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PHYSIOLOGICAL AND NUTRIENT CHANGES IN SWEET PEPPER (*Capsicum annuum* L.) SEEDLINGS CAUSED BY CADMIUM

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ABSTRACT

Cadmium (Cd) is a highly toxic element to living organisms and represents a potential environmental pollution problem. Some crops are severely damaged when grown in the presence of toxic Cd levels, although the extent of the damage varies among species, even among varieties. In this study two Cd concentrations (15 and 30 μM) added to the nutrient solution used for growing Yolo Wonder variety sweet pepper (*Capsicum annuum* L.) seedlings were evaluated. As a control, seedlings were kept in a nutrient solution without Cd. The seedlings showed high sensitivity to Cd. Plant height decreased by 22.43 and 36.24 % with the addition of 15 and 30 μM compared to the control treatment, respectively. Also, the foliar area of seedlings treated with 30 μM Cd was reduced by about 80 % compared to control plants. Chlorophyll content (a, b and total) also decreased with the presence of Cd in solution. The accumulation of N, P and K *in planta* decreased with increasing Cd concentration in the nutrient solution, while Cd accumulation in plant tissue was directly proportional to the Cd level added to the nutrient solution. The accumulation by plant organs in descending order was as follows: roots > leaves > stems.

Palabras clave: toxicidad por metales pesados, toxicidad por Cd, tolerancia a Cd, Solanaceae

RESUMEN

El cadmio (Cd) es un elemento altamente tóxico para los organismos vivos y representa un problema potencial de contaminación ambiental. Algunos cultivos son severamente dañados cuando crecen en presencia de niveles tóxicos de Cd, aunque el grado del daño varía entre especies e incluso entre variedades. En este estudio se evaluaron dos concentraciones de Cd (15 y 30 μM) agregadas a la solución nutritiva de plántulas de pimiento morrón (*Capsicum annuum* L.) de la variedad Yolo Wonder. Como testigo, se crecieron plántulas en solución nutritiva sin Cd. Las plántulas mostraron alta sensibilidad al Cd. La altura de planta disminuyó entre 22.43 y 36.24 % con la aplicación de 15 y 30 μM, respectivamente, comparado con el testigo. Además, el área foliar de las plántulas tratadas con 30 μM Cd se redujo en alrededor del 80 % comparado con la

plántulas testigo. El contenido de clorofila (a, b y total) también disminuyó con la presencia de Cd en la solución. La acumulación de N, P y K in planta se redujo con el incremento de la concentración de Cd en la solución nutritiva, mientras que la acumulación de Cd en los tejidos vegetales fue directamente proporcional al nivel de Cd agregado a la solución nutritiva. La acumulación por órganos de la planta en orden descendente fue como sigue: raíces > hojas > tallos.

INTRODUCTION

Cadmium (Cd) is a toxic metal to living organisms when certain limits are exceeded in their cells and tissues. Once this metal exceeds these tolerance limits, disorders of physiological, biochemical and molecular nature can occur, including imbalances in the uptake and in the transport of nutrients, which may ultimately inhibit plant growth, development and reproduction. Since this metal shows high mobility and low affinity for soil colloids (Akoumianakis *et al*. 2008, Erdem *et al*. 2012), it easily enters the trophic chain and has been implicated in various diseases including anemia, pulmonary edema, liver, nerve and brain damage, cancer and even death (ATSDR 2012). In fact, Cd is the most dangerous of all metals since it is the only element that can damage human and animal cells at concentrations that are not toxic in plants (Peijnenburg *et al*. 2000, Peralta-Videa *et al*. 2009).

Despite the danger that cadmium poses to human health, Mexico does not have a plan or strategy for either managing it or reducing its use in the manufacture of toys and industrial products such as batteries and fertilizers (Godoy 2011). In addition, the country produces more than 1600 t of cadmium annually and exports fertilizers as well as chemical, plastic and non-corrosive products containing this metal. Mexico also imports considerable quantities of nickel-cadmium batteries that when discarded, exacerbate environmental pollution. Hence, recent studies have found the presence of this metal in some foods at concentrations far exceeding the levels allowed by international standards for human consumption, which represents a huge public health risk (Gama-Flores *et al*. 2007, Peralta-Videa *et al*. 2009, Lango-Reynoso *et al*. 2010, Godoy 2011). As a result, some research groups in the country have focused their attention on studying this matter in greater detail.

In plants, the toxic effects of Cd include oxidative stress, genotoxicity, oxidation of the photosynthetic apparatus, and inhibition of root metabolism (Andresen and Küpper 2013), through its interaction with soil minerals and microbial populations, which in turn alters the uptake of nutrients. Stomatal opening, transpiration and photosynthesis are other processes also affected by Cd. Chlorosis, leaf curls and stunting are the most visible symptoms of Cd toxicity. Chlorosis can be caused by deficiencies in Fe and P or by reduced transport of Mn, and in general, toxic Cd levels interfere with the uptake, transport, mobility and use of nutrients and water in the plant. Cd also affects membrane permeability because it causes lipid peroxidation, which reduces the water content in the cell and alters the organism's water balance. Cd also reduces ATPase activity and causes changes in chloroplast metabolism by inhibiting chlorophyll biosynthesis and reducing the activity of enzymes involved in CO_2 fixation. Oxidative stress caused by toxic Cd levels is determined by inducing the production of free radicals derived from the transport of membrane electrons, chloroplasts and mitochondria, and the decrease in antioxidant system activity, which leads to senescence and cell death in extreme conditions (Benavides *et al*. 2005, Erdem *et al*. 2012, Nazar *et al*. 2012), and which depends on the homeostatic mechanisms that the plant has developed through evolution, making it possible to observe different responses between genotypes. As pepper is one of the most cultivated vegetable and spice crops worldwide, and Mexican sweet pepper production is facing serious environmental problems including heavy metal pollution, this research aimed to analyze the effect of Cd on growth, chlorophyll concentration and the accumulation of some nutrients and Cd in different parts of Yolo Wonder variety sweet pepper (*Capsicum annuum* L.) seedlings.

MATERIALS AND METHODS

Experiment location

The experiment was conducted in a zenithal-type greenhouse covered with milky-white plastic at Colegio de Postgraduados in Montecillo, State of Mexico, at an altitude of 2250 m, 19º 29' LN and 98º 54' LW.

Nocturnal temperatures ranged from 9.03 to 24.4 ºC,

TABLE I. NUTRIENT SOLUTION CONCENTRATIONS USED IN YOLO WONDER VARIETY SWEET PEPPER SEEDLINGS TREATED WITH DIFFERENT Cd CONCENTRATIONS

Experimental phase	NO_3^-	$H_2PO_4^-$	SO_4^{2-}	K^+	Ca^{2+}	Mg^{2+}
	mol_c/m^3					
25 % Steiner (acclimation phase)	3.00	0.25	1.75	1.75	2.25	1.00
50 % Steiner (treatment phase)	6.00	0.50	3.50	3.50	4.50	2.00

with an average of 18.42 ºC. Diurnal temperatures ranged from 10.21 to 41.52 ºC, with an average of 25.09 ºC. Nocturnal relative humidity ranged from 44.7 to 100 %, with a mean of 72.9 %; meanwhile during the day, relative humidity ranged from 96.1 to 21.7 %, with an average of 49.98 %. The luminosity averaged 600.49 mmol/m^2/s.

Plant material

Twenty day old Yolo Wonder variety sweet pepper (*Capsicum annuum* L.) seedlings were used as plant experimental material. At the time of transplantation, the seedlings had a height of 7 cm and ten leaves on average.

Prior to treatment application and as an acclimation strategy, seedlings were established in floating plates, for which 3-L plastic buckets fitted with lids, were used. The buckets were first disinfected with sodium hypochlorite (1 mL/L) and then filled with 25 % Steiner nutrient solution (Steiner 1984; **Table I**), supplemented with micronutrients.

Treatments evaluated

After an eight-day acclimation period, the seedlings established in nutrient solution received the Cd treatments, and the Steiner nutrient solution concentration was increased to 50 % (**Table I**), supplemented with micronutrients at the concentrations indicated in **Table II**. Two Cd concentrations (15 and 30 µM) using $3CdSO_4$ $8H_2O$ as a source of this element were used. As a control treatment, plants were irrigated with a nutrient solution without Cd. Cd treatments were applied for 15 days, with the nutrient solution changed every seven days, and every 48 h the water lost through evapotranspiration was replenished and the pH adjusted to 5.3. The nutrient solution was oxygenated using air pumps of 19 L of capacity and timers set to go off every 3 h to provide aeration for 15 min.

TABLE II. MICRONUTRIENT CONCENTRATION IN THE NUTRIENT SOLUTIONS USED IN YOLO WONDER VARIETY SWEET PEPPER SEEDLINGS TREATED WITH DIFFERENT Cd CONCENTRATIONS

Concentration	Nutrients					
	Fe	Cu	Zn	Mn	B	Mo
mg/L	4.988	0.186	0.466	2.328	0.423	0.173

Four replicates were used for each treatment and the experimental unit consisted of four seedlings in a 3-L plastic bucket fitted with lids. The experimental units were distributed in a completely randomized design.

Variables evaluated

At the experiment's harvest time (15 days after the treatments began), the following growth parameters were assessed: plant height, stem diameter, number of leaves, foliar area, root length and volume.

All fresh leaf material of two seedlings of each one of the four experimental units were collected and stored at –80 º C for chlorophyll determination. Chlorophyll content was determined in ethanol extracts using the method described by Quick *et al*. (1991) and read at wavelengths of 665 and 645 nm.

In parallel, fresh material of the rest two seedlings of each experimental unit were collected and divided into leaves, stems and roots, and dried separately at 70 ºC in a forced convection oven (Riossa brand, HCF-125D model; Monterrey, N. L., Mexico) for 72 h. Once the material was dry, each plant organ was individually weighed. In the dry leaf, stem and root material, concentrations of N, P, K and Cd were determined. Total nitrogen concentration was determined by the Semimicro-Kjeldhal method (Bremner 1965) using a catalyst mixture, adding sulfuric-salicylic acid for digestion. The P, K and Cd concentrations were determined using the wet digestion of dry material method by adding perchloric and nitric acids (Alcántar and Sandoval 1999). Readings were made using an inductively coupled plasma atomic emission spectrometer (Agilent ICP-OES, 725-ES model; Mulgrave, Victoria, Australia). With the concentration values of each element and the dry matter weights of each organ, nutrient accumulation was estimated.

Statistical analysis

An analysis of variance and a Tukey test ($P \leq 0.05$) were performed using the PROC ANOVA procedure for a completely randomized experiment in the SAS statistical software (SAS Institute 2011) Version 9.3.

RESULTS

Cd influence on growth parameters

The Cd caused negative effects on the growth of sweet pepper seedlings, even in the lowest concentration tested. As the Cd concentration in the nutrient solution was increased, stem diameter gradually decreased. Plant height was reduced by 22.43 and 36.24 % when seedlings were exposed to 15 and 30 µM Cd in the nutrient solution, respectively, compared to the control treatment (**Fig. 1A**).

Similarly, Cd application in the two concentrations evaluated (15 and 30 µM Cd) significantly reduced root length and root volume, compared to the control (**Fig. 1B**).

The Cd-exposed seedlings had fewer leaves, and they were smaller than those of the control plants. In the treatments with 15 and 30 µM Cd, foliar area was smaller by 44.48 and 79.77 % respectively, compared to the control.

Cd effect on chlorophyll concentration

Chlorophyll concentrations were negatively correlated with the Cd concentration added to the nutrient solution. In plants treated with the higher Cd concentration (30 µM), the levels of chlorophyll a, chlorophyll b and total chlorophyll decreased by 49.45, 52.04 and 49.99 %, respectively, considering the control treatment as a reference (**Fig. 2**).

Accumulation of macronutrients and Cd

Accumulation of P and K in leaves showed no statistical differences between the two Cd levels tested, but the accumulation observed in control plants was lower. By contrast, foliar N accumulation was statistically different both between the Cd levels added to the nutrient solution and between them and the control.

In stems, the macronutrient accumulation decreased significantly in the presence of Cd in the nutrient solution.

Similarly, the accumulation of N, P and K in roots also decreased in plants treated with both 15 and 30 µM Cd in the nutrient solution. In these tissues, the N concentration had greater variation as a function of the Cd concentration in the nutrient solution (**Fig. 3A**, **B** and **C**).

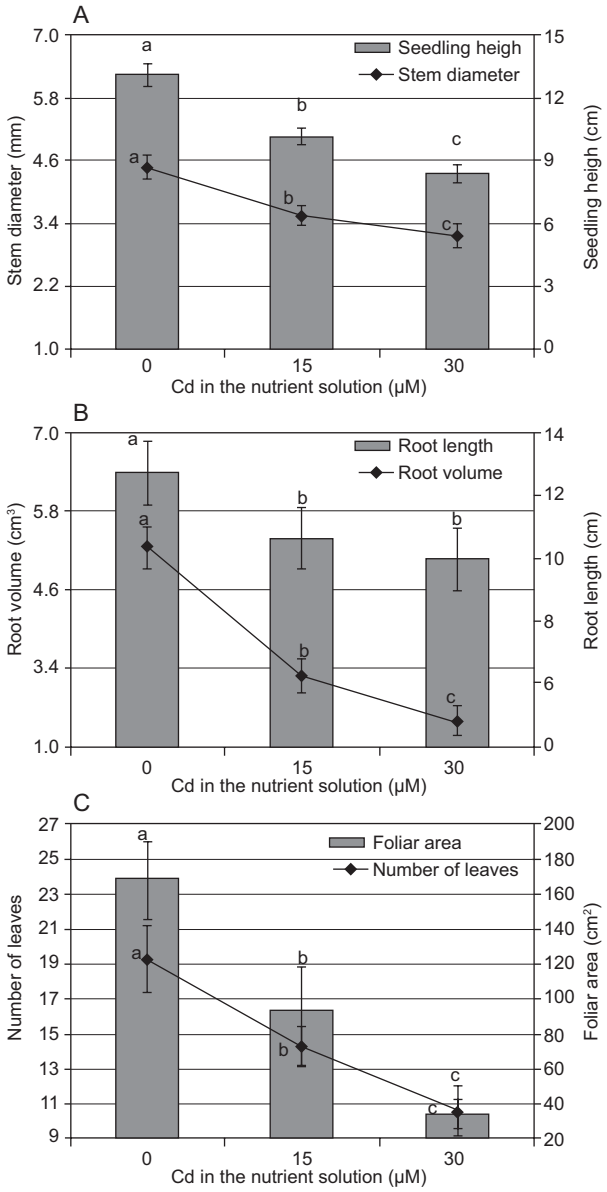

Fig. 1. Growth parameters of Yolo Wonder variety sweet pepper seedlings treated with different Cd concentrations in the nutrient solution. (A). Stem diameter and root length; (B). Root volume and root length, (C). Number of leaves and foliar area. Means ± SD with different letters in each variable are significantly different (Tukey $P \leq 0.05$)

On the other hand, the accumulation of Cd in leaves, stems and roots was positively correlated with its concentration in the nutrient solution. In treatments consisting of Cd supplied at concentrations of 15 and 30 µM, the accumulation of this element in leaves was 91.46 and 64.69 mg, respectively. The Cd quantities in stems were slightly lower than those recorded in leaves, with averages of 77.15 and 46.44 mg Cd in the treatments with 30 and 15 µM Cd,

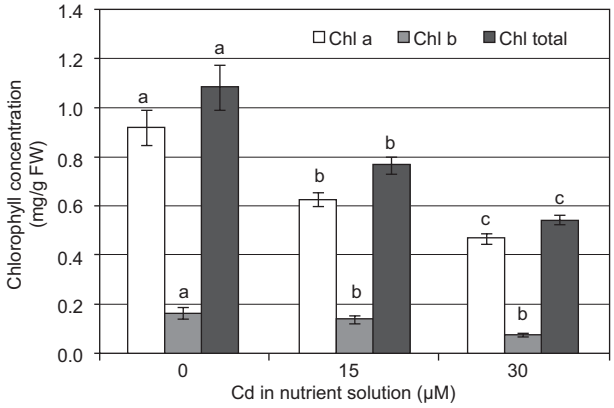

Fig. 2. Foliar chlorophyll (a, b and total) concentration in Yolo Wonder variety sweet pepper seedlings treated with different Cd concentrations in the nutrient solution. Means ± SD with different letters in each variable are significantly different (Tukey $P \leq 0.05$)

respectively. As in leaf tissue and in stems, roots show a greater accumulation of Cd when its concentration is higher in the nutrient solution (**Fig. 3D**).

DISCUSSION

In general, the growth of sweet pepper plants decreased with the increased Cd levels in the nutrient solution. These results coincide with those that have been reported by Chen *et al.* (2011), who observed gradual reductions in growth and development in mustard (*Sinapis alba*) and Chinese cabbage (*Brassica rapa* L. var. rosularis) treated with Cd added to the soil at concentrations of 0-24 mg/kg. These authors reported that the application of 24 mg/kg Cd to the soil, significantly reduced chlorophyll a and b contents compared to the control treatment: chlorophyll a fell by 32.29 and 20.56 % in cabbage and mustard, respectively, while the reductions in chlorophyll b were 21.54 y 21.55 %, respectively.

In sorghum, Liu *et al.* (2011) observed decreased root activity when Cd was added to the soil at concentrations of 50 and 100 mg/kg, and concluded that Cd decreases primary root elongation and lateral root growth. Meanwhile, Dong *et al.* (2005) reported that

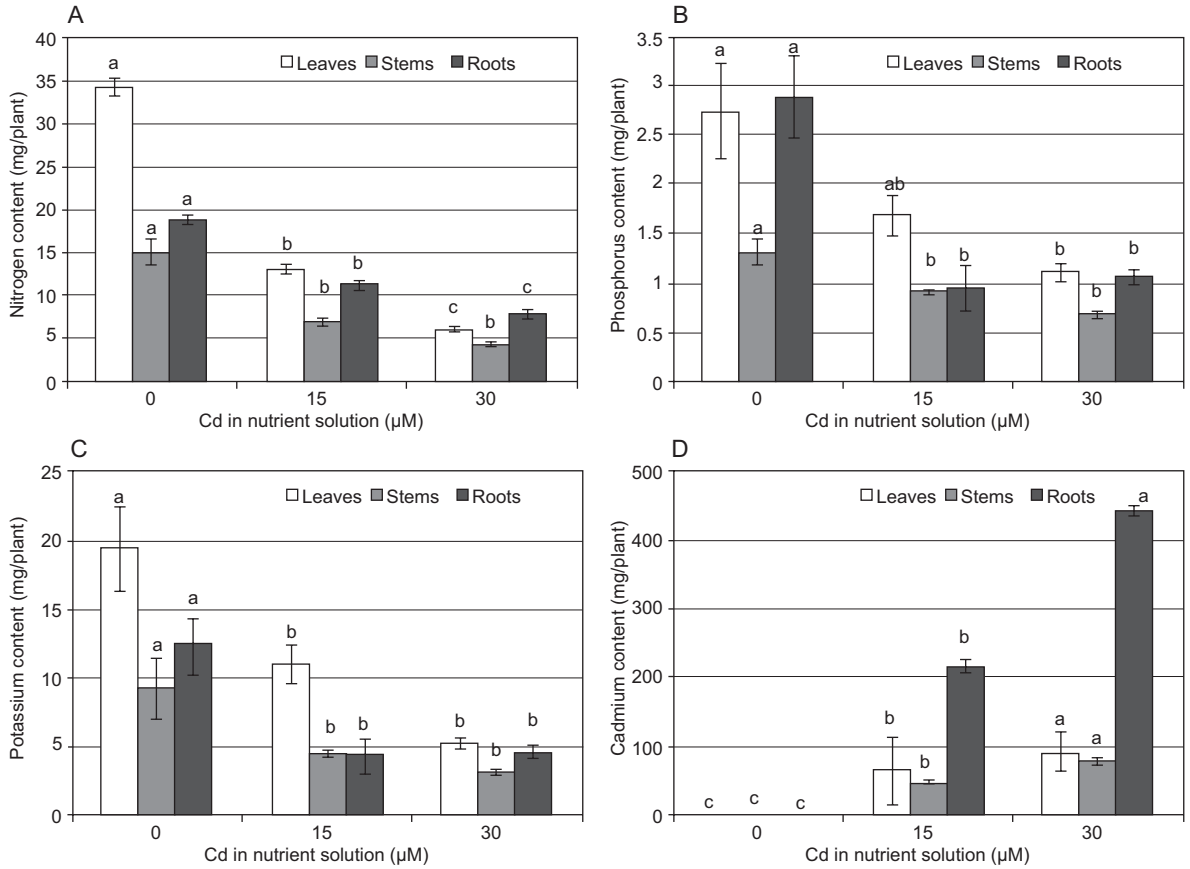

Fig. 3. Nitrogen (A), phosphorus (B), potassium (C) and cadmium (D) content in leaves, stems and roots of Yolo Wonder variety sweet pepper seedlings treated with different Cd concentrations in the nutrient solution. Means ± SD with different letters in each nutrient by plant organ are significantly different (Tukey $P \leq 0.05$)

the addition of 1 and 10 mmol/L Cd in the nutrient solution of tomato decreased plant height by 18.9 and 46.4 %, respectively, root length by 25.8 and 41.1 %, respectively, and root volume by 45.2 and 63.7 %, respectively, compared to the control.

Blum (1997) noted that the reduction in foliar area is an evasion mechanism that minimizes water loss under stress conditions due to toxic metals. Likewise, in the case of this study, foliar area decreased with increased Cd levels in the nutrient solution.

Astolfi *et al*. (2005) observed that Cd treatment produces an inhibition in plasma membrane H^+ATPase activity. Also, Ali *et al*. (2000) and Maksymiec *et al*. (2007) pointed out that as a result of the presence of Cd in the plant, there are imbalances in chloroplast metabolism, which inhibits chlorophyll synthesis and reduces the activity of enzymes involved in CO_2 fixing. These findings are also consistent with the results in this study, since the presence of Cd in the growth medium significantly decreased the chlorophyll content in pepper leaves.

Nazar *et al*. (2012) reported that high Cd levels in the soil alter the uptake and translocation of nutrients, which leads to nutrient deficiencies, oxidative stress, and decreased plant growth and development. In the case of the present study, the accumulation of all the nutrients assessed (N, P and K) decreased in the presence of Cd.

Cadmium accumulates preferentially in the root, and is sequestered in the vacuole of the cells, while only a small fraction is transported to the plant shoot, concentrating in descending order in the stems, leaves, fruits and seeds (Chan and Hale 2004). Likewise, Jidesh and Kurumthottical (2000) found that the highest Cd accumulation occurred in roots compared to other plant organs of the sweet pepper. In our study, the order of Cd accumulation *in planta* was as follows: roots > leaves > stems (**Fig. 3**).

Comparatively, the Yolo Wonder variety evaluated is a genotype very sensitive to Cd toxicity, since in preliminary studies (data not shown), seedlings subjected to more than 50 µM Cd in solution did not tolerate more than a day without presenting severe defoliation and visible morphological damage. Similarly, Abdel-Latef (2013) showed that the application of 100 µM Cd severely affected physiological and biochemical variables in pepper plants variety Zhongjiao. Conversely, Ramachandran and Vincent (2013) demonstrated that five different pepper genotypes could tolerate concentrations ranging between 125 and 500 µM Cd in solution, although differential responses were also observed among genetic materials. Moreover, in comparison to other plant species, Sereno *et al*. (2007) reported that sugarcane can tolerate up to 500 µM of Cd without showing any signs of toxicity. This behavior has also been recently confirmed by Trejo-Téllez *et al*. (2014), as sugarcane cultivar CP 72-2086 showed no effects of 1 mM Cd on K, Ca, Fe and Mn concentrations in leaves. Therefore, screening for more pepper genotypes would increase our understanding of how this genetic diversity possesses different reservoirs of physiological, biochemical and molecular mechanisms to tolerate Cd.

CONCLUSIONS

This research demonstrated that the addition of 15 and 30 µM Cd to the nutrient solution significantly decreases plant height, foliar area, chlorophyll content in leaves and the accumulation of N, P and K in different plant tissues. Additionally, the Cd showed the highest accumulation in roots, followed by leaves and stems.

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