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Effect of organic amendments on cadmium stress to pea: A multivariate comparison of germinating vs young seedlings and younger vs older leaves

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ABSTRACT

Despite significant recent advancement in research, biogeochemical behavior of heavy metals with respect to their applied form is still topical. Moreover, metal toxicity to plants may vary with their stage of development/maturity. Therefore, this study for the first time evaluated the influence of ethylenediaminetetraacetic acid (EDTA) and citric acid (CA) on cadmium (Cd) accumulation and toxicity to germinating and young pea seedlings as well as in younger and older leaves. The experimental setup of current study consisted of two separate studies. The first study was performed on germinating seedlings grown in a Cd-contaminated sand media. Pea seeds were treated with two levels of Cd (Cd-25 and Cd-100) alone and combined with different levels of EDTA and CA. The second study was carried out in hydroponic solution. The influence of organic amendments on Cd accumulation and toxicity to pea plants was evaluated by determining Cd contents in pea seedlings, H_2O_2 contents, chlorophyll contents and lipid peroxidation in younger and older leaves.

Cadmium stress caused overproduction of H_2O_2 in roots and leaves of pea seedlings. Cadmium-induced overproduction of H_2O_2 caused a decrease in the pigment contents and increased lipid peroxidation. Application of EDTA at higher levels (81 and 200 μM) increased Cd accumulation by pea plants. However, CA did not affect Cd accumulation by pea. Both EDTA and CA increased Cd-induced H_2O_2 production and lipid peroxidation. Younger pea leaves showed more sensitivity to Cd stress compared to older leaves. Similarly, Cd toxicity was more pronounced in germinating seedlings than young seedlings. Moreover, Pearson correlation and principal component analysis (PCA) showed very interesting correlations between treatments and stress responses of germination and young seedlings as well as younger and older leaves. Based on multivariate analysis, it is proposed that the Cd toxicity to pea plants greatly vary with its growth stage and the maturity of organs (younger or older leaves).

1. Introduction

Cadmium (Cd) is one of the important environmental contaminant, which has profound effects on biodiversity and human health (Sandbichler and Höckner, 2016; Sigel et al., 2013). This metal is ranked 7th in the priority list of pollutants released by the Agency for Toxic Substances and Disease Registry (ATSDR, 2014). Cadmium is a non-essential element that may not be toxic at low concentrations, but it is highly toxic to plants at higher levels (Manquián-Cerda et al., 2016; Mombo et al., 2016). It can induce harmful effects to biochemical, physiological and morphological processes in plants (Lu et al., 2018). At cellular level, Cd disturbs cell redox status, damages biological molecules (lipids, proteins, DNA and RNA) and provokes over-production of ROS by inhibiting enzymes involved in ROS control (Shahid et al., 2017a). Cadmium toxicity negatively affects membranes permeability as well as the activity of membrane-bound enzymes (Sandbichler and Höckner, 2016; Shahid et al., 2017b).

Nowadays, there is an increasing evidence that biogeochemical behavior of metals including Cd greatly depends on their chemical speciation (Khalid et al., 2017c; Kroukamp et al., 2016; Xiong et al., 2016). It is known that different forms of a metal have different bioavailabilities and phytotoxicities (Rafiq et al., 2017b; Shahid et al., 2017c). Studies showed that the soil-plant transfer of Cd depends mainly on its free form (Cd^{2+} level) (Meighan et al., 2011). Therefore,

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it is highly important to understand the role of Cd speciation towards Cd remediation and risk assessment studies.

Synthetic and natural chelating agents have been used in various studies to increase, or sometimes decrease, the bioavailability of metals in soil (Meighan et al., 2011; Shahid et al., 2012b). These compounds form complexes with metals, and thereby may increase or decrease metal bioavailability and phytotoxicity (Meighan et al., 2011; Shahid et al., 2012a). Among these compounds, EDTA has been found to be the most popular and effective chelating ligand to enhance metal solubility in soil, and therefore, it has been widely applied for the removal of metals from contaminated sites (Bloem et al., 2017; Shahid et al., 2014a). According to the literature, EDTA can release Cd from soil exchange sites to solution phase and thus causes increase in phyto-availability (Eissa, 2016; Meighan et al., 2011). This increased dissolution of Cd is due to high binding constant of EDTA for Cd (log K_f Cd–EDTA is 17.4) (Wei et al., 2012). Similarly, Low molecular weight organic acids (LMWOAs) can modify metal speciation and its biogeochemical behavior in soil-plant system. These compounds can alter metals uptake by plants. Among LMWOAs, citric acid (CA) is the most studied organic acid. It is reported that CA can enhance metals uptake and their accumulation in plants (Khalid et al., 2017b; Shahid et al., 2015).

In addition to metal speciation, data regarding the toxic effects of metals at different growth stages of plants is scarce. There are numerous studies which indicate metal toxicity to plants either at germination stage or vegetative growth stage. However, studies comparing metal stress on germinating and young seedlings under same experimental conditions are scarce. Similarly, risk assessment studies in which different types of plants are used as bioindicators do not fully indicate the part of plant organ (younger, older or all) taken for analysis. Plant biochemical and physiological responses can vary greatly with plant maturity. Therefore, maturity of a plant and its organ (younger or older) must be taken into consideration in risk assessment and remediation studies.

To the best of our knowledge, there exists very rare data regarding the effect of Cd on plants under different applied levels of Cd as well as at different maturity and growth stages of plants. Therefore, this study was intended to evaluate the influence of Cd speciation (in the presence and absence of CA and EDTA) on its accumulation and toxicity to germinating and two weeks older pea seedlings as well as younger and older pea leaves.

2. Materials and methods

2.1. Growth conditions, treatment level and composition

The current study was comprised of two experiments. The first experiment was performed on germinating pea seedlings grown in sand culture (sand was sterilized with 0.1 M HCl and oven-dried at 100 °C). The germinating pea seedlings were treated with fifteen Cd treatments (Table 1): control with nutrient solution only, two levels of Cd alone (25 and 100 μM, T_1-T_2), chelation of 25 μM Cd by four levels of EDTA (T_3-T_6), chelation of 100 μM of Cd with four levels of EDTA (T_7-T_{10}), and chelation of 100 μM of Cd with four levels of CA (T_{11}-T_{14}). The applied levels of Cd were selected based on previous studies as reviewed in Shahid et al. (2017a).

The applied levels of CA and EDTA were selected based on calculations of Cd speciation using Visual Minteq (version 2.60). The results of Visual Minteq showed that at these applied levels, CA chelates 12%, 25%, 37% and 50% of Cd, whereas EDTA chelates 25%, 50%, 75% and 100% of Cd in nutrient solution at pH 6. It was found that the same concentration of CA was required to chelate 12%, 25%, 38% and 50% of Cd for both levels of Cd (Cd-25 and Cd-100). Therefore, only the higher level of Cd (100 μM Cd) was used for CA treatment. After 10 days of treatment exposure, pea seedlings were harvested to determine the following parameters: (i) H_2O_2 contents (Shahid et al., 2014b), (ii) TBARS contents (Hodges et al., 1999), and (iii) chlorophyll contents (Lichtenthaler, 1987).

Table 1
Composition and experimental design of Cd treatments.

Treatments	Composition	Cd-chelated (%)	Cd-free (%)
Control	Hoagland solution (HS)	-	-
Cd alone			
T_1	HS + 25 μM Cd	0	90
T_2	HS + 100 μM Cd	0	90
Cd-25 + EDTA			
T_3	HS + 25 μM Cd + 7.5 μM EDTA	25	67
T_4	HS + 25 μM Cd + 15 μM EDTA	50	46
T_5	HS + 25 μM Cd + 25 μM EDTA	75	23
T_6	HS + 25 μM Cd + 100 μM EDTA	98	2
Cd-100 + EDTA			
T_7	HS + 100 μM Cd + 27 μM EDTA	25	68
T_8	HS + 100 μM Cd + 53 μM EDTA	50	45
T_9	HS + 100 μM Cd + 81 μM EDTA	75	23
T_{10}	HS + 100 μM Cd + 200 μM EDTA	99	1
Cd-100 + citric acid			
T_{11}	HS + 100 μM Cd + 900 μM CA	12	79
T_{12}	HS + 100 μM Cd + 1800 μM CA	25	68
T_{13}	HS + 100 μM Cd + 2800 μM CA	38	56
T_{14}	HS + 100 uM Cd + 3900uM CA	50	45

In second experiment, pea seeds were germinated in peat culture for one week. The healthy and uniform seedlings were transferred to nutrient solution for vegetative growth (Shahid et al., 2011). After one week of vegetative growth, pea seedlings were treated with different Cd treatments used in germination experiment (Table 1) for eight days. Each treatment was replicated four times. After treatment exposure, pea plants were harvested and separated into roots and shoots. Younger (top most) and older (the lowest) leaves were harvested and analyzed separately.

2.2. Cadmium analysis in root and shoot

After harvest, pea roots were washed with 0.01 M HCl to remove metals adsorbed at root surface. After oven drying, a mechanical grinder was used to grind root and shoot samples of pea to powder form. Pea samples were digested with a 1:1 mixture (10 mL) of hydrogen peroxide (v/v 30%) and nitric acid (v/v 65%) at 200 °C for about 2–3 h until the solution became colorless (Shahid et al., 2011). After filtration, Cd contents were measured by atomic absorption spectrophotometer (AAS, Thermo AA®, Solar-Series). Quality assurance was performed using standard reference material. Moreover, Cd solutions of known concentration were run after every 15th measure of samples by AAS.

2.3. Analysis of physiological attributes

In case of physiological attributes, plant samples after harvest were immediately frozen in liquid nitrogen and stored in refrigerator. Plant physiological attributes were analyzed separately in younger and older leaves for hydroponic experiment.

2.4. Hydrogen peroxide analysis

For hydrogen peroxide (H_2O_2) contents analysis, 500 mg of frozen pea samples were homogenized with 0.1% trichloroacetic acid by grinding in liquid nitrogen followed by centrifugation at 12,000 g for 20 min. The absorbance mixture contained 0.5 mL of the supernatant, 0.5 mL of potassium phosphate buffer (10 mM with pH 7.0) and 1 mL of potassium iodide (1 M). Spectrophotometer (AA, Solar–Series) was used to measure absorbance at 390 nm.