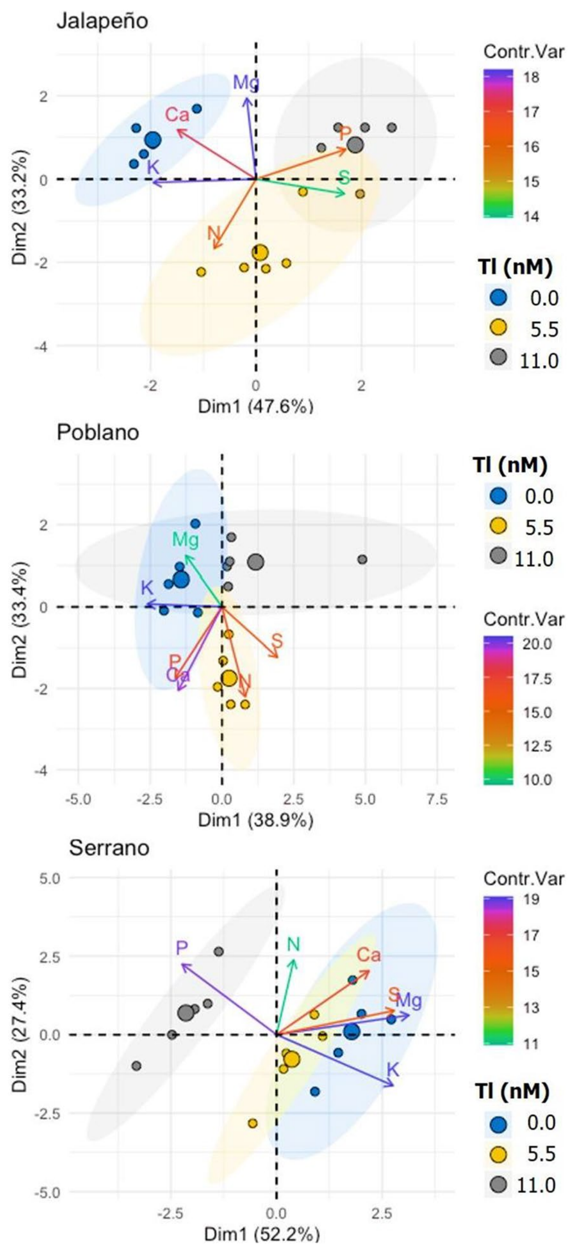
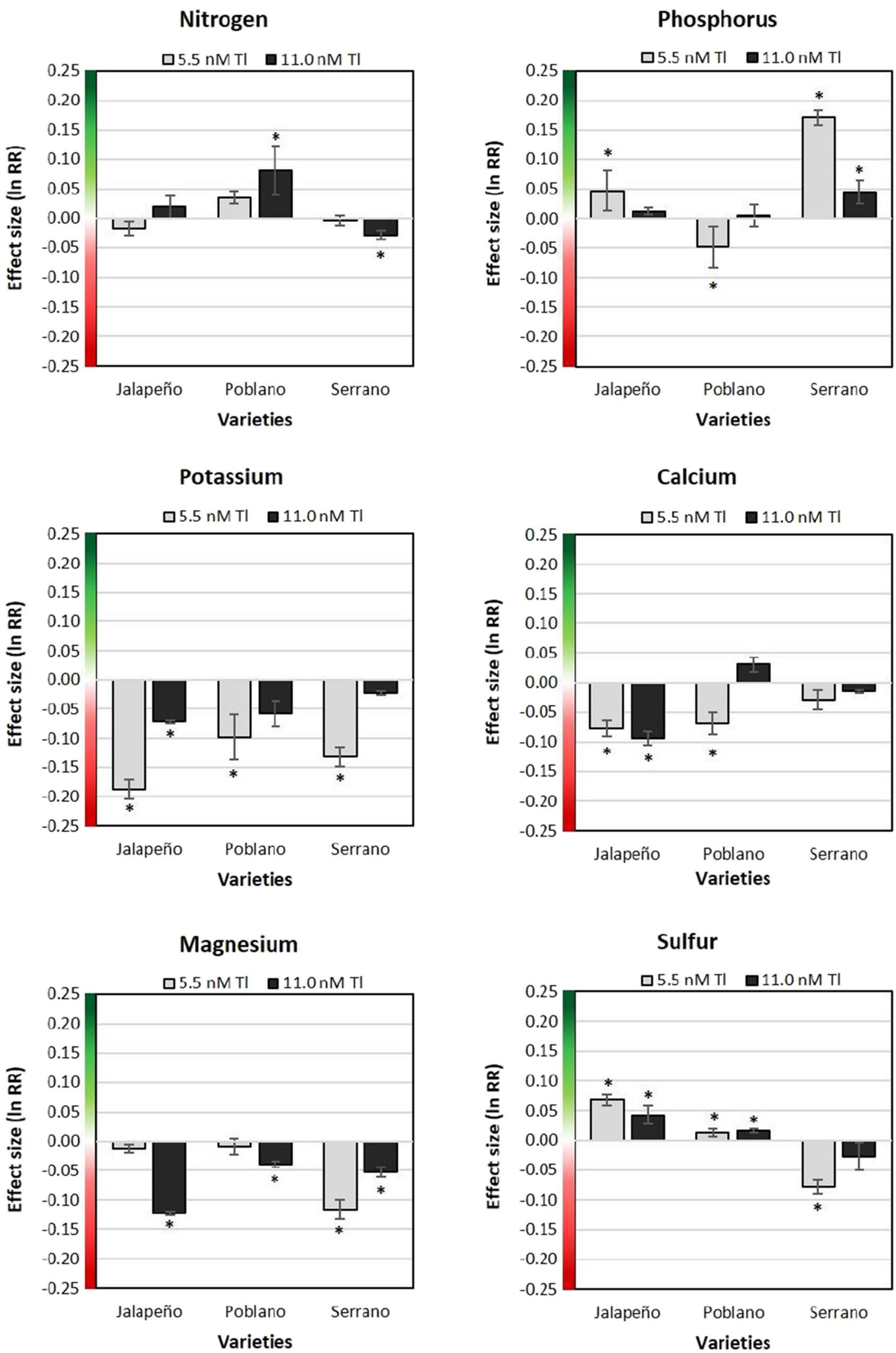


Fig. 2 Principal component analysis for leaf nutrients concentrations in three chili pepper varieties treated with thallium (0, 5.5, or 11 nM Tl) in the nutrient solution


Nitrogen
Phosphorus
Potassium
Calcium
Magnesium
Sulfur
5.5 nM Tl
11.0 nM Tl
Effect size (ln RR)
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
*
Jalapeño
Poblano
Serrano
Varieties

◂**Fig. 3** Effect size and direction of thallium on macronutrient concentrations in the leaves of three chili pepper varieties. Positive and negative values indicate positive and negative effects, respectively. The asterisk indicates significant effects ($n=6$)

Regarding N, P, and S, the size and direction of the effect of Tl was dose dependent and different among varieties. The 11 nM Tl dose significantly affected leaf N concentration in Poblano and Serrano, being positive in the former and negative in the latter.

Significant and positive effects of 5.5 nM Tl on the leaf P concentration in Jalapeño and Serrano were recorded; contrarily, this same dose caused a significant negative effect on the P concentration in Poblano. Both 5.5 and 11 nM Tl caused effects of significant size and of positive direction in the leaf S concentration in Jalapeño and Poblano (Fig. 3). The significant positive effects recorded may be related to the stimulation of mechanisms aimed at mitigating negative effects of Tl, and thus being indicators of tolerance in the varieties.

3.3 Macronutrient Ratios in Leaves

The interaction among nutrients in plant tissues is of great importance as it is a valuable diagnostic tool that helps us to know and maintain optimal concentrations. Any imbalance in nutritional ratios can alter cell homeostasis and limit crop yield (Sardans et al., 2017; Sinclair et al., 1997). The ratio between foliar nutrient concentrations of N and the rest of the nutrients for the Jalapeño, Poblano, and Serrano varieties treated with Tl is shown in Table 1.

The N:K ratio increased with the 5.5 nM Tl dose in Jalapeño and Serrano, with respect to the control, while in Poblano, both evaluated Tl doses (5.5 and 11 nM Tl) increased it. The N:Ca ratio increased in Jalapeño with 5.5 and 11 nM Tl, while in Poblano, it increased only with the 5.5 nM Tl dose, in both cases with respect to the ratio recorded in the absence of this element. In the N:S relationship, effects of Tl were also observed, where the 11 nM Tl dose increased this ratio in Jalapeño and Serrano, and the 5.5 nM Tl dose increased it in Serrano (Table 1).

The Jalapeño and Serrano varieties showed a positive and significant interaction between the Tl concentration in the nutrient solution and the N:K and N:Mg leaf concentration ratios (Table 1). This may suggest a competition for absorption sites between these two ions, since these elements can associate synergistically or antagonistically in the plant (Kam et al., 2019; Mazur et al., 2016). The interaction between nutrients is directly related to the absorption of metals present in the soil solution, which depends on the internal mechanisms of each species or variety studied (Smical et al., 2008). This may explain, at least in part, the different behavior of the chili pepper varieties evaluated in response to Tl.

Similar results have been observed in wheat seedlings exposed to 1 mg Cd L^{-1}, revealing that varieties exposed to Cd increased the nutritional concentration of N, P, K, and Mn, and decreased that of Mo. This response could be attributed to the genotypic difference of the plants and their tolerance capacity to Cd (Zhang et al., 2002).

In the present study, varieties evaluated displayed different responses to Tl exposure. The nutritional status was affected by Tl in the following order: Jalapeño > Serrano > Poblano. Coincidentally, Jalapeño, Serrano, and Poblano seeds treated with 25 μM Tl decreased germination by 7.7, 7.5, and 5.3%, respectively, as compared to the control (Buendía-Valverde et al., 2018).

Plants are generally sensitive to the presence of non-essential metals, but at low concentrations, these could stimulate proper growth and development (Arif et al., 2016). Due to its high toxicity, we tested low concentrations of Tl in the present study. This could explain in part the relatively low variation in leaf concentrations of macronutrients.

Considering that Tl is a non-essential element in plant nutrition, and that it produces different effects among the evaluated varieties, it can be inferred that this element interferes with the absorption of nutrients, probably by affecting the permeability of the plasma membrane. However, more studies are needed to explore the mechanisms that this element alters within the plant.

3.4 Foliar Concentration of Thallium

A few studies have quantified specific concentrations of Tl in plant tissues, although it is reported that this element can be found in concentrations lower than 0.1 mg kg^{-1} on a dry basis (Kazantzis, 2000). In this study, foliar concentrations of Tl in control plants ranged between 0.7 and 1.8 mg kg^{-1} on a dry basis. In Tl-treated plants, Tl concentrations ranged from 12

Table 1 Nutrient concentration ratios between N and the rest of the macronutrients analyzed in chili pepper leaves treated with thallium (Tl) for 80 days

Jalapeño					
Tl (nM)	N:P	N:K	N:Ca	N:Mg	N:S
0.0	15.30 ± 0.61a	0.75 ± 0.01c	1.67 ± 0.01c	2.99 ± 0.04b	27.41 ± 0.26a
5.5	14.11 ± 0.26a	0.86 ± 0.01a	1.78 ± 0.03b	2.94 ± 0.04b	25.14 ± 0.47b
11.0	15.43 ± 0.31a	0.82 ± 0.01b	1.90 ± 0.02a	3.47 ± 0.03a	26.68 ± 0.48a
Poblano					
Tl (nM)	N:P	N:K	N:Ca	N:Mg	N:S
0.0	15.48 ± 1.16a	0.97 ± 0.05 c	1.63 ± 0.05 b	3.15 ± 0.04 b	26.97 ± 0.38b
5.5	16.63 ± 1.08a	1.06 ± 0.02 b	1.79 ± 0.04 a	3.28 ± 0.04 b	27.71 ± 0.30ab
11.0	16.89 ± 0.79a	1.13 ± 0.05 a	1.71 ± 0.06 ab	3.59 ± 0.11 a	28.82 ± 0.82 a
Serrano					
Tl (nM)	N:P	N:K	N:Ca	N:Mg	N:S
0.0	26.96 ± 0.17 a	0.92 ± 0.02 b	2.10 ± 0.03 a	2.74 ± 0.06 b	24.36 ± 0.03 a
5.5	22.94 ± 0.53 b	1.06 ± 0.03 a	2.17 ± 0.03 a	3.15 ± 0.10 a	26.66 ± 1.38 a
11.0	24.64 ± 1.09 b	0.92 ± 0.01 b	2.09 ± 0.03 a	2.84 ± 0.06 b	25.00 ± 0.85 a

Means ± SD with different letters in each variable indicate significant differences ($n = 6$, $\alpha = 0.05$ by Tukey's multiple test)

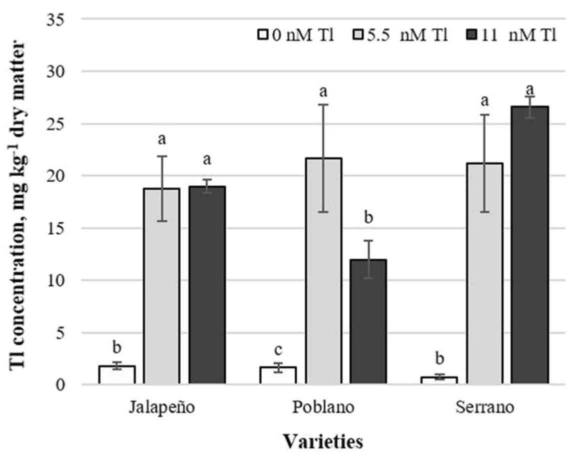


Fig. 4 Leaf concentration of Tl in three chili pepper varieties treated with thallium ($n = 6$, $\alpha = 0.05$ by Tukey's multiple test)

to 26.6 mg kg^{-1} on a dry basis (Fig. 4). These values are lower than those reported for barley (*Hordeum vulgare*) and sunflower (*Helianthus annuus*), with concentration of 30–246 and 89–637 mg kg^{-1} of dry matter of stems, respectively (Kim et al., 2016). In five cultivars of green cabbage (*Brassica oleracea* L. var. capitata L.), a Tl hyperaccumulating plant, established in soil, the concentrations of this element in leaves ranged between 101 and 192 mg kg^{-1} of dry matter (Ning et al., 2015). Likewise, in *Tessaria absinthioides* growing in the soil and in the wild, in mining areas contaminated with Tl, foliar concentration ranges of Tl between 0.01 and 0.07 mg kg^{-1} were reported. The highest concentrations were recorded in areas where the level of Tl in water was 120 ng L^{-1} (0.587 nM Tl) (Queirolo et al., 2009). These results show the differences in foliar concentration of Tl not only depend on plant genotype, but also on the production system. In edible parts of chili peppers, Tl concentration has been reported among 0.17 mg kg^{-1} on a dry basis (D'Orazio et al., 2020) and 2.91–5.31 mg kg^{-1} on a dry basis (Jiang et al., 2020).

3.5 Shoot Biomass Production

Exposure to Tl did not significantly affect the dry biomass of shoots in any of the three chili varieties evaluated (Table 2). Coincidently, biomass productions as not affected by the doses of Tl tested, neither. Interestingly, no visual symptoms revealing nutrient deficiencies or toxicities caused by Tl were observed. In white mustard (*Sinapis alba* L.), Tl toxicity was observed in leaves when its concentration in the nutrient solution increased from 0.489 to 4.89 µM, after 4 weeks of treatment (Mazur et al., 2016). Such Tl levels are very high compared to those evaluated in our study. Roots are the best Tl bioaccumulators, as compared to shoots (leaves and stems) (Queirolo et al., 2009). Furthermore, the Tl bioconcentration capacity among plant species follows the following gradient: rhizome vegetables > leafy vegetables > fruit vegetables (chili in this category) > cereal (Jiang et al., 2020).

Table 2 Shoot dry biomass production in chili pepper plants treated with thallium (Tl) for 80 days

Tl (nM)	Varieties		
	Jalapeño	Poblano	Serrano
0.0	28.10 ± 10.07a	30.30 ± 7.86a	65.25 ± 10.87a
5.5	19.37 ± 7.42a	43.52 ± 21.02a	47.57 ± 8.81a
11.0	19.20 ± 6.73a	28.95 ± 4.37a	78.3 ± 13.75a

Means ± SD with different letters in each variety indicate significant differences ($n = 6$, $\alpha = 0.05$ by Tukey's multiple test)

4 Conclusions

Herewith, we demonstrated that the exposure to Tl has different effects on the nutrient status of three chili pepper varieties: Jalapeño, Poblano, and Serrano. Jalapeño was the most affected variety, while Poblano was the least. The susceptibility of the Jalapeño variety to Tl was evidenced in the significant negative correlations between this metal and the foliar concentrations of Ca and Mg, as well as with the Ca:S and Mg:S concentration ratios. Also in Jalapeño, the Tl concentration in the nutrient solution was positively related to the leaf N:K, N:Ca, N:Mg, P:Ca, and P:Mg concentration ratios. On the contrary, in Poblano, Tl only correlated significantly and positively with the leaf Mg:S concentration ratio. The principal component analysis demonstrated that the sum of principal component 1 and principal component 2 represented 80.8, 72.3, and 79.6% of the total variance of macronutrient concentration in leaves of the Jalapeño, Poblano, and Serrano varieties, respectively, with principal component 1 being the dominant one. Tl did not affect shoot dry biomass in any of the three varieties evaluated, though plants exposed to this element concentrated 12.0 to 26.6 mg kg^{-1} on a dry basis. Thus, different genotypes of chili pepper exhibit different responses to Tl.

Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

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