The Effects of Soil Amendments On Leaf Anatomical Characteristics of Marigolds Cultivated In Cadmium-spiked Soils

Alapha Thongchai
Yala Rajabhat University
Weeradej Meeinkuirt (weeradej.mee@mahidol.ac.th)
Mahidol University
Puntaree Taeprayoon
Mahidol University
Isma-ae Chelong
Yala Rajabhat University

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Abstract

The marigolds (*Tagetes* spp.) in this study, were classified as excluders for cadmium (Cd); however, their leaves also accumulated substantial Cd content. Among the experimental growth media (*i.e.*, control, cattle, pig manure, and leonardite served as soil amendments), pig manure was found to result in significantly increased growth performance for all marigold cultivars, as seen by relative growth rates (119-132.3%) and showed positive effects on leaf anatomy modifications *e.g.*, thickness of spongy and palisade mesophyll, size of vein area and diameter of xylem cells. This may be due to substantially higher essential nutrient content, *e.g.*, total N and extractable P, in pig manure that aided all marigold cultivars particularly the French cultivar which exhibited the highest relative growth rate (132.3%). In the Cd only treatment, cell disorganization was observed in vascular bundles as well as in palisade and spongy mesophyll, which may have been causal in returning the lowest plant growth performances recorded in this study, particularly in the American and Honey cultivars (RGR = 73% and 77.3%, respectively).

Introduction

Several reports indicate that organic amendments can improve physicochemical factors and immobilize heavy metals in soil through adsorption and isolation of cationic compounds by forming stable complexes. Soil properties affecting the availability of cadmium (Cd) within the medium include; Cd adsorption and desorption on iron (Fe) and manganese (Mn) oxides, redox potential (Eh), calcium carbonate, oxyhydroxides, pH, organic carbon (OC), ionic strength, ligand anions, cation exchange capacity (CEC), phosphorus (P), organic matter (OM) and the proportion of clay minerals and percentage of sorbing sites occupied by Cd. Control of at least some of these properties can thereby be employed to reduce Cd phytoavailability and decrease the transfer of the metal into the food chain[1,2]. Furthermore, the OM content of animal manure (*e.g.*, cattle, chicken, horse and bat manures) forms strong complexes with Cd resulting in the reduced mobility of Cd in soil media and reduced leach rate into different soil layers, decreasing both its toxicity and the phytobioavailability of the soil. In one study the application of cattle manure to contaminated soil was found to have resulted in a reduction of Cd leaching by approximately 63%[3]. Many researchers have indicated that leonardite serves as an exceptional substitute for mineral-based fertilizer given that it is rich in humic acid, falvic acid and organic matter (50-75%). Application of leonardite (400 kg ha^{-1}) and inorganics (*e.g.*, zeolite) or organic (*e.g.*, animal manure) materials can substantially improve the physicochemical properties of soil, leading to increased crop yield and quality[4,5].

Leaves are the major site of photosynthesis in most vascular plants, and leaf morphology and anatomy play crucial roles in photosynthesis efficiency; other important leaf traits such as thickness and stomatal density directly influence metal tolerance and sensitivity[6]. Physiological function, such as leaf photosynthesis and respiration, are closely associated with the changes in leaf structure, chemical function and carbon balance; the character of the changes depends on plant species and functional types[7]. Cadmium is a non-essential element for plants that can be easily taken up by root systems and transported predominantly into the aerial parts marigolds (*Tagetes* spp.). Elevated Cd concentrations reduce plant growth by limiting the rate of photosynthesis, reducing stomatal length in the lower epidermis and mesophyll thickness[6,8]. At high levels of Cd in plants, the concomitant toxicity is clearly visible; notably there are severe reductions in stomatal density, also in the size of abaxial epidermis and stomatal apertures, similarly in trichome length and density for both leaf surfaces (adaxial and abaxial)[9].

Studying anatomical modifications in leaves can help with the understanding of biological processes in plants regarding heavy metal tolerance and accumulation mechanisms; for instance, elevated heavy metal accumulations in leaves has been known to inhibit the leaf transpiration rate, resulting in reduced plant growth and yields[10,11]. However, the deposition and desorption of heavy metals on plant leaves is known to vary by; plant species, specific heavy metals content, speciation and some key environmental factors e.g., pH, cation exchange capacity (CEC) and organic matter (OM)[12] in which, comparison of the leaf anatomical characteristics of plant species grown in heavy metal-enriched soil may help

explain heavy metal accumulation and patterns of distribution in different plant species and cataloguing the effects of heavy metal toxicity and tolerance in plant species. Many plant species exposed to Cd toxicity exhibit alterations in leaf anatomical structures, examples include; Indian mustard (*Brassica juncea*), tomato (*Solanum lycopersicum*), wheat (*Triticum aestivum*) and willow (*Salix viminalis*)[13]. Furthermore, many reports indicate that the presence of certain essential nutrients in plant media can affect the modification of leaf anatomical characteristics, for example; Fe deficiency induces more numerous but smaller stomatal apertures in both upper and lower leaf surfaces. Similarly, considerable amounts of Cu and Cd in combination have been found to cause significant reductions in leaf width, total thickness and thickness of mesophyll parenchyma in the leaf midrib[14].

The present study has been carried out to investigate the effects of soil amendment applications on Cd accumulation in leaf materials, in particular the anatomical modifications in leaves of four marigold cultivars (*Tagetes erecta* L. *e.g.*, Honey, American and Sunshine and *T. patula* L. *e.g.*, French) grown in pot systems in a greenhouse environment.

Results And Discussion

Selected physicochemical properties of soils utilized for this study are shown in Table 1. The pH values of the Cd-enriched soils ranged from 6.8-7.1 and that of the control soil measured 6.5. The desirable soil pH for optimum plant cultivation varies in the range of 5.5-7.0[15]. Soil pH is also a key variable for plant growth because it can influence several physicochemical properties of soil impacting plant traits such as height, lateral spread, mass, flower size and shape, and pollen production[16]. The application of organic amendments slightly increased the soil CEC from 28.3 cmol kg^{-1} in the Cd-Alon treatment to 28.4-30.8 cmol kg^{-1} in the Cd-enriched soil amended with pig manure, cattle manure and leonardite. The control soil amended with Osmocote^{Ò} showed a remarkably low CEC value; a factor of 1.6-1.7 times lower than any of the other treatments. The elevated CEC content in Cd-enriched soils led to Cd immobilization via sorption and precipitation mechanisms[17]. When comparing the differences in essential nutrients between the Cd-Alon soil and Cd soils with amendments, it is apparent that organic amendments increased total N, Ext. P, and Ext. K contents by approximately 1.5-2.5, 1.1-4.9, and 1-4.7 times, respectively. The highest total N and Ext. P contents were found in the Cd-Pig treatment (0.5% and 1,483.2 mg kg^{-1}). The Cd-Catt treatment also showed enhanced levels of Ext. P and Ext. K at 400.2 mg kg^{-1} and 2,444.3 mg kg^{-1}, respectively.

Substantial OM contents were found in the Cd-Leo and Com-Ctrl treatments (10.2% and 11.7%, respectively), whereas the lowest OM content was found in the Cd-Alon treatment (3.4%). Humic substances originating from organic matters in leonardite can improve the physicochemical properties of soil and stimulate *plant growth performance*[18]. Rehman et al.[19] indicated that increased OM in amendments, applied in different combinations could increase rice growth under high Cd-contaminated effluent water added to the soil surface during planting.

In this study, the spiked Cd resulted in significantly higher Cd contents in Cd-soils when compared with the control soil ($p < 0.05$). After plant harvest, Cd-soils showed slight decrease in Cd content; however, there appears to be no statistically significant difference ($p > 0.05$). Several researches have reported that organic soil amendment practices in Cd and Zn co-enriched soils have been used in order to immobilize Cd as stable forms thus enhancing Cd removal from crop plants, thereby markedly safeguarding human health and conserving the living environment[2]. Furthermore, Cd content slightly decreased to 2.1 times the level in the Com-Ctrl treatment ($p < 0.05$), which falls within the soil quality criteria for agricultural soil (< 1 mg kg^{-1})[20].

The order of dry biomass plant tissue quantities was as follows: stems > roots > leaves. Significantly differences in dry biomass were recorded from specimens raised in the Cd-Pig treatment across all marigold cultivars ($p < 0.05$; Table 2). The Sunshine cultivar showed a higher dry biomass content in comparison to other marigold cultivars by factors of 1.5 to 47.2 times. These results are consistent with a previous study[21]. Substantial increase in dry biomass content was also

measured in the Cd-Catt treatment for Sunshine cultivar (24.6 g). The nutrient additive content of organic amendments such as pig and cattle manure, enhance N considerably, which helps induce both plant growth and yield. Therefore, available N from animal manures is considered to have substantial potential and is highly necessary for optimum plant growth in contaminated soils. This essential element is also usually added as a supplement in various types of organic fertilizers, either alone or in combination with inorganic fertilizers[22]. The substantial OM content in the Cd-Leo treatment did not exhibit a commensurate influence on plant growth performances in this study. The descending order of dry biomass production in marigold cultivars for all treatments was as follows: Sunshine > Honey > American > French, whereas the Cd-Catt treatment showed a slightly different pattern: Sunshine > American > Honey > French. The observed trends were slightly different for the highest %RGR values for the study plants as follows: French > Sunshine > American > Honey (Fig. 1). Furthermore, significant differences in mean values of percentage relative growth rate were observed in specimens grown in the Cd-Pig treatments for all marigold cultivars ($p < 0.05$).

Among Cd treatments, all marigold cultivars accumulated high quantities of Cd in whole plants, particularly in the Cd-Alon treatment for the French and Sunshine cultivars, and the Cd-Catt treatment for Honey and American treatments; ranging from 27.6 to 49.1 mg kg^{-1} (Table 3). However, marigold tissues (roots, stems, leaves and whole plant) in the Com-Ctrl treatment possessed the lowest Cd contents in all marigold cultivars ($p < 0.05$). Furthermore, all marigold cultivars accumulated the lowest levels of Cd in the Cd-Pig treatment. Results of Cd quantities in plant tissues also showed that marigold roots accumulated the highest content of Cd followed by stems and then leaves, respectively, which is consistent with a previous study[21]. This ornamental plant exhibited excluder potential, that is – the propensity to accumulate Cd in lower concentrations in above ground parts compared to roots[23].

In this study, leaves of four marigold cultivars showed slight anatomical modification in the Com-Ctrl and amended treatments (Table 4). A high accumulation of Cd in leaves from the hyperaccumulating plants (*e.g.*, marigold cultivars) varies according to plant genotype, Cd speciation and Cd quantities; potentially, however, leaves can be seriously injured as a result of Cd toxicity[24]. Typically, elevated heavy metal content causes decreases in the diameter and number of xylem cells, which has also been reported and shown similar studies of some terrestrial plant species *e.g.*, results from heavy metal toxicity in leaves of *Triticum aestivum* cv. Ekiz under chromium (Cr) stress[25]. Such phenomena are somewhat consistent with the present study, as leaf anatomical characteristics in the Cd-Alon treatment for some marigold cultivars showed remarkably smaller major vein areas (*e.g.*, in the French, American and Sunshine cultivars) and diameter of xylem cells (*e.g.*, in the American and Sunshine cultivars), compared to other treatments.

The largest major vein area and xylem cell diameters were found in the study plants cultivated in the Cd-Pig treatment for all marigold cultivars (15,580-33,910 mm^2 and 15.2-17.4 mm, respectively) ($p < 0.05$). Comparisons of plant characteristics between specimens grown in the Cd-Pig and Cd-Alon treatments, revealed significant differences, particularly, the major vein areas and diameter of xylem cells of American and Sunshine cultivars which were 2.2 and 2.1 times different, respectively. Cadmium accumulation in plant leaves causes increased mesophyll cell size and thickness of sclerenchyma, phloem, and mesophyll, as seen in the similar studies in peas (*Pisum sativum* L. cv. Lincoln)[26]. Vollenweider et al.[24] indicated that leaf mesophyll in the willow tree (*Salix viminalis* L.) could store Cd at higher concentrations when compared to veins. In this study, greater mesophyll thickness was also generally found in the study plants cultivated in the Cd-Pig treatment for all the marigold cultivars (235.9-351.2 mm); however, the highest value was found in the Cd-Catt treatment for Honey cultivar (371.5 mm) ($p < 0.05$). To some extent, remarkable mesophyll thickness in the Cd-Pig treatment may be linked with large palisade parenchyma and spongy parenchyma cells in the mesophyll[27], as seen clearly in Fig. 2C for Honey cultivar, and compared to the Com-Ctrl treatment (Fig. 2A). As such, substantial total N and extractable P contents in pig manure may be key nutrients for growth of the study plants and their development into mature plants and may also indirectly help enhance the size and number of vacuoles in mesophyll cells, thereby alleviating Cd toxicity for the study plants since Cd can be sequestered and stored in the vacuole of leaf cells[28].

In this study, French and Sunshine cultivars grown in the Com-Ctrl treatment revealed significantly lower epidermis thickness on the adaxial side when compared to those grown in the amended treatments ($p < 0.05$). Similar results were observed in Sunshine cultivar grown in the Cd-Alon treatment. Furthermore, epidermis thickness on the abaxial side in the Cd-Pig and Cd-Catt treatments for Honey cultivar and in the Cd-Pig treatment for American cultivar showed slight differences in comparison to the Com-Ctrl treatment ($p < 0.05$). Some evidence indicates that heavy metals present in the growing medium can lead to increased epidermal cell size, which also helps magnify heavy metal concentrations absorbed in to the epidermal layer from contaminated soil and subsequently adsorbed on the cell wall of the epidermis. This strategy of the plants helps prevent heavy metal mobility, thus reducing absorbed and/or adsorbed into chloroplast cells, thereby decreasing rates of photosynthesis[10].

Remarkably small major vein areas and diameters of xylem cells were typical of all marigold cultivars grown in the Cd-Alon treatment, whereas these two characteristics were clearly visible in the study plants grown in the Cd-Pig treatment across all marigold cultivars. A measure of disorganization and shrinkage of epidermal cells and disorganization of mesophyll and vascular bundle material were observed in Honey cultivar grown in the Cd-Alon treatment (Fig. 2B); although similar developments were observed in American cultivar, they were more modest; slight disorganization in vascular bundle and a small amount of shrinkage in epidermal cells. Such phenomena have caused severe decline of cell viability and breakdown of palisade and spongy mesophyll layer mainly as a result of Zn or Cd stress[24,28]. Among cultivars grown in amended treatments, only French cultivar in Cd-Catt treatment developed small major vein areas, phloem degradation, a small degree of shrinkage in epidermal cells and slight disorganization of mesophyll. To some extent, a disorganized and degraded mesophyll layer caused by exposure to Cd can eventually interfere with photosynthetic efficiency, photosystems and other multiprotein complexes (MPCs) in thylakoids, resulting in decreased plant growth and productivity[29].

In this study, venation patterns of the study plants were also observed with a light microscope. The marigold cultivars were found to have slightly different densities and numbers of minor veins; specimens of the Sunshine cultivar grown in the Cd-Leo treatment exhibited the highest number. Ranked in order of decreasing minor vein density the list of marigold cultivars is as follows: Sunshine > American > Honey >American; whilst the list for the treatments is as follows; Cd-Leo > Cont > Cd-Catt > Cd-Pig > Cd. Minor veins play a major role in heavy metal mobility and the contaminants primarily assimilate in the leaf mesophyll at the mature stage; however, higher quantities of heavy metals (*e.g.*, Cd) can be detected in the major veins lying within the leaf mesophyll during the development period[30,31]. However, the rate of water and solute (including heavy metal) uptake and transport from veins across the mesophyll to the point of evaporation from aerial parts, depends on plant genotype, hydraulic capacity, and photosynthetic mechanism[32].

Notably specialized structures of the epidermis *i.e.*, trichomes were found in Honey cultivar specimens grown in Cd-Pig and Cd-Leo treatments as were American cultivar specimens grown in the Cd-Alon treatment; whereas no such structures developed in any specimens of the French and Sunshine cultivars regardless of treatment type. Trichome structures can act as barriers preventing heavy metal adsorption and accumulation via leaf surfaces; however, there is still no clear evidence/understanding of the mechanism by which trichomes detoxify heavy metal from leaf surfaces. The presence of trichomes, their structure and number on leaf surfaces probably depends on several physicochemical and biological factors, including the presence of heavy metals[33,34].

Conclusions

In this study, we conclude that organic amendments (particularly pig manure) could reduce the effects of Cd toxicity and support plant growth performance on the study plants, as clearly seen by the leaf anatomical changes. The obvious results *i.e.*, cell disorganization in vascular bundle and palisade and spongy mesophyll were found in the Cd-Alon treatment that contained high quantities of Cd without any organic amendments.

Methods

Plant materials: Seeds of four marigold cultivars, including *T. erectae.g.*, Honey, American and Sunshine, and *T. patulae.g.*, French, were germinated in acid-washed sand with total and extractable Cd levels below detectable limits. All seeds were transferred to a greenhouse at the Nakhonsawan Campus, Mahidol University. The environmental conditions in the greenhouse were within 27–32 °C, ~ 65% relative humidity and ~ 16,000 lx.

Greenhouse experiments: All experiments were carried out according to the ethical principles on plant usage. Prior to planting, all soil samples were oven-dried and passed through a 2-mm sieve. Then, each organic amendment (10% w/w) *i.e.*, pig, cattle and leonardite was mixed thoroughly with Cd-enriched soil that was collected from Mae Sot, Tak Province; designated as Cd-Pig, Cd-Catt, Cd-Leo, and a Cd-enriched soil with no amendment was designated as Cd-Alon. Furthermore, commercial soil amended with Osmocote® (slow-release inorganic fertilizer; 0.15%) also served as control soil; designated as Com-Ctrl. Cd-enriched soils were spiked with $Cd(NO_3)_2$ to attain measured values of 8.1±1.6, 8.3±0.7, 8.3±1.0 and 7.4±1.0 mg Cd kg^{-1} for Cd-Alon, Cd-Pig, Cd-Catt and Cd-Leo treatments, respectively, whereas the control soil, purchased from an agricultural supplies shop, contained low concentrations of Cd (1.9±0.2 mg Cd kg^{-1}). All pots were then saturated with deionized water (DI water) for four weeks at 70% water holding capacity. Selected physicochemical properties of the soil samples were analyzed following the standard methods e.g., soil texture, soil pH, CEC, OM, total nitrogen (total N), extractable phosphorus (Ext. P) and extractable potassium (Ext. K)[35,36].

The seedlings of marigolds (~ 10 cm height at 7 days) were transferred to 3.5-kg size plastic pots and placed on greenhouse benches in a randomized complete block design (RCBD). Five replicate pots were used for each treatment, and each replicate comprised a single healthy seedling. The pot treatment systems were designed and modified by following the methods described by Thongchai et al.[21].

Cadmium determination: At 3 months after planting, plant samples were harvested and oven-dried at 70°C for 3 days. For each sample, shoots, roots, leaves and whole plant tissues were then ground to a fine powder with an IKA® A11 basic mill. Plant materials were digested with concentrated 70% HNO_3 and 37% HCl (1:3)[35]. Cd contents in solution were analyzed using a flame atomic absorption spectrophotometry (FAAS; Perkin Elmer AAnalyst 200, USA) after filtering with Whatmann® No. 42 filter paper.

Two standard reference materials (NIST SRM® 2710a Montana soil and NIST SRM® 1515 apple leaves) for soil and plant samples were used for the verification of the accuracy of analytical measurements. Percentage recoveries of Cd for the soil and plant materials were in the range 90-110% of the stated content, whereas the relative standard deviation (RSD) ranged from 1.14–4.01%.

Light microscopy: The middle portions of the leaf at the plant maturity stage were cut into small pieces (0.5 X 0.5 cm^2) and immediately fixed in the fixative FAA (formaldehyde: glacial acetic acid: 95% ETOH: distilled water; 5:5:50:40 mL) for 48 h as described by Johansen[37]. The specimens were gradually dehydrated using a graded series of tertiary butyl alcohol (TBA) and then embedded in a paraffin wax. Sections were cut at 5 mm with a motorized rotary microtome (ERM 4000, HESTON). The sections were subsequently counterstained with fast green and safranin[37]. After staining, slides were dehydrated, cleaned with fresh xylene and mounted with Permount® for examination and photographing structural changes of leaf cells under a Leica DM1000 LED microscope with the Leica MC170 HD camera. Measurement of the lumen diameter of xylem cell, mesophyll thickness, major vein area, epidermal cell size on both adaxial and abaxial sides of the leaf were carried out using the ImageJ program (version 1.52v).

Data analyses: Dry weight of shoots, roots, leaves and whole plant tissues were recorded. Furthermore, relative growth rate (RGR) index data were calculated as described by Phusantisampan et al.[38], affording a metric of plant growth tolerance in

extreme environmental conditions.

The statistical analysis was carried out using SPSS® (SPSS, Chicago, IL) on a Windows-based PC. A one-way ANOVA and least significant difference (LSD) post hoc comparison were used to detect significant differences in the mean values ($p <$ 0.0) are presented as mean±standard deviation.

Declarations

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Author contributions

A.T. and W.M. conceived the idea and designed experiments in this study. A.T., W.M., P.T. and I.C. were involved in data collection, analysis and to the interpretation of results. A.T. and W.M. were involved in the writing up of the manuscript. W.M. gave supervision to this study and approved the final version of the manuscript. All authors reviewed the manuscript.

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Completing interests

The authors declare no completing interests.

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Tables

Table 1 Physicochemical properties of the soils used in this study

Soil Properties	Treatment				
	Com-Ctrl	Cd-Alon	Cd-Pig	Cd-Catt	Cd-Leo
Soil texture	Loam	Clay	Clay	Clay	Clay
Soil pH	6.5	7.1	6.8	7.1	6.8
CEC (mol kg^{-1})	18.2	28.3	28.6	28.4	30.8
OM (%)	10.2	3.4	8.3	8.1	11.7
Total N (%)	0.4	0.2	0.5	0.4	0.5
Ext. P (%)	318.6	300.4	1,483.2	400.2	322.4
Ext. K (%)	1,121.4	521.4	1,228.4	2,444.3	5,438
Before planting					
Total Cd (mg kg^{-1})	1.9±0.2bA	8.1±1.6aA	8.3±0.7aA	8.3±1.0aA	7.4±1.0aA
After plant harvest					
Total Cd (mg kg^{-1})	0.9±0.1bB	7.9±2.4aA	7.8±1.2aA	7.9±1.1aA	7.0±1.1abA

CEC cation exchange capacity, *OM* organic matter, *N* nitrogen, *Ext.* extractable, *P* phosphorus, *K* potassium, *Cd* cadmium

Values followed by the same letter are not significantly different; lower-case letters show the difference of treatments (LSD, $p < 0.05$); capital letters indicate the difference of Cd concentrations in soils before planting and after plant harvest (LSD, $p < 0.05$)

Table 2 Dry biomass production of the study plants (n = 5)

Plant	Treatment	Dry biomass production (g dw)			
		Stem	Leaf	Root	Total
French	Com-Ctrl	3.9±1.1bB	0.8±0.2abA	1.8±1.0bB	6.4±0.6bB
	Cd-Alon	1.9±0.8bA	0.1±0.0bA	0.3±0.2cA	2.3±0.9cB
	Cd-Pig	27.2±3.2aB	2.3±0.5aA	6.9±4.2aAB	36.3±4.3aB
	Cd-Catt	3.2±0.7bB	0.1±0.0bB	0.3±0.1cB	3.6±0.8bcB
	Cd-Leo	1.9±0.4bA	0.1±0.0bA	0.2±0.1cA	1.7±0.4bcB
Honey	Com-Ctrl	10.3±1.3abA	1.1±0.3abA	2.4±0.2bAB	13.8±1.7bA
	Cd-Alon	3.0±0.7bA	0.3±0.1bA	0.4±0.2cA	3.7±0.9cA
	Cd-Pig	35.3±8.8aB	3.0±0.5aA	4.5±2.3aB	42.7±10.5aAB
	Cd-Catt	1.1±0.5cB	0.1±0.0bB	0.2±0.1cB	1.4±0.5dC
	Cd-Leo	3.4±0.8bA	0.1±0.0bA	0.4±0.1cA	3.9±0.9cA
American	Com-Ctrl	7.3±1.9bA	1.2±0.2abA	3.6±1.3bA	12.1±3.2bA
	Cd-Alon	2.1±0.8cA	0.1±0.0bA	0.2±0.1cA	2.4±0.9cB
	Cd-Pig	31.3±6.7aB	2.6±0.4aA	8.1±1.8aA	42.0±5.9aAB
	Cd-Catt	3.6±2.4cB	0.3±0.1bB	0.4±0.2cB	4.3±2.5cB
	Cd-Leo	1.7±0.9bcA	0.2±0.1bA	0.3±0.1cA	2.2±1.1cB
Sunshine	Com-Ctrl	11.7±1.0bA	1.8±0.3abA	2.2±1.5bAB	15.6±2.3bcA
	Cd-Alon	4.4±1.9cA	0.2±0.0bA	0.4±0.2cA	5.1±2.1cA
	Cd-Pig	55.5±17.2aA	3.5±0.5aA	7.1±5.8aA	66.1±22.8aA
	Cd-Catt	21.3±8.9abA	1.6±0.5abA	1.7±1.1abA	24.6±9.6bA
	Cd-Leo	3.7±2.0cA	0.2±0.1aA	0.3±0.1cA	4.2±2.2cA

Values followed by the same letter are not significantly different; lower-case letters show the difference of treatments (LSD, $p < 0.05$); capital letters indicate the difference in growth performance among cultivars within the same treatment (LSD: $p < 0.05$)

Table 3 Cd accumulation in different plant tissues from the four marigold cultivars ($n = 5$)

Marigold Cultivar	Treatment	Cd accumulation in plant (mg kg^{-1})			
		Root	Stem	Leaf	Whole Plant
French	Com-Ctrl	4.0dA	1.7cA	2.3cA	2.5cA
	Cd-Alon	50.4aAB	37.4aA	38.2aA	39.0aA
	Cd-Pig	28.5cA	24.0bA	25.6bA	22.4bA
	Cd-Catt	40.1bAB	22.3bA	25.3bAB	34.0abA
	Cd-Leo	39.4bB	25.6bA	26.9bA	30.9bA
Honey	Com-Ctrl	3.7dA	1.4cA	1.8cA	2.1cA
	Cd-Alon	39.7abB	17.4abC	23.5aB	30.8aB
	Cd-Pig	19.9cB	10.1bBC	16.6bB	18.0bAB
	Cd-Catt	46.4aAB	20.7aA	26.8aAB	34.5aA
	Cd-Leo	32.1bC	13.8bB	19.0bB	22.5abB
American	Com-Ctrl	3.9cA	1.6dA	1.9dA	2.3dA
	Cd-Alon	50.5aA	27.5abB	37.0aA	41.5aA
	Cd-Pig	18.8bB	12.8cB	16.3cB	17.7cB
	Cd-Catt	58.1aA	29.5aA	37.8aA	49.1aA
	Cd-Leo	52.3aA	23.6bA	29.5bA	33.5bA
Sunshine	Com-Ctrl	4.3cA	1.6cA	2.1cA	2.9cA
	Cd-Alon	48.3aAB	18.6aC	23.2aB	27.6aB
	Cd-Pig	20.5bB	8.0bC	9.2bC	15.8bB
	Cd-Catt	23.1bB	10.7bB	13.0bB	17.3bA
	Cd-Leo	19.4bD	9.6bB	11.7bC	14.5bC

Values followed by the same letter are not significantly different; lower-case letters show the difference of treatments of the same cultivar (LSD, $p < 0.05$); capital letters indicate the difference in Cd accumulation performance among cultivars within the same treatment (LSD: $p < 0.05$)

Table 4 Leaf anatomical characteristics among five marigold cultivars ($n = 5$)

Marigold cultivar	Treatment	Mid-vein area (μm^2)	Adaxial-epidermis thickness (μm)	Abaxial-epidermis thickness (μm)	Mesophyll thickness (μm)	Diameter of xylem cell (μm)
French	Com-Ctrl	7731.1±6488.6bB	13.7±1.5cAB	17.6±2.2aA	289.7±79.0aA	12.7±1.0bA
	Cd-Alon	3042.9±2079.7bC	18.0±1.5bA	23.8±3.0aA	223.3±66.8abA	11.3±10.7bA
	Cd-Pig	24694.5±4212.1aAB	21.3±2.8aA	23.0±10.8aA	283.9±67.4abAB	15.2±2.5aA
	Cd-Catt	4723.9±2496.2bB	15.7±1.0bcA	16.5±1.6aA	199.6±14.6abBC	9.9±4.1cA
	Cd-Leo	8742.1±2090.1bB	14.9±1.1bcA	16.8±5.6aAB	182.2±43.8bB	13.7±3.3abA
Honey	Com-Ctrl	7191.4±1333.3bB	18.3±4.0aA	16.2±3.1abA	219.8±24.1bAB	11.4±3.7abA
	Cd-Alon	7877.0±3700.7bB	13.7±2.0aAB	12.2±2.6abC	154.4±34.3bB	12.0±2.5abA
	Cd-Pig	25043.4±10954.3aAB	18.8±7.3aA	17.4±5.6aA	341.4±83.6aA	17.1±7.1aA
	Cd-Catt	12205.9±5786.5bB	13.9±1.5aA	17.9±6.4aA	371.5±82.4aA	14.7±5.8abA
	Cd-Leo	4009.3±2894.0bC	7.3±1.9bB	11.0±2.5bB	189.4±37.7bB	9.4±2.2bA
American	Com-Ctrl	7713.7±2121.1cB	17.7±5.4aA	15.4±5.7abA	151.6±31.0bcB	11.0±1.2bcA
	Cd-Alon	7005.2±1026.8cBC	17.0±4.3aA	18.6±4.5aAB	166.2±25.0bB	9.0±3.0cB
	Cd-Pig	15582.9±5516.9aB	13.9±4.8aA	19.1±4.1aA	235.9±24.3aB	15.8±4.2aA
	Cd-Catt	12208.9±3700.8abB	13.5±2.8aA	15.9±2.8abA	132.9±14.0cC	13.7±4.1abA
	Cd-Leo	8194.7±2526.4bcB	13.0±2.7aA	12.8±2.9bAB	173.4±15.6bB	9.8±3.2bcA
Sunshine	Com-Ctrl	21444.5±5608.7bA	12.1±3.8bB	14.9±3.4aA	225.5±77.9bAB	13.6±2.9abcA
	Cd-Alon	15969.8±2671.7bA	11.5±3.9bB	14.1±2.9aBC	262.8±19.9bA	11.3±2.6cA
	Cd-Pig	33914.2±7148.8aA	17.4±4.9aA	17.0±3.0aA	351.2±97.3aA	17.4±3.2aA
	Cd-Catt	35071.8±10772.7aA	18.4±2.8aA	15.2±2.1aA	270.2±32.6abAB	15.3±3.4abA
	Cd-Leo	19947.3±3052.6bA	14.7±4.3abA	18.8±7.1aA	254.2±46.7bA	12.6±2.8bcA

Values followed by the same letter are not significantly different; lower-case letters show the difference of treatments in the same plant species (LSD, $p < 0.05$); upper-case letters indicate the difference of leaf anatomical characteristics among marigold cultivars within the same treatment (LSD, $p < 0.05$)

Figures

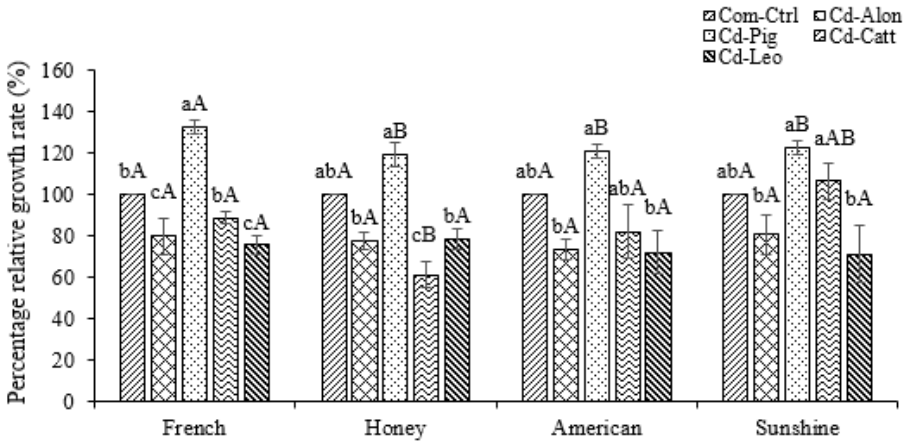

Figure 1

Percentage relative growth rate values of the study plants (n = 5)

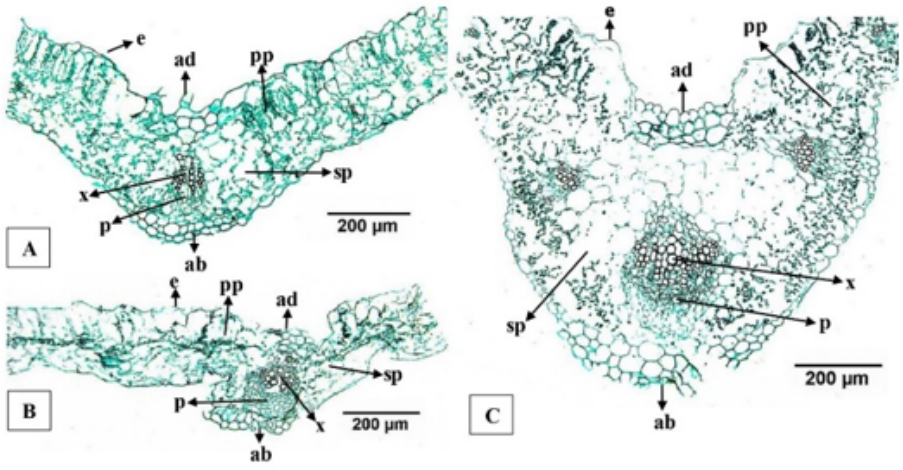

Figure 2

Leaf anatomy modifications occurred in Honey cultivar grown in different soil treatments (A) low Cd: Com-Ctrl treatment (B) soil without organic amendment: Cd-Alon treatment (C) amended soil: Cd-Pig treatment. ad = adaxial epidermis; ab = abaxial epidermis; e = epidermis; pp = palisade parenchyma; sp = spongy parenchyma; x = xylem; p = phloem