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Cadmium affects the regeneration of the leafy vegetable *Talinum portulacifolium* stem cuttings in nutrient solution

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Resumen

El cadmio afecta a la regeneración de los esquejes en solución nutritiva de la verdura de hoja Talinum portulacifolium

Investigamos el efecto de varias concentraciones (0,0-5,0 ppm) de cadmio (Cd) en la capacidad de regeneración; las características morfológicas y la acumulación de Cd en los esquejes de tallo de la verdura de hoja *Talinum portulacifolium* cultivada en cultivo hidropónico. El Cd retrasó la brotación de los esquejes en un 7%, la callosidad en un 8% y el enraizamiento en un 38%. Las diferentes concentraciones de Cd afectaron significativamente a los pesos fresco y seco de las partes de la planta, excepto las raíces. La acumulación de Cd fue mayor en los tallos que en las hojas (2,22 vs 0,57 ppm). El índice de tolerancia calculado osciló entre el 59% y el 88%. Basándose en las observaciones, se concluyó que el Cd interfiere con la regeneración de los esquejes de tallo de *T. portulacifolium* e implica preocupación sobre el consumo y el uso terapéutico de esta hortaliza de hoja que crece en suelos contaminados.

Palabras clave: Callo; Crecimiento; Metales pesados; Hidropónico; Enraizamineto; Índice de tolerancia.

Abstract

We investigated the effect of various concentrations (0.0-5.0 ppm) of cadmium (Cd) on the regeneration ability; morphological characteristics and Cd accumulation in the leafy vegetable *Talinum portulacifolium* stem cuttings grown in hydroponic culture. Cd delayed sprouting of stem cuttings by 7%, callusing by 8% and rooting by 38%. Different Cd concentrations significantly affected fresh and dry weight of plant parts except roots. Accumulation of Cd was more in the stems than in leaves (2.22 vs 0.57 ppm). The calculated tolerance index ranged from 59% to 88%. Based on the observations it was concluded that Cd interferes with the regeneration of *T. portulacifolium* stem cuttings and imply concerns on the consumption and therapeutic use of this leafy vegetable growing on polluted soils.

Key words: Callus; Growth; Heavy Metals; Hydroponics; Rooting; Tolerance index.

Introduction

Pollution of natural resources like water and soil by heavy metals (HMs) is a major concern for plant and human health because of their longtime persistent effect in the environment. HMs is one of the most dangerous environmental pollutants as most of these HMs are toxic even at very low concentrations. Pollution of the environment by toxic HMs has undisputedly become an issue of worldwide concern after industrialization due to its toxic and long lasting effects (Friberg *et al.* 2019). Humans are directly exposed to HMs due to the consumption of crops and vegetables grown on HM contaminated soils and even ground water are contaminated with HMs (Jackson & Alloway 2017).

Among the various HMs that pollute the environment cadmium (Cd) with no known biological function is toxic for both plants and humans (Jackson & Alloway 2017, Shahid *et al.* 2016). Low concentrations of Cd occurs naturally in the soil and Cd originating from metal industry, plastics, sewage and phosphate fertilizers increases the presence of this HM in the soil (Shahid *et al.* 2016). The high mobility of Cd in the soil renders it 2 to 20 times more toxic than other HMs (Friberg *et al.* 2019, Khan *et al.* 2017). Even at low concentrations, Cd can negatively affect plant growth, metabolism of sugars, assimilation of sulphate and activities of different enzymes (Shahid *et al.* 2016) and cellular activities like cell proliferation and their differentiation in humans and animals (He *et al.* 2017, Waisberg 2003). In India, soils and ground water affected by various anthropogenic activities have Cd concentrations of 12.8-90.0 mg/kg and 40-280 µg/L respectively (Kubier *et al.* 2019).

De-contamination of Cd from soils is receiving increasing attention from the public as well as governmental bodies, particularly in developing countries (Khan *et al.* 2017). Plant based technologies are applicable for removing HMs from areas of low concentrations with shallow soils and water, although longer treatment time may be required (Clemens *et al.* 2013). However, when using a plant based system it is important to understand the toxic effects of Cd on the plants and also the ability of plants to grow or regenerate in such toxic environments.

Hydroponics not only reduces the period of plant growth and the duration of the experiments but also lowers the space required for carrying the experiment. Other advantages of hydroponics include maneuverability due to the distinct characteristics of the liquid medium, easy observation of the intact root system and undisturbed monitoring of changes in the root zones (Zhi-xin *et al.* 2007). The bioavailability of an HM in the soil is mainly affected by the total content of other HMs in the soil, chemical and physical properties of the soil and also the plant species (Shahid *et al.* 2016). So in order to get a clear understanding of the effects of a particular HM, researchers have focused their attention on the hydroponic system (McBride *et al.* 2016, Wang *et al.* 2016). Hydroponics help to understand the direct and exact effect of Cd or other HMs on plants as there is no interference from other soil factors. Moreover, we will also have an idea on the ability of plants in absorbing, concentrating or precipitating toxic metals from polluted effluents (Dushenkov *et al.* 1995).

Talinum Adans, belonging to the plant family Portulaceae, consists of herbaceous succulent plants. The leaves of several *Talinum* species are edible and are widely cultivated in tropical regions. The genus *Talinum* consists of 15 species of which five occur in India (Swarna *et al.* 2015).

Talinum species like *Talinum fruticosum* (L.) Juss. (Adefemi *et al.* 2012, Babyemi *et al.* 2017, Ebong *et al.* 2018, Kumar & Prasad 2015), *Talinum paniculatum* (Jacq.) Gaertn. (de Souza *et al.* 2018) and *Talinum portulacifolium* (Forssk.) Asch. ex Schweinf. (Sekhar *et al.* 2007) were examined for their ability to accumulate or tolerate different HMs.

In the present study, the model plant *T. portulacifolium* Asch. ex Schweinf., is an erect undershrub and the leaves of this plant are cooked and consumed as a vegetable or used raw in salads (Jansen 2004, Nair & Henry 1983). Moreover, the leaves of this plant are also used for treating eye diseases; roots are used for curing cough and gonorrhea and the whole plant is believed to possess aphrodisiac properties and relieve constipation (Jansen 2004). In India, *T. portulacifolium* is found in Assam, Western and North-Western India, and Peninsular India (Nair & Henry 1983, Sankara Rao *et al.* 2019). Further *T. portulacifolium* is popularly propagated through stem cuttings for cultivation in home gardens and

Kumar and Prasad (2010) suggested that this plant species in hydroponic media could serve as a tool to study various aspects of environmental pollution. As a multiutility plant, the influence of Cd on the regeneration of *T. portulacifolium* and its efficacy to accumulate the heavy metal would enable us to understand the risks associated with the collection and consumption of this leafy vegetable from Cd contaminated/rich soils. Therefore, in this study, we focused on the effect of different concentrations of Cd on the regeneration and morphological characteristics of *T. portulacifolium*. In addition, the ability of *T. portulacifolium* to accumulate Cd from the hydroponic solution without the interference of other soil factors was also examined.

Material and methods

Plant material and experimental design

Herbaceous stem cuttings (~120 mm long and ~10 mm thick) of *T. portulacifolium* were collected from plants growing in pots containing uncontaminated Alfisol soil at Bharathiar University Campus (11º2'20.4792"N, 76º52'35.1084"E), Coimbatore, India. The unrooted stem cuttings (120 mm long and 10 mm thick) were prepared using a sharp stainless steel knife. The two-way factorial experiment consisted of 11 concentrations of Cd and five growth periods with five replicates. The observations for growth periods one to four were non-destructive and there were 55 (11×5) experimental units. The cuttings were placed in glass containers filled with full-strength Hoagland nutrient solution (KNO_3, $Ca(NO_3)_2{\cdot}4H_2O$, $MgSO_4{\cdot}7H_2O$, $NH_4H_2PO_4$, $MnCl_2{\cdot}4H_2O$, H_3BO_3, MoO_3, $ZnSO_4{\cdot}7H_2O$, $CuSO_4{\cdot}5H_20$, $FeSO_4{\cdot}7H_2O$) (Hoagland and Arnon 1950), that was either unspiked or spiked with a known concentration of Cd. Two stem cuttings were placed in each glass container containing 150 mL of the Cd unspiked or spiked nutrient solution. The glass containers were covered with aluminium foil to prevent the nutrient solution from exposure to light. The cuttings were placed in such a way that only the lower part of the twig (approximately one to two cm) was immersed in the metal solution. The solutions were replaced every 7th day throughout the experiment. The cuttings were placed at room temperature (28 ± 2 ºC) with 14 h day/10 h dark period at a light intensity of 300 to 350 µmol/m^{2}s.

Preparation of the Cd solution

The concentrations of Cd used in the present study was based on a trial run where stem cuttings of *T. portulacifolium* were grown in a wide range of Cd spiked nutrient solutions (0, 5, 10, 15 and 20 ppm) for 20 days. As the stem cuttings raised in Cd concentrations beyond 5.0 ppm failed to develop and even cuttings raised in this Cd concentration exhibited toxicity symptoms like browning and reduced regeneration. Hence the Cd concentrations for the present study were fixed between 0 and 5.0 ppm. Cadmium chloride ($CdCl_2$) formed the sources of Cd and the solution containing Cd was prepared by dissolving known quantities of $CdCl_2$ in Hoagland nutrient solution. Required concentrations (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 ppm) of Cd was prepared individually by diluting the stock solution (1000 ppm; 2.036 g of $CdCl_2$ in 1L) using Hoagland nutrient solutions. The treatments are hereafter coded as Cd0.0, Cd0.5, Cd1.0, Cd1.5, Cd2.0, Cd2.5, Cd3.0, Cd3.5, Cd4.0, Cd4.5 and Cd5.0. Hoagland nutrient solution without $CdCl_2$ (0.0 ppm) served as the medium for growth of control plants.

Plant growth measurements and harvest

The duration (in days) for sprouting, root initiation and callus formation after exposure to Cd solutions were observed visually. The number of shoots, shoot length, leaf number, root number and total root length (length of all individual roots) was measured non-destructively at 7, 14, 21 and 28 (termed as D7, D14, D21 and D28) and destructively at 35 days (D35) after the initiation of the experiment. The cuttings were finally harvested destructively at D35, washed in distilled water and the leaves, stems and roots were separated. After recording the fresh weights, the plant parts were dried at 78 ºC for 48 hours in a hot air oven to record their dry weight. The magnitude of stem decay and toxicity symptoms was assessed visually.

Heavy metal analysis in cuttings

Dried plant samples (leaves and stem) were ground into a fine powder and sieved through a 0.2 mm sieve. The plant samples were wet acid digested with HNO_3, H_2SO_4, and $HClO_4$ in the ratio of 9:2:1 (Antosiewicz 1993, Piper 1966) for quantifying their total Cd. All the plant digests were analyzed for Cd concentration using Atomic

Absorption Spectrophotometer (Varian Techtran Spectr AA 10/20 BQ, Australia). Due care was taken to avoid metal contamination during the entire process of harvesting, washing, drying and grinding. Cd analysis could not be performed for roots due to inadequate sample in certain treatments.

Tolerance index (Ti)

The Ti was calculated to measure the ability of the plant to grow in the presence of a given concentration of metal, according to Wilkins (1978) using the formula:

Ti = [Dry weight of plants grown in Cd solution / Dry weight of plants grown in Cd free solution] × 100

Statistical analysis

Mean values of morphological growth parameters of plants were calculated from five replicates in each concentration. Analysis of Variance (one-way or two-way) was performed after analyzing the data for normality (Levene's test). When the F values were significant, Duncan's Multiple Range Test (DMRT) was used to compare the variation among treatments. Pearson's correlation or regression analysis was used to assess the relation of Cd concentration to different regeneration, growth and Cd accumulation parameters. All statistical analysis was performed using Statistical Package for Social Science (SPSS, Version 9.0).

Results

Effect of Cd on the regeneration of *T. portulacifolium*

Stem cuttings sprouted between D2 and D3 after their exposure to different concentrations of Cd, whereas, root initiation was delayed up to the D12 at the highest concentration Cd5.0 (Fig. 1). Though the differences in time taken for sprouting in different concentrations of Cd were not significant ($F_{8,36}$=<1; p>0.05), the differences in duration for root initiation ($F_{8,36}$=6.84; p<0.01) and callus formation ($F_{8,36}$=3.12; p<0.05) were significant. As concentrations of Cd was significantly and positively correlated to days for root initiation (r=0.898; p<0.001; n=11), no such correlation (p>0.05; n=11) was found for days taken for sprouting (r=0.492) or callus formation (r=0.250) (Fig. 1).

Different concentrations of Cd and growth

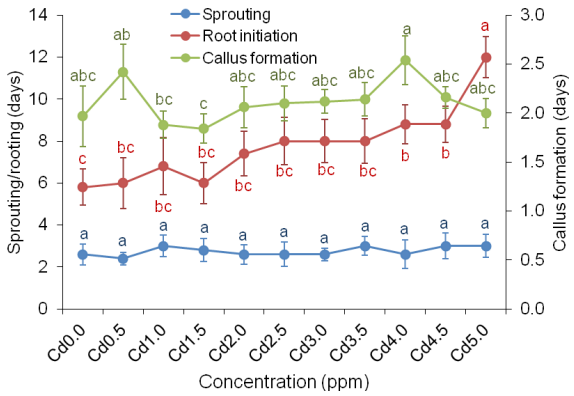

Figura 1. Influencia de diferentes concentraciones de cadmio (Cd) en la formación del callo, germinación y enraizamiento de esquejes de *Talinum portulacifolium* en cultivo hidropónico. Las barras de error indican error estándar. Puntos de una variable con la misma(s) letra(s) no son significativamente diferentes (p>0,05) según la prueba de rango múltiple de Duncan.

Figure 1. Influence of different concentrations of cadmium (Cd) on callus formation, sprouting and rooting of *Talinum portulacifolium* stem cuttings in hydroponic culture. Error bars indicate ± standard error. Points for a variable bearing same letter(s) are not significantly different (p>0.05) according to Duncan's Multiple Range Test

period failed to significantly influence the number of shoot apices (Table 1). The two-way interaction for the main factors (day × treatment) was also not significant for shoot apices. Shoot lengths and leaf numbers of *T. portulacifolium* stem cuttings significantly varied with growth period and were significantly influenced by the concentrations of Cd. However, the interaction day × treatment was not significant for these variables. Root numbers and total root length significantly varied with growth period and was significantly influenced by different concentrations of Cd in the nutrient solution. Though the interaction day × treatment was significant for total root length it was insignificant for root numbers (Table 1).

The numbers of shoot apices were almost similar over a period of 35 days and were not influenced by the increasing concentrations of Cd. On D7, maximum shoot length was observed in Cd0.0 and there was a 72% decrease in Cd4.5 which recorded the minimum shoot length. As the number of roots was maximum in Cd0.5, roots were absent in Cd3.0-Cd5.0. Moreover, root elongation was inhibited by different concentrations of Cd, maximum root length was observed in Cd0.5. Different concentrations of Cd was significantly and negatively correlated to shoot length, root numbers and total root length at the D7 (Table 2).

On D14, shoot length ranged between 3.78 cm (Cd0.0) and 1.9 cm (Cd4.5). More number of

Days(D)	Cd concentration (ppm)	Shoot apex (per cutting)	Shoot length (cm)	Leaf number (per cutting)	Root number (per cutting)	Total root length (cm)
D7	Cd 0.0	2.8 ± 0.37 a	1.16 ± 0.08 a	10.6 ± 0.75 a	19.6 ± 0.81c	0.64 ± 0.05 b
	Cd 0.5	3.0 ± 0.45 a	0.90 ± 0.13 b	9.0 ± 0.71 abc	27.6 ± 0.93 a	1.58 ± 0.11 a
	Cd 1.0	2.2 ± 0.37 a	0.72 ± 0.04 bc	5.2 ± 0.37 d	2.0 ± 0.71 e	0.18 ± 0.02 cd
	Cd 1.5	2.6 ± 0.24 a	0.92 ± 0.07 b	9.8 ± 0.86 ab	22.4 ± 1.21 b	0.26 ± 0.02 c
	Cd 2.0	2.6 ± 0.24 a	0.68 ± 0.07 c	7.8 ± 0.58 bc	7.6 ± 1.12 d	0.14 ± 0.02 d
	Cd 2.5	2.8 ± 0.20 a	0.68 ± 0.06 c	8.6 ± 0.51 abc	6.0 ± 0.71 d	0.10 ± 0.03 ef
	Cd 3.0	2.8 ± 0.20 a	0.68 ± 0.04 c	9.4 ± 0.87 abc	0.0 ± 0.00 f	0.00 ± 0.00 f
	Cd 3.5	2.4 ± 0.24 a	0.60 ± 0.04 c	7.8 ± 0.58 bc	0.0 ± 0.00 f	0.00 ± 0.00 f
	Cd 4.0	2.8 ± 0.58 a	0.74 ± 0.05 bc	10.4 ± 0.87 a	0.0 ± 0.00 f	0.00 ± 0.00 f
	Cd 4.5	3.0 ± 0.63 a	0.32 ± 0.07 d	7.2 ± 0.58 c	0.0 ± 0.00 f	0.00 ± 0.00 f
	Cd 5.0	2.6 ± 0.24 a	0.56 ± 0.05 c	9.2 ± 0.58 abc	0.0 ± 0.00 f	0.00 ± 0.00 f
D14	Cd 0.0	2.8 ± 0.37 a	3.78 ± 0.24 a	19.8 ± 0.86 a	34.6 ± 1.81 a	0.80 ± 0.07 b
	Cd 0.5	3.0 ± 0.32 a	3.46 ± 0.22 a	18.2 ± 1.24 ab	28.2 ± 1.28 b	0.50 ± 0.07 cde
	Cd 1.0	2.2 ± 0.49 a	2.64 ± 0.17 bc	16.4 ± 0.93 bcd	16.2 ± 0.86 de	0.38 ± 0.06 def
	Cd 1.5	2.6 ± 0.40 a	2.80 ± 0.13 b	16.0 ± 1.22 bcd	21.6 ± 0.93 c	0.62 ± 0.09 cd
	Cd 2.0	2.8 ± 0.49 a	2.76 ± 0.21 b	17.8 ± 0.37 abc	19.2 ± 1.07 cd	0.48 ± 0.06 cde
	Cd 2.5	3.0 ± 0.32 a	2.52 ± 0.08 bc	16.0 ± 1.05 bcd	17.2 ± 0.80 de	0.52 ± 0.04 cd
	Cd 3.0	2.8 ± 0.49 a	2.50 ± 0.11 bc	14.8 ± 0.86 cde	17.8 ± 1.07 de	0.30 ± 0.07efg
	Cd 3.5	2.4 ± 0.24 a	2.50 ± 0.15 bc	12.6 ± 0.93 e	17.8 ± 1.24 de	0.14 ± 0.02g
	Cd 4.0	2.8 ± 0.37 a	2.14 ± 0.14 cd	14.0 ± 1.14 de	14.6 ± 0.93 e	0.38 ± 0.07 def
	Cd 4.5	2.8 ± 0.37 a	1.91 ± 0.12 d	16.0 ± 0.63 bcd	16.2 ± 0.97 de	1.08 ± 0.11 a
	Cd 5.0	2.6 ± 0.24 a	2.14 ± 0.14 cd	15.8 ± 0.86 bcd	11.2 ± 0.80 f	0.24 ± 0.04 fg
D21	Cd0.0	2.8 ± 0.20 a	5.34 ± 0.16 a	21.4 ± 0.60 a	48.4 ± 1.08 a	1.62 ± 0.07 a
	Cd 0.5	3.0 ± 0.32 a	5.56 ± 0.11 a	20.0 ± 1.14 ab	36.4 ±0.93 b	0.64 ± 0.02 de
	Cd 1.0	2.2 ± 0.49 a	4.68 ± 0.12 b	15.8 ± 0.37 e	20.8 ± 0.86 f	0.44 ± 0.05 ef
	Cd 1.5	2.6 ± 0.40 a	4.82 ± 0.14 b	17.2 ± 0.58 b-e	31.2 ± 0.97 c	0.64 ± 0.09 de
	Cd 2.0	2.8 ± 0.58 a	5.22 ± 0.14 a	16.4 ± 0.93de	27.6 ± 1.03 de	1.41 ± 0.11 b
	Cd 2.5	2.8 ± 0.20 a	4.76 ± 0.12 b	20.6 ± 0.81 a	38.6 ±1.36 b	0.64 ± 0.05 de
	Cd 3.0	2.8 ± 0.58 a	3.76 ± 0.14 cd	15.0 ± 0.89 e	29.4 ± 1.08 cde	0.40 ± 0.09 f
	Cd 3.5	2.6 ± 0.68 a	3.58 ± 0.12 d	16.0 ± 0.71 de	27.8 ± 0.86 de	0.66 ± 0.05 d
	Cd 4.0	2.8 ± 0.58 a	4.06 ± 0.11 c	16.8 ± 1.07 cde	26.6 ± 0.93 e	0.71 ± 0.06 d
	Cd 4.5	3.0 ± 0.32 a	4.78 ± 0.16 b	19.4 ± 1.08 abc	30.2 ± 0.86 cd	1.08 ± 0.07 c
	Cd 5.0	2.8 ± 0.37 a	3.16 ± 0.11 e	18.8 ± 1.36 a-d	28.4 ± 0.93 cde	0.51 ± 0.05 def
D28	Cd0.0	2.8 ± 0.37 a	5.68 ± 0.09 be	22.8 ± 0.66 a	48.4 ± 1.44 a	1.82 ± 0.09 a
	Cd 0.5	3.0 ± 0.55 a	6.38 ± 0.25 a	21.6 ± 0.81 a	39.4 ± 1.25 b	0.68 ± 0.06 cd
	Cd 1.0	2.2 ± 0.37 a	5.28 ± 0.14 d	16.8 ± 1.24 bcd	23.8 ± 1.16 e	0.44 ± 0.05 e
	Cd 1.5	2.6 ± 0.24 a	5.48 ± 0.09 cd	17.8 ± 1.20 bc	31.2 ± 1.07 d	0.70 ± 0.07 bcd
	Cd 2.0	2.8 ± 0.20 a	5.88 ± 0.09 b	19.8 ± 0.66 ab	33.2 ± 1.39 cd	0.52 ± 0.04 de
	Cd 2.5	2.8 ± 0.37 a	5.58 ± 0.07 bcd	21.8 ± 1.07 a	45.4 ± 1.81 a	0.68 ± 0.06 cd
	Cd 3.0	2.8 ± 0.20 a	4.28 ± 0.13 e	16.4 ± 0.93 cd	31.0 ± 1.30 d	0.44 ± 0.09 e
	Cd 3.5	2.6 ± 0.24 a	4.18 ± 0.09 e	17.6 ± 1.08 bc	32.8 ± 1.24 cd	0.74 ± 0.05 bc
	Cd 4.0	2.8 ± 0.37 a	4.28 ± 0.08 e	13.8 ± 0.97 d	23.8 ± 1.07 e	0.90 ± 0.05 b
	Cd 4.5	3.0 ± 0.32 a	3.92 ± 0.11 ef	22.0 ±1.41 a	30.2 ± 1.28 d	2.00 ± 0.08 a
	Cd 5.0	2.8 ± 0.49 a	3.72 ± 0.13 f	21.0 ± 1.38 a	35.8 ± 0.86 bc	0.62 ± 0.09 cde
D35	Cd 0.0	2.8 ± 0.20 a	6.38 ± 0.04 b	23.0 ±1.00 a	49.4 ± 1.12 a	1.88 ± 0.13 ab
	Cd 0.5	3.0 ± 0.32 a	6.86 ± 0.07 a	22.2 ± 0.80 ab	39.4 ± 1.08 c	0.68 ± 0.04 ef
	Cd 1.0	2.2 ± 0.37 a	5.70 ± 0.15 cd	16.2 ± 1.66 cd	24.2 ± 1.07 e	0.48 ± 0.04 f
	Cd 1.5	2.6 ± 0.24 a	5.80 ± 0.13 c	17.8 ± 0.66 cd	32.8 ± 1.07 d	0.70 ± 0.07 ef
	Cd 2.0	2.8 ± 0.37 a	6.46 ± 0.09 b	20.0 ± 1.00 abc	33.4 ± 1.29 d	0.58 ± 0.05 f
	Cd 2.5	2.8 ± 0.49 a	5.84 ± 0.11 c	22.0 ± 1.00 ab	45.4 ± 1.63 b	0.90 ± 0.07 e
	Cd 3.0	2.8 ± 0.37 a	4.96 ± 0.14 f	17.4 ± 0.93 cd	31.0 ± 1.30 d	1.70 ± 0.14 bc
	Cd 3.5	2.6 ± 0.24 a	5.20 ± 0.14 ef	17.8 ± 1.07 cd	32.8 ± 0.97 d	2.04 ±0.08 a
	Cd 4.0	2.8 ± 0.37 a	5.38 ± 0.09 de	18.4 ± 1.08 cd	24.8 ± 0.86 e	1.30 ± 0.07 d
	Cd 4.5	3.0 ± 0.32 a	4.14 ± 0.13 g	19.0 ± 1.00 bcd	30.8 ± 0.73 d	1.62 ± 0.06 c
	Cd 5.0	2.8 ± 0.37 a	4.24 ± 0.09 g	20.4 ± 0.93 abc	29.8 ± 1.71 d	1.54 ± 0.08 c

F statistics

Source	df					
Days (D)	4,160	<1 ns	193.51**	24.94**	58.47**	9.41**
Treatment (T)	10,160	1.66 ns	10.35**	4.80**	11.29**	2.30*
D × T	40,160	<1 ns	<1 ns	<1 ns	<1 ns	1.61*

Tabla 1. Número de ápices de brotes, hojas, taíces regeneradas y longitud del tallo y la raíz de *Talinum portulacifolium* expuestos a diferentes concentraciones de cadmio (Cd) a lo largo de un periodo de 35 días. *, **: Significativo a p<0,05 y p<0,01, respectivamente; ns: no significativo. Las medias de una columna seguidas de la misma(s) letra(s) no son diferentes significativamente (p>0,05) según la prueba de rango múltiple de Duncan.

Table 1. Number of shoot apices, leaves, roots regenerated and length of shoot and root of *Talinum portulacifolium* exposed to different concentrations of cadmium (Cd) over period of 35 days. *, **: Significant at p< 0.05 and p< 0.01 respectively; ns, not significant. Means in a column for a day followed by a same letter(s) are not significantly (p>0.05) different according to Duncan's Multiple Range Test.

Days (D)	Shoot apex (per cutting)	Shoot length (cm)	Leaf number (per cutting)	Root number (per cutting)	Total root length(cm)
D7	0.075 ns	-0.820**	-0.019 ns	-0.772**	-0.656*
D14	-0.050 ns	-0.908***	-0.694*	-0.818**	-0.085 ns
D21	-0.246 ns	-0.244 ns	-0.224 ns	-0.460 ns	-0.303 ns
D28	-0.313 ns	-0.889***	-0.210 ns	-0.381 ns	-0.232 ns
D35	0.175 ns	-0.873***	-0.296 ns	-0.510 ns	0.441 ns

Tabla 2. Correlación de Pearson para la concentración de cadmio y el número de ápices de brotes, longitud del brote, hoja, número y longitud total de raíces durante diferentes puntos temporales (n=10). *,**,***: Significativo a p<0,05, p<0,01, p<0,001; ns: no significativo.

Table 2. Pearson's correlation for the cadmium concentration and the number of shoot apices, shoot length, leaf and numbers and total root length during different time points (n=10). *,**,***: Significant at p<0.05, p<0.01, p<0.001 respectively. ns, not significant.

Cd	Fresh weight (mg)			Dry weight (mg)		
(ppm)	Leaf	Stem	Root	Leaf	Stem	Root
Cd0.0	1582.31±119.22 a	5351.62±52.10 a	421.35±72.09 a	1012.83 ± 142.76 a	3302.43 ± 316.81a	102.43 ± 17.12 a
Cd 0.5	1363.71± 52.91ab	5802.74 ± 75.64 a	243.62 ± 47.21 a	779.54 ± 104.28 b	3005.48 ± 30.76 ab	101.35 ± 7.91 a
Cd 1.0	881.51± 114.66 cd	3681.77 ± 32.62 b	278.93 ± 58.46 a	542.73 ± 44.10 cd	2378.16 ± 193.25 cd	101.41 ± 7.80 a
Cd 1.5	1024.41± 180.59 bc	3724.15 ± 271.27 b	379.40 ± 70.17 a	557.18 ± 83.15 bcd	2215.51 ± 9.61 d	101.92 ± 6.36 a
Cd 2.0	1011.74 ± 169.76 bc	4293.59 ± 488.70 b	440.28 ± 102.74 a	598.43 ± 77.59 bcd	2501.62 ± 177.36 bcd	102.24 ± 6.38 a
Cd 2.5	1213.21± 108.04 bc	4401.93 ± 389.15 b	457.93 ± 115.70 a	660.31 ± 48.77 bcd	2806.67 ± 157.47 abc	102.17 ± 8.58 a
Cd 3.0	564.16 ± 10.74 bc	3563.73 ± 109.94 b	165.42 ± 71.68 a	462.12 ± 20.71 d	2135.08 ± 19.34 d	100.83 ± 12.44 a
Cd 3.5	631.82 ± 17.35 d	3402.17 ± 529.39 b	305.81 ± 84.59 a	564.88 ± 40.05 bcd	2581.91 ± 21.45 bcd	101.69 ± 8.23 a
Cd 4.0	850.14± 98.41 cd	3807.25 ± 418.35 b	204.17 ± 63.87 a	436.18 ± 13.00 d	2303.14 ± 335.88 cd	101.41 ± 8.66 a
Cd 4.5	632.88 ± 9.73d	3958.83 ± 162.38 b	236.15 ± 64.70 a	442.54 ± 2.68 d	2842.28 ± 27.59 abc	101.24 ± 8.73 a
Cd 5.0	854.32 ± 68.19 cd	4370.18 ± 460.65 b	203.84 ± 65.75 a	713.65 ± 72.56 c	3140.94 ± 30.23 a	101.27 ± 8.70 a
$F_{10,44}$	9.516 ***	5.336 ***	1.870 ns	5.768 ***	5.420 ***	0.003 ns
r=11	-0.744 **	-0.529 ns	-0.507 ns	-0.578 ns	-0.102 ns	-0.490 ns

Tabla 3. Pesos freso y seco de *Talinum portulacifolium* de partes de plantas expuestos a diferentes concentraciones de cadmio (Cd) y su relacion. *, **: Significativo a p<0,05 y p<0,01, respectivamente; ns: no significativo. Las medias de una columna seguidas de la misma(s) letra(s) no son diferentes significativamente (p>0,05) según la prueba de rango múltiple de Duncan.

Table 3. Fresh and dry weights of *Talinum portulacifolium* plant parts exposed to different concentrations of cadmium (Cd) and their relationship. *, **: Significant at p< 0.05 and p< 0.01 respectively; ns, not significant. Means in a column for a day followed by a same letter(s) are not significantly (p>0.05) different according to Duncan's Multiple Range Test.

leaves and roots were observed in Cd0.0 compared to other concentrations of Cd. Total root length in Cd4.5 decreased by 49% compared to Cd0.0 (Table 1). Shoot length, leaf and root numbers were significantly and negatively correlated to the concentration of the Cd in the nutrient solution (Table 2).

At D21, maximum shoot length was in Cd0.5 and more number of leaves was observed in Cd0.0. Root numbers of Cd1.0 showed a 57% decrease over Cd0.0 (Table 1). Total root length ranged between 1.62 cm (Cd0.0) and 0.40 cm (Cd3.0) among the different concentrations of Cd. No significant correlation existed between the concentrations of Cd and different growth parameters observed on D21 (Table 2).

Cuttings in Cd0.5 had the maximum shoot length and minimum shoot length was observed in Cd5.0 on D28. There was no significant variation in the leaf number among the various treatments.

The number of roots ranged from 48.4 (Cd0.0) to 23.8 (Cd1.0 and Cd4.0) and the root numbers in Cd1.0 and Cd4.0 were 51% lower than Cd0.0. Total root length was maximum in Cd0.0 and minimum in Cd1.0 and Cd3.0. Increasing concentrations of Cd significantly and inversely influenced the shoot length (Table 2).

On D35 shoot numbers were higher in Cd0.5 compared to Cd0.0, and it gradually decreased with increasing concentrations of Cd. The maximum number of leaves and roots was observed in Cd0.0, and minimum occurred in Cd1.0. The root length ranged between 2.04 cm (Cd3.5) and 0.48 cm (Cd1.0) (Table 1). A significant negative correlation existed between the shoot length and increasing concentrations of Cd (Table 2).

Plant fresh and dry weights

Different concentrations of Cd significantly influenced the fresh and dry weights of stem and leaf but not root (Table 3). Changes in the fresh and dry weights of plant parts were not linear except for the leaf fresh weight which was significantly and negatively correlated to Cd concentrations. Leaf fresh weight was maximum in Cd0.0 and minimum in Cd3.0 ppm (0.56 g). The decline in leaf fresh weight in Cd spiked solutions ranged from 14-65%. Maximum fresh weight of stems was recorded in Cd0.5 cuttings which were 8% higher than Cd0.0 (Table 3). While the fresh

weight of stems in other Cd spiked solutions was 18-36% lower than that of Cd0.0. Though the 10-62% decline in root fresh weighs among Cd treatments were not significant, a 5% and 10% in root fresh weight was evident in cuttings raised in Cd2.0 and Cd2.5 respectively. Not much variation was observed in root fresh weight among different treatments. The maximum dry weight of leaves and shoots occurred in Cd0.0 compared to different concentrations of Cd. The dry weights of leaf, stem and roots in different concentrations of Cd spiked solutions were 5-35%, 23-56% and 0.20-1.56% lower than Cd0.0 (Table 3).

Morphological changes

At higher concentrations of Cd (Cd5.0) leaves weathered off and chlorosis of matured leaves was evident. Cadmium also caused stem decay, browning of roots and stunted root and shoot growth in *T. portulacifolium*, and necrotic spots on the leaves. Pink colouration of the metal solution was observed after four days, in which the stem cuttings of *T. portulacifolium* were immersed. The intensity of colouring increased over a period of the next seven days and gradually disappeared during the later stages of plant growth.

Accumulation of Cd in plant parts

Significant variation ($p<0.001$) existed between the accumulation of Cd in the stems ($F_{10,22}$ = 28.718) and leaf ($F_{10,22}$=12.810) of *T. portulacifolium* at various concentrations of Cd (Fig. 2). Accumulation of Cd in the *T. portulacifolium* stem increased linearly with the increasing concentrations of Cd in the nutrient solution, with the highest accumulation at Cd4.0 ppm. In contrast changes in Cd accumulation in the *T. portulacifolium* leaves were not linear with concentrations of Cd in the nutrient solution and maximum accumulation occurred at Cd3.0 (Fig. 2). Cadmium concentrations in leaves was not correlated to Cd concentration in stems (r=-0.071; p>0.05; n=10).

Tolerance index

The Ti of *T. portulacifolium* ranged from 59.09% (Cd3.0) to 87.72% (Cd0.5) (Fig. 3) and was not related to the concentrations of Cd in the nutrient solution (r=0.118; p>0.05; n=10). The Ti of plants in Cd0.5 was 0.51%-32.64% higher than plants in other Cd treatments. The Ti was significantly and negatively correlated to Cd content in leaves

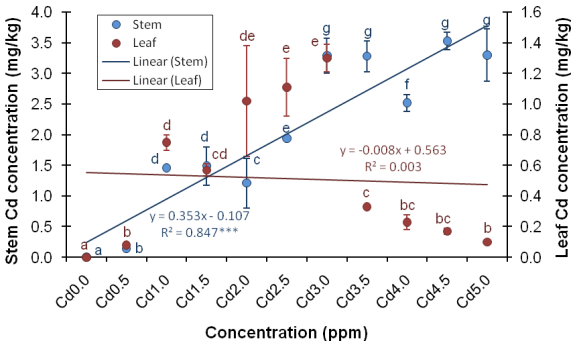

Figura 2. Contenido de cadmio en hojas y tallos de *Talinum portulacifolium* esquejes expuestos a diferentes concentraciones de Cd en cultivo hidropónico y la relación entre ambos. Puntos ± error estándar con la misma(s) letra(s) no son diferentes significativamente (p>0,05) según la prueba de rango múltiple de Duncan. *** Significatico a p<0,001.

Figure 2. Cadmium (Cd) content in leaves and stems of *Talinum portulacifolium* stem cuttings exposed to different concentrations of Cd in hydroponic culture and their relationship. Points ± standard error for a variable bearing same letter(s) are not significantly different (p>0.05) according to Duncan's Multiple Range Test. ***Significant at p<0.001.

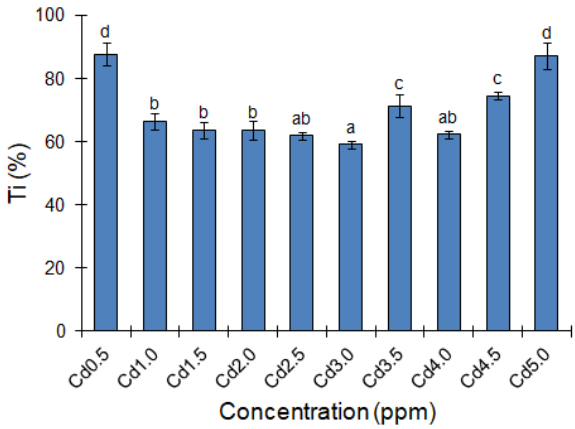

Figura 3. Índice de tolerancia (Ti) de esquejes de *Talinum portulacifolium* creciendo a distintas de cadmio (Cd) en cultivo hidropónico. Las barras de error indican error estándar. Barras con la misma(s) letra(s) no son significativamente diferentes (p>0,05) según la prueba de rango múltiple de Duncan.

Figure 3. Tolerance index (Ti) of *Talinum portulacifolium* stem cuttings grown in different concentrations of cadmium (Cd) in hydroponic culture. Error bars indicate ± standard error. Bars bearing same letter(s) are not significantly different (p>0.05) according to Duncan's Multiple Range Test.

(r=-0.758; p<0.01; n=10) and not in stems (r=-0.081; p>0.05; n=10).

Discussion

Effect on the regeneration of stem cuttings

The results of the present study show that different concentrations of Cd affect the regeneration of the stem cuttings of *T. portulacifolium*. Once sep-

arated from the mother plant, hormones such as auxins, jasmonates, and ethylene start accumulating at the cut region and eventually play varied roles in the development of the adventitious roots and regeneration of the cuttings (Steffens & Rasmussen 2016). The cuttings first physiological response to the detachment from the mother plant is first by the formation of the callus and then by the roots. However, these two are independent activities (Dole & Gibson 2006). Though rootless stem cuttings of *T. portulacifolium* developed callus within three days in all the Cd concentrations, this physiological process was not observed in previous studies examining the influence of Cd on the stem cuttings of *Talinum* (Rajkumar *et al.* 2009) or *Portulaca* L. species (Mohanapriya *et al.* 2006, Thangavel & Subburam 1998). Nevertheless, callus formation in response to exposure to different concentrations of zinc (Zn) has been recently reported in *T. portulacifolium* (Muthukumar *et al.* 2018). However, later changes in hormone signalling with the increased presence of cytokinin and strigolactone prevent callus proliferation and initiate adventitious root development (Steffens & Rasmussen 2016).

The initiation of roots within seven days in the nutrient solutions that were unspiked or spiked with low concentrations of Cd (up to Cd1.5) is in accordance with the observations of Kumar & Prasad (2010) where *T. portulacifolium* stem cuttings grown in hydroponic medium developed roots within a week. Nevertheless, the delay in root initiation with increasing concentrations of Cd in the solution is in line with the observations of Rajkumar *et al.* (2009) who also observed a delay in the initiation of roots in *T. fruticosum* (as *Talinum triangulare* (Jacquin) Willdenow) with increasing concentrations of Cd (0.5-3.0 mg/L). Moreover, in contrast to the observations of Rajkumar *et al.* (2009), where stem cuttings of *T. fruticosum* failed to initiate roots beyond 3.0 mg/L of Cd, root initiation in *T. portulacifolium* stem cuttings occurred even in the concentration of 5.0 mg/L tested. Increasing concentrations of other heavy metals like copper (Cu) and Zn also delays root initiation in *Portulaca oleracea* L. and *T. portulacifolium* respectively (Mohanapriya *et al.* 2006, Muthukumar *et al.* 2018). Degradation of auxin may be liable for the Cd-induced differences in the rooting ability of *T. portulacifolium* stem cuttings. For instance, exposure of poplars (*Populus* ×*canescens* (Aiton) Sm.) stem cuttings to Cd upregulated the GH3 enzyme activities responsible for the removal of auxins from the dynamic pool through conjugation which in turn reduced the concentration of auxins in the stem (Elobeid *et al.* 2011). The delay in root initiation can also be due to the reduction in the synthesis of cytokinin, the hormone responsible for the initiation of adventitious roots (Vysotskaya *et al.* 2007), thereby affecting the supply of nutrients consequent to the inhibitory effect of metals on the metabolic pathways (Hashem 2014).

Compared to roots leaves sprouted without much delay in all concentrations of Cd and emerged on the stem cuttings on the 2nd or 3rd day. A similar response has also been noted in *P. oleracea* where leaves sprouted on the 3rd day on the stems cuttings exposed to different sources and concentrations of Cu (Mohanapriya *et al.* 2006). In contrast, sprouting happened on the 4th day after exposure to Cd in *T. fruticosum* stem cuttings (Rajkumar *et al.* 2009). In spite of high mobility of Cd in plants, sprouting occurred even in the highest concentration of Cd tested (Eze *et al.* 2014). This implies the lack of buildup of critical concentrations of Cd in the stems are adequate enough to affect the regeneration of the cutting during the early stages of exposure. Moreover, as roots are in direct contact with the metal source they exhibit early symptoms to metal exposure than the shoots (Mohanapriya *et al.* 2006). Unrooted stem cuttings take up a minimal amount of water and nutrients directly through the cut surface (Alem 2010, Santos *et al.* 2009). This could also be true for the uptake of HMs like Cd by unrooted stem cuttings resulting in weak response to metal exposure during the initial stages.

The reduction in the number of shoot buds and leaves with increasing concentrations of Cd in *T. portulacifolium* stem cuttings may be due to the inhibition of cytokinin synthesis by Cd (Hashem 2014) since the role of this phytohormone on cell division and shoot morphogenesis are well established (Kieber & Schaller 2018). Similar observations were also reported for *P. oleracea* and *T. portulacifolium* for other HMs like Cu and Zn (Mohanapriya *et al.* 2006, Muthukumar *et al.* 2018). The reduction of leaf number and area was also evident in response to Cd toxicity in hydroponic experiments done by Zacchini *et al.* (2009) with poplar and willow clones. The reduction in shoot and root lengths of *T. portulacifolium* stem cuttings in response to Cd exposure is similar to

the observations of Lai *et al.* (2016) where different concentrations of Cd reduced the growth of *Impatiens walleriana* Hook.f. rooted stem cuttings exposed to different concentrations of Cd. The reduced plant elongation may be due to the direct toxic effect of Cd on the membrane integrity and cell division. Moreover, a reduction in the root respiration and protein synthesis in addition to plant photosynthesis might have also contributed to the slow growth of *T. portulacifolium* roots and shoots in response to Cd toxicity (Agarwal *et al.* 1987). The slow development of roots indicates metal penetration and accumulation in roots (Mohanapriya *et al.* 2006). Browning of roots and stunned root and shoot growth was also reported by Koleva *et al.* (2010) where Cd inhibited root length and induced browning in durum wheat.

Effect of Cd on plant fresh and dry weights

Exposure to Cd affected the fresh and dry weights of stem cuttings corroborating the observations in *I. walleriana* and *Salix triandroides* W.P. Fang stem cuttings exposed to Cd (Lai *et al.* 2017, Yao *et al.* 2018). Plant biomass can reduce at an organ level or whole plant level when the toxic elements are absorbed by plants (Emamverdian *et al.* 2015). This decline in response to the uptake of toxic elements could be due to the inhibition of the metabolic process that is related to plant growth. For instance, recent studies have shown that Cd can alter the auxin (IAA) homeostasis in the root system through its detrimental effects on the IAA biosynthetic genes as well as the distribution of the hormone can affect root growth (Bruno *et al.* 2017, Ronzan *et al.* 2018).

Tolerance to Cd

Tolerance often circumscribed as the extent to which a plant can resist exposure to increasing concentrations of HMs without displaying any phytotoxicity (Zha *et al.* 2004). One way of assessing tolerance to HMs is through observing changes in plant growth with increasing contaminant exposure i.e., Ti. Though *T. portulacifolium* exhibited a certain degree of tolerance for all the fitness parameters measured within the tested Cd concentrations, a decline in plant growth was evident with increasing levels of Cd. The negative relation between Ti and concentration of Cd in *T. portulacifolium* leaves is in accordance with Henson *et al.* (2013) who also reported a negative correlation between Ti and shoot Cd concentrations in *Chamaecrista fasciculata* (Michx.) Greene partridge pea. Normally metal hyperaccumulators are characterized by improved tolerance accomplished through internal detoxification (Pollard *et al.* 2002). Nevertheless, *T. portulacifolium* could not be designated as a hyperaccumulator as the plants accumulated less than 100 mg/kg of Cd under the present set of growth conditions (Henson *et al.* 2013). The phytotoxic effects of Cd on *T. portulacifolium* were well pronounced as the plants showed various morphological changes like wilting and withering of leaves in addition to the necrotic spots. The accelerated senescence caused by Cd in leaves may be attributed to the increased cell membrane permeability (Langille & MacLean 1976).

Morphological changes

Exposure to high concentrations of Cd-induced chlorosis in leaves as Cd can affect the biosynthesis of chloroplasts as well as the chloroplast structure resulting in a decreased number of chloroplasts per cell and reduced photosynthesis (Sun *et al.* 2015). This is in accordance with Koleva *et al.* (2010) where Cd-induced chlorosis in the leaves of *Triticum durum* L. has been reported. Moreover, excess concentrations of Cd are known to induce changes in cell size and interfere with the cell division (Baryla *et al.* 2001). The toxicity symptoms of Cd are indicated by the appearance of necrotic spots on the leaves, poor branching of roots and root browning (Das *et al.* 1997).

The pink colouration of the metal solution during early stages of cutting regeneration indicates the exudation of plant pigments as noted by Rajkumar *et al.* (2009). Earlier studies suggest that *Talinum* accumulate greater concentrations of anthocyanins in response to heavy metal exposure (Kumar *et al.* 2012). Anthocyanins are dominant secondary metabolites that protect plants against oxidative stresses by quenching the free radical ions through the donation of phenolic hydrogen atoms (Hernández *et al.* 2009). Furthermore, anthocyanins can also react with metals forming complexes thereby protecting plants from various abiotic stresses (Castaneda-Ovando *et al.* 2009).

Cd accumulation and its effect on consumption and therapeutic value

According to FAO/WHO (2014), the permissible limit for Cd is 0.05 mg/kg for vegetables and 0.2 mg/kg for leafy vegetables. Based on the ob-

servations of the present study *T. portulacifolium* accumulated up to 18 folds higher Cd in the stems and six folds higher Cd in the leaves than the permissible limits for leafy vegetables. This is accordance with studies where species of *Talinum* growing in soils contaminated with Cd like the dumpsites accumulated enormous levels of Cd (2-72 mg/kg) in their shoots (Obasi *et al.* 2009, 2013, 2015). The concentration of Cd in *T. portulacifolium* is also several times higher compared to the concentration of Cd reported for other leafy vegetables from different parts of the globe (Huang *et al.* 2017). As the major source of Cd exposure in humans occurs through the consumption of leafy vegetable either in cooked or raw forms and leafy vegetables acquire and accumulate more Cd than other crops. Chronic exposure to low concentrations of Cd from non-dietary sources can also result in organ toxicity especially nephrotoxicity in mammals (Thévenod & Lee 2013). Moreover, some recent studies have shown that the raw materials used in the preparation of herbal drugs are the major source of HMs in plant-based pharmaceutical products (Dghaim *et al.* 2015, Nessa *et al.* 2016). Hence it is important to grow this leafy vegetable in uncontaminated soils or collect the plant materials from unpolluted areas for therapeutic preparations (Girisha & Ragavendra 2009, Huang *et al.* 2017).

Conclusions

Pollution of soil and water by Cd is a global problem that affects both plant and human health mainly in Africa and Asia. Our results show that Cd interferes with the regeneration potential of *T. portulacifolium* stem cuttings by affecting the root initiation and plant development. From the results, it is also clear that *T. portulacifolium* can accumulate great concentrations of Cd if available in the growing medium. Despite the accumulation of appreciable quantity of Cd than those prescribed for leafy vegetables, *T. portulacifolium* is not a hyperaccumulator of Cd under the studied conditions. Therefore caution is necessary while consuming shoots of *T. portulacifolium* originating from unknown sources. The same is also true for using *T. portulacifolium* as a therapeutic agent.

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