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ECOTOXICOLOGICAL RESPONSES OF MORPHOLOGICAL AND PHYSIOLOGICAL PARAMETERS OF CADMIUM-STRESSED MAIZE SEEDS

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Abstract

The destructive effect of cadmium (Cd) on morphological, anatomical and physiological parameters of maize seeds stressed with different concentrations were studied. Cd inhibited germination and seedling growth of maize in parallel with increasing concentrations. Increasing concentrations of Cd resulted in decreased stomatal responses in maize leaves compared with control, leaf area also diminished with Cd stress, photosynthetic pigment contents decreased, the chlorophyll degradation also increased. The changes on germination, growth and development of maize seed exposed to Cd stress were determined as more sensitive parameters to detect its damage.

Introduction

Heavy metals polluted soil and water are serious environmental problem. Plants exposed to various stresses, and intensity and duration of stress may occur slowly and it gradually changes plant growth conditions. Cadmium, a non-essential element for plants, is potentially toxic for higher plants, animals and humans, and is one of the most dangerous heavy metals. Even its smallest concentrations (1 μM) can have toxic effects on all organisms (Seregin *et al*. 1998). Furthermore, Cd has a higher solubility than other metals (Xie *et al*. 2013) and so it is present in higher amounts in the environment and quickly absorbed and accumulated in plants.

Cd cannot be degraded, even at low concentration, its uptake by root and transportation to the all plant parts can adversely effect water uptake imbalance (Clemens 2006), macro and micronutrients uptake distribution and photosynthesis rate (Dias *et al*. 2013). The destructive effects are constantly increased because of its accumulation. Even, strength and duration of stress exposure can also cause irreversible changes in plant growth and development (Fitter and Hay 2002). As a result of all these negative effects, Cd causes significant degradations in germination and seedling growth (Shaikh *et al*. 2013), root, stem (Salvatore *et al*. 2008) and leaf (Baryla 2001) parameters of plants, and gradually eradicated plants from the environment. However, with the investigation of modifications in germination and growth parameters of plants under various stresses which leads to changes on biochemical and physiological processes, anatomical and morphological anomalies that may be the visible signs of these changes can be determined (Anastasov 2010). Therefore, in this study, ecotoxicological effects of Cd were determined via investigated that morphological, anatomical and physiological changes in seed and seedling of maize induced Cd stress. This is the first comprehensive study by employing morphological, anatomical and physiological approaches in order to have a deeper look about stomatal movements in maize leaves under Cd stress.

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Materials and Methods

The experiment was performed to determine the effects of different concentrations of Cd^{2+} on maize seeds (*Zea mays* L. cv. caramelo). Seeds were surface sterilized with sodium hypochlorite 0.5% (v/v). They were soaked in 100 ml $CdCl_2H_2O$ solutions with different Cd^{2+} concentrations of 0 (control), 20, 40, 60, 80, 100 and 120 µM in beakers for 96 hrs. Subsequently, for each experiment, 25 seeds were placed on a Whatman paper soaked with 20 ml distilled water and then incubated in 20ºC for 7 days. Each treatment was replicated three times. The edge of radicle through the seed coat was taken as the criteria of seed germination. On the 7th day after sowing, required parameters were calculated to determine germination index (GI) (Tiquia 2010) and vigor index (VI) (Hangarter 1997). These indexes were calculated using the following equations; GI = (% relative seed germination × % relative root growth)/100. [% relative seed germination: (Number of seeds germinated in Cd concentration/number of seeds germinated in control) × 100; % relative root growth: Mean root length in Cd concentration/Mean root length in control × 100]. VI = Seedlings length (cm) × Germination percentage/100. Seedlings were transplanted into pots with perlit, and in each pot Hoagland's nutrient solution was regularly added for 45 days. Stomatal index was calculated by the number of stomata and epidermal cells counted in each field (1 mm^2) at independent measurement by superficial sections taken from adaxial and abaxial surfaces of leaves (Rengifo *et al*. 2002), based on average of 50 microscopic field. Stomatal sizes were defined using an ocular micrometer. The leaf area of plants was determined using the following equations (Pandey and Singh 2011). LA: × /y (*x:* Graph paper weight of the leaf surface; *y*: Similar graph paper weight of 1 cm^2 area).

Cd contents of seeds and pH amount of Cd absorption in seeds treated with different concentrations of Cd for 96 hrs were determined by ICP-OES analysis (SDU Environmental Engineering Lab.). After 96 hrs, the medium pH was measured with pH meter (ADWA AD1020). Total chlorophyll contents of 25 plants belonging to each treatment were calculated using a chlorophyll meter (Minolta SPAD-502). Pigment degradation was calculated according to Um and Kirdmanee (2009).

Data were analyzed using one-way ANOVA followed by Duncan's multiple range test for post-hoc (SPSS, version 14.0) ($p \leq 0.05$).

Results and Discussion

Heavy metals exerted adverse affect on germination and growth satages of seeds (Hu *et al*. 2015). In the present study it had been found that Cd treatment led to different morphological and physiological responses with the increase concentrations of Cd in maize seeds and seedlings. Cadmium content also increased in seeds with in increased Cd concentration the treatment. At the highest Cd concentration (120 µM) with the lowest medium pH (pH: 5.05) (Fig. 1a), amount of absorbed Cd in the seeds were found to be the highest (35.79 µg/g) ($p \leq 0.05$). We can say that the pH had shown an effect on Cd absorption by maize plant. In this study, we found that seed weights decreased with increasing Cd concentrations (Fig. 1b). Seed weights decreased by 2, 4, 7, 8, 15 and 23% in 20, 40, 60, 80, 100 and 120 µM concentrations, respectively ($p \geq 0.05$), but at 80 µM and higher concentrations we also observed statistically significant decreases ($p \leq 0.05$). We think that the reduction of seed weights as Cd concentration increases could be caused by a decrease in water imbibition rate.

It was observed that the harmful effects of Cd stress on GI and VI decreased by 9, 56, 65, 83, 92, 98% and 45, 73, 81, 87, 92, 98% in 20, 40, 60, 80, 100 and 120 µM Cd concentrations, respectively in compared to control (Fig. 2a, b). Cadmium caused significant decreases in growth parameters (Fig. 2c) ($p \leq 0.05$). Especially, root length decreased by 58% at 80 µM and the decrease continued as the concentration increased. The effects on coleoptile length were similar to root length. It is thought that the decrease in this parameters with increasing Cd concentrations is probably proportional to chromosomal and mitotic abnormalities (Hemachandraand and Pathiratne 2015).

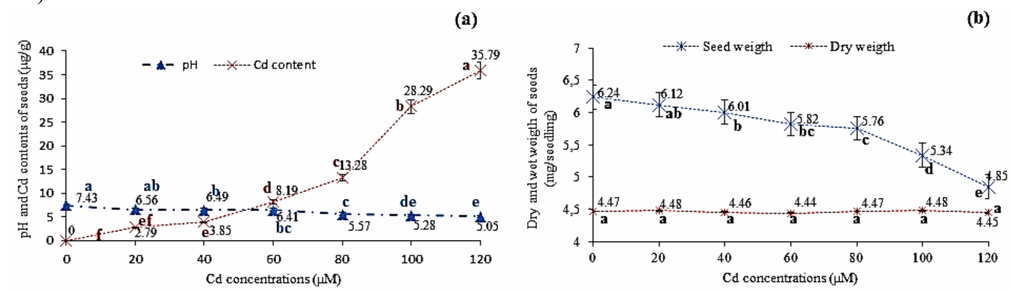

Fig. 1. Cd contents, pH levels (a) and weights of maize seeds before and after pre-treated with different concentrations of Cd. ($p \leq 0.05$).

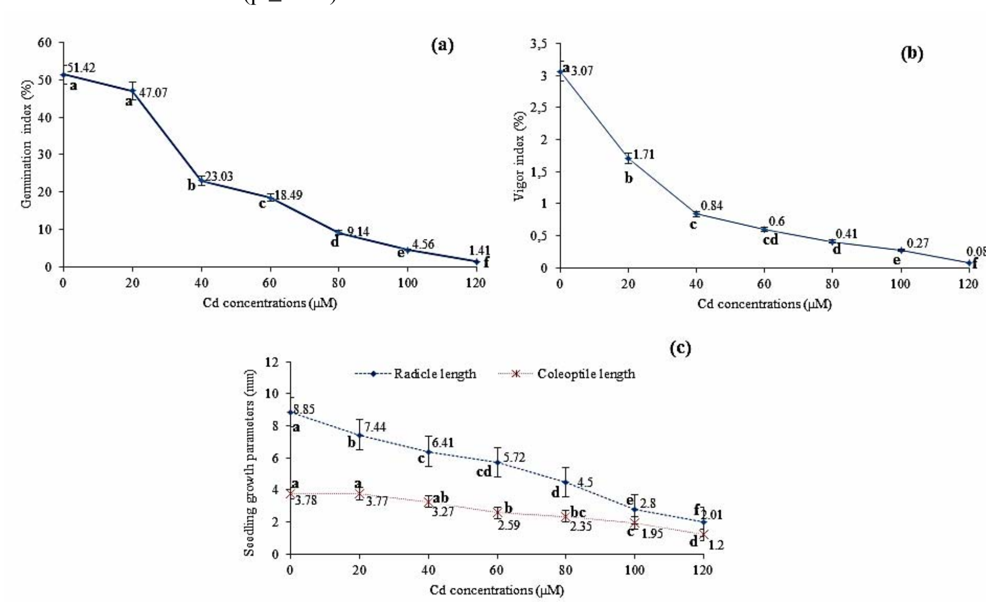

Fig. 2. Effects of different concentrations of Cd on germination and seedling growth; GI (a), VI (b) and seedling growth (c) for 7 days. ($p \leq 0.05$).

Stomata are apertures that have an important role in transpirational control, and stomatal parameters such as stomata density and stomata sizes are thought to be at the center of gas exchange (Fu *et al*. 2014). In the present study, stomata and epidermis number decreased with increasing Cd concentrations on both adaxial (Fig. 3a) and abaxial (Fig. 3b) surfaces, compared to the control. Furthermore, inhibitory effects of Cd stress on stomata and epidermis numbers on both surfaces were shown by decreased stoma index (Fig. 3a, b).

Stomata length and width on abaxial surface of maize leaf reduced by 28 and 51%, compared with the control, at the highest concentrations of Cd (120 µM) (Fig 4a, b). Decline in stomata index and size per unit area with increasing Cd concentrations resulted in a reduction in leaf surface area (Fig. 4c). Significant reductions in transpiration by the toxic effect of stress reduces photosynthesis rate (Li *et al*. 2015) and negatively affects the growth of the plants. The decreases in stomata and epidermis counts and sizes on both leaf surfaces were reflected by a parallel decrease in leaf surface area, as increased Cd stress, compared to control ($p \leq 0.05$). We think that the decrease of leaf parameters with increasing Cd concentrations can affect CO_2 assimilation and evoporation negatively, and growth of plants will be inversely related to increasing Cd concentrations.

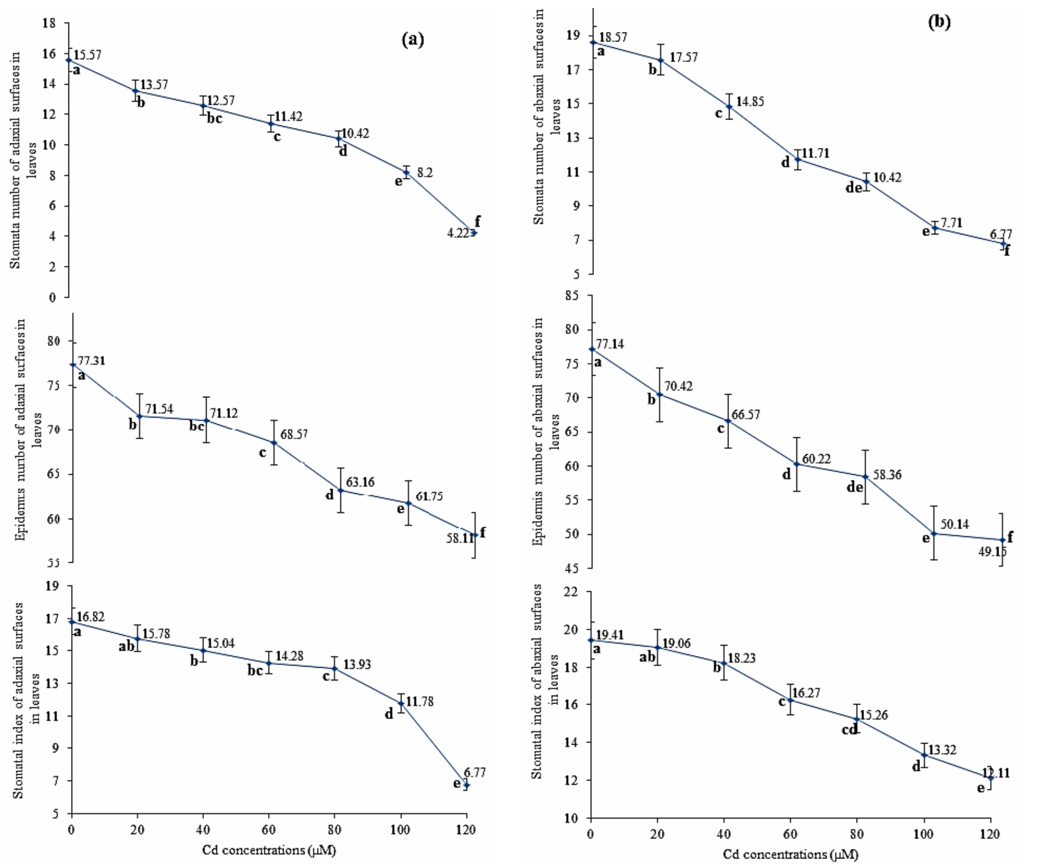

Fig. 3. Comparison of stomatal properties on adaxial surfaces (a) and abaxial surfaces (b) of maize seedling leaves exposed to different levels of Cd stress ($p \leq 0.05$).

Total chlorophyll contents of maize seeds exposed to increasing Cd stress were investigated and the most total chlorophyll contents was observed on the plant tissues grown at 0 µM (control) (pH: 7.6) while the least were on the plant tissues exposed to the highest concentration of Cd (pH: 1.2) (Fig. 5a). Photosynthetic pigments is one of the important indicators determining photosynthesis rate (Xu *et al*. 2013), but photosynthetic pigment content are inhibited in the presence of stress (Meier *et al*. 2011).

Chlorophylls are known to be easily degraded by various environmental conditions. Although, chlorophyll degradation is an important parameter in determining plants' responses to environmental stress, the mechanism is less well understood (Hörtensteiner and Kräutler 2011). Total chlorophyll content showed inverse relation with chlorophyll degradation with increasing concentration of Cd (Fig. 5b) ($p \leq 0.05$). If a phytotoxic effect is present, cells carrying this biomolecule die because inhibition of Chl biosynthesis or degradation can lead to cell death (Tanaka and Tanaka 2006). The fact that chlorophyll contents decrease as concentration of Cd increases in maize seeds grown under Cd stress indicated that photosynthesis rate of these plants can also be affected negatively.

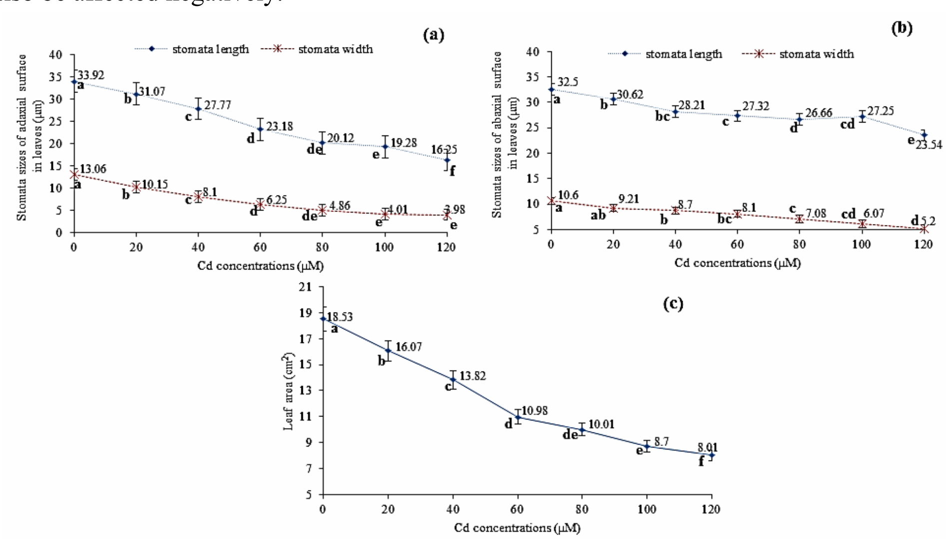

Fig. 4. Effects on stomata sizes (width/length) on both surfaces of maize leaves exposed to Cd stress; adaxial surfaces of leaves (a), abaxial surfaces in leaves (b), leaf surface areas (c). ($p \leq 0.05$).

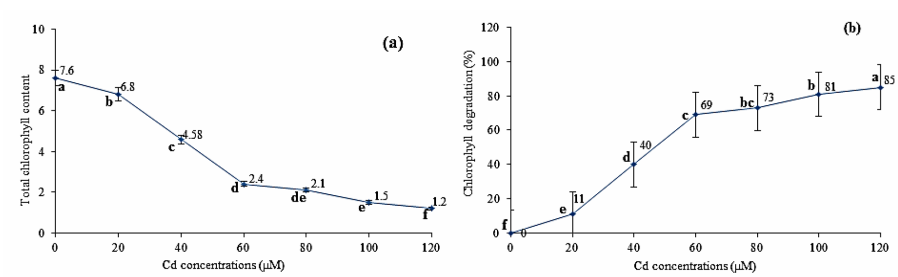

Fig. 5. Comparison of total chlorophyll content (a) and chlorophyll degradation (b). ($p \leq 0.05$).

The results presented, here clearly indicated the effect of Cd on all growth parameters of maize plant depended on its dose, and they can be considered as a sensitive indicator for Cd toxicity. In addition, have it is thought to adversely affect all livings nurtured with plants exposed to chemicals, and also negative effects to environmental factors such as, soil and water, as accumulation of chemical compounds increased.

References

Anastasov H 2010. Influence of oxyfluorfen on some anatomic indices in the leaves of *Virginia tobacco* plant *(Nicotiana tabacum* L.*)*. Biotechnol. Biotec. Eq. **24**: 33, doi: 10.1080/13102818.2010.10817805.

Baryla A, Patrick C, Franck F, Coulomb C, Sahut C and Havaux M 2001. Leaf chlorosis in oilseed rape plants (*Brassica napus*) grown on Cd-polluted soil: causes and consequences for photosynthesis and growth. Planta **212**: 696-709.

Clemens S 2006. Evolution and function of phytochelatin synthases. J. Plant Physiol. **163**: 319-332.

Dias MC, Monteiro C, Moutinho-Pereira J, Correia C, Goncalves B and Santos C 2013. Cd toxicity affects photosynthesis and plant growth at different levels. Acta Physiol. Plant. **35**: 1281-1289.

Fitter AH and Hay RKM 2002. Environmental physiology of plants, 3rd Edition, Academic Press, London. pp. 245-251.

Fu J, Yongfang S, Xitong C, Yuefei X and Tianming H 2014. Exogenous 5-aminolevulenic acid promotes seed germination in *Elymus nutans* against oxidative damage induced by cold stress. Plos one **9**: 1-9.

Hangarter RP 1997. Gravity light and plant form. Plant Cell and Environ. **20**: 796-800.

Hemachandraand CK and Pathiratne A 2015. Assessing toxicity of copper, cadmium and chromium levels relevant to discharge limits of industrial effluents into Inland surface waters using common onion, *Allium cepa* Bioassay. Bull. Environ. Contam. Toxicol. **94**: 199-203.

Hortensteiner S and Kräutler B 2011. Chlorophyll breakdown in higher plants. Biochimica et Biophysica Acta (BBA) - Bioenergetics **1807**: 977-988.

Hu J, Deng Z, Wang B, Zhi Y, Pei B, Zhang G, Luo M, Huang B, Wu W and Huang B 2015. Influence of Heavy Metals on Seed Germination and Early Seedling Growth in *Crambe abyssinica*, a Potential Industrial Oil Crop for Phytoremediation. Amer. J. Plant Sci. **6**: 150-156.

Li S, Yang W, Yang T, Chen Y and Ni W 2015. Effects of cadmium stress on leaf chlorophyll fluorescence and photosynthesis of *Elsholtzia argy* - A cadmium accumulating plant. Int. J. Phytoremediation **17**: 85-92.

Meier S, Tzfadia O, Vallabhaneni R, Gehring C and Wurtzel ET 2011. A transcriptional analysis of carotenoid, chlorophyll and plastidial isoprenoid biosynthesis genes during development and osmotic stress responses in *Arabidopsis thaliana*. BMC Systems Biol. **5**: 1-19.

Pandey SK and Singh H 2011. A simple, cost-effective method for leaf area estimation. J. Bot. **2011**: 1-6.

Rengifo E, Urich R and Herrera A 2002. Water relations and leaf anatomy of the tropical species, *Jatropha gossypifolia* and *Alternanthera crucis*, grown under elevated CO_2 concentration. Photosynthetica **40**: 397-403.

Salvatore MD and Carafa AM, Carratù G 2008. Assessment of heavy metals phytotoxicity using seed germination and root elongation tests: A comparison of two growth substrates. Chemosphere **73**: 1461-1464.

Seregin I, And V and Ivanov VB 1998. The transport of Cd and lead ions through root tissues. Russ. J. Plant Physl. **45**: 780-785.

Shaikh IR, Shaikh PR, Shaikh RA and Shaikh AA 2013. Phytotoxic effects of heavy metals (Cr, Cd, Mn and Zn) on wheat (*Triticum aestivum* L.) seed germination and seedlings growth in black cotton soil of Nanded, India. Res. J. Chem. Sci. **3**: 14-23.

Smiri M 2011. Effect of Cd on germination, growth, redox and oxidative properties in *Pisum sativum* seeds. J. Environ. Chem. Ecotox. **3**: 52-59.

Tanaka A and Tanaka R 2006. Chlorophyll metabolism. Curr. Opin. Plant Biol. **9**: 248-255.

Tiquia SM 2010. Reduction of compost phytotoxicity during the process of decomposition. Chemosphere **79**: 506-512.

Um C and Kirdmanee C 2009. Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. Pakistan J. Bot. **41**: 87-98.

Xie WY, Huang Q, Li G, Rensing C and Zhu YG 2013. Cadmium accumulation in the rootless macrophyte *Wolffia globosa* and its potential for phytoremediation. Int. J. Phytoremediation **15**: 385-397.

Xu D, Chen Z, Sun K, Yan D, Kang M and Zhao Y 2013. Effect of cadmium on the physiological parameters and the subcellular cadmium localization in the potato (*Solanum tuberosum* L.). Ecotox. Environ. Safe. **97**: 147-153.

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