



Research article

In situ immobilization of Cd by organic amendments and their effect on antioxidant enzyme defense mechanism in mung bean (*Vigna radiata* L.) seedlings



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ABSTRACT

In situ immobilization of Cd is desirable due to the damaging effects of *ex situ* remediation techniques on soil. In this greenhouse study, the role of biochar (BC), chitosan (CH), and green waste (GW) was studied for *in-situ* Cd immobilization and alleviating Cd toxicity in mung bean seedlings. Amendments were applied at rates of 0.5% and 1% (w/w). The minimum mean value of Cd, in root, shoot, and soil (DTPA-Cd) (12.2, 4.7, and 0.7 mg kg⁻¹, respectively), occurred in the Cd + 1% CH treatment compared to all Cd amended treatments. Shoot dry weight (21%) increased significantly in Cd + 1% BC amended soil compared to the control. Reactive oxygen species were affected significantly, with the lowest increased value of hydrogen peroxide (4%) in the Cd + 1% CH treatment while the minimum increase in the value of superoxide (O₂⁻) occurred in the Cd + 1% BC soil compared to the control. Malondialdehyde (20%) increased lowest with Cd + 1% CH treatment. Protein, ascorbate (AsA) contents, and catalase (CAT) activity increased the most (3, 2, and 15%, respectively) in the Cd + 1% BC treatment while dehydroascorbate reductase (DHAR) and superoxide dismutase (SOD) activity increased the most (9 and 234%, respectively) in the Cd + 1% CH soil compared to the control. Glutathione reductase (GR), ascorbate peroxidase (APX), and glutathione peroxidase (GPX), activity were reduced the most in the Cd + 1.0% BC treatment while dehydroascorbate (DHA) and glutathione S-transferase (GST) activity decreased the most in the Cd + 1% CH soil. Overall, *in situ* immobilization by amendments improved growth and antioxidant defense mechanisms of mung bean seedlings and was reflected by tolerance to Cd-toxicity.

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1. Introduction

Soil pollution with heavy metals has become a serious issue in developed and developing countries (Bolan et al., 2014; Ramzani et al., 2016a). Rapid industrialization, sewage sludge, raw city

effluents, manures, and excessive application of fertilizers (especially phosphatic fertilizer) are the major sources of Cd contamination of soil (Nahar et al., 2016). Phosphate fertilizers such as single super phosphate contain 2 to more than 40 mg kg⁻¹ Cd while triple super phosphate contain from less than 10 to over 100 mg kg⁻¹ Cd contents (Van Kauwenbergh, 2001). Cadmium concentration in crops increased by application of phosphatic fertilizers containing high levels of Cd (Grant, 2011). Cadmium accumulates readily in plants and its toxicity damages plant infrastructure by necrosis, altered stomatal movement, ion homeostasis, limited availability of water and nutrients, changed metabolism, and affecting activities of several key enzymes (Dias et al., 2013; Ehsan et al., 2014; Nahar et al., 2016). Reactive oxygen species (ROS) (i.e. superoxide, O₂⁻; hydrogen peroxide, H₂O₂)

Abbreviations: BC, biochar (0.5% or 1.0% w/w); CH, chitosan (0.5% or 1.0% w/w); GW, green waste (0.5% or 1.0% w/w); BCF, biological concentration factor; BAC, biological accumulation coefficient; TF, translocation factor; GPX, glutathione peroxidase; GST, glutathione S-transferase; H₂O₂, Hydrogen peroxide; O₂⁻, superoxide; MDA, malondialdehyde; CAT, catalase; APX, ascorbate peroxidase; DHAR, dehydroascorbate reductase; SOD, superoxide dismutase; AsA, ascorbic acid (ascorbate); DHA, dehydroascorbate; GR, glutathione reductase.

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and malondialdehyde (MDA, a product of lipid peroxidation) produced in response to Cd toxicity cause damage to biomolecules and biomembranes including nucleic acids by oxidizing proteins, and peroxidizing lipids (Chen et al., 2010; Nahar et al., 2016; Ramzani et al., 2016a). Plants prevent oxidative stress and ROS production by its antioxidant defense system involving multiple enzymes and antioxidants (Ramzani et al., 2017). Antioxidant enzymes may include glutathione peroxidase (GPX), superoxide dismutase (SOD), glutathione S-transferase (GST), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) (Gill and Tuteja, 2010). An antioxidant system is well equipped with different antioxidant components to scavenge over-produced ROS and thus protects plants from oxidative injury (Hasanuzzaman et al., 2011; Ramzani et al., 2017).

Microbial and chemical activities are not able to degrade metals. One way to ensure food safety in metal-contaminated soil is to use amendments to manipulate metal bioavailability (Bolan et al., 2014; Venegas et al., 2016). The basic purpose of soil remediation techniques is to modify heavy metal mobility in soil and their translocation into plants parts (Bolan et al., 2014; Ramzani et al., 2016a).

Many different *ex situ* and *in situ* remediation techniques are available. *Ex situ* remediation techniques for heavy metals (e.g. Cd, Ni, Pb) include soil excavation, washing, and electrokinetics (Alkorta et al., 2004; Peng et al., 2009). However, these *ex situ* metal immobilization techniques are not feasible due to their expense and soil health hazard problems (Ghosh and Singh, 2005; Dermont et al., 2008). A promising alternative to *ex situ* remediation techniques is the *in situ* immobilization of metals (Hmid et al., 2015; Ramzani et al., 2016a). Plants have various mechanisms such as chelation, active excretion, binding, compartmentalization, and exclusion to avoid toxic effects of Cd and other heavy metals (Zagorchev et al., 2013). Recently, many studies on *in situ* remediation with different soil amendments have been made (Herath et al., 2015; Ramzani et al., 2016a). In heavy metal-contaminated soil, different soil immobilizing agents like liming materials, organic amendments, clay, and phosphate minerals have been studied for remediation and to reduce metal toxicity (Ok et al., 2010; Hmid et al., 2015; Puga et al., 2015). Several different mechanisms such as cation exchange, metals ions binding at specific sites, adsorption and complexation are involved, that influence heavy metal immobilization (Lee et al., 2013; Herath et al., 2015).

In this context, for *in situ* immobilization of Cd, various organic amendments such as chitosan, biochar, and green waste have been used. Chitosan, a deacetylated product of chitin, is a polysaccharide biopolymer with high binding capacity and unique functionalities (Dalida et al., 2011; Tripathi et al., 2016). Amino and hydroxyl groups on chitosan surfaces act as binding sites for metals and may possess high adsorption sites for heavy metals (Nghah et al., 2011; Wang et al., 2016).

Organic amendments like biochar and green waste compost have also been tested in Cd remediation strategies, although different conclusions about these amendments for heavy metal immobilization have been reported (Beesley et al., 2010; Venegas et al., 2016). For example, Beesley et al. (2010) found that biochar is more effective than green waste for Cd immobilization in soil pore water while Venegas et al. (2016) found larger decreases in Cd extractability from soil with green waste compared with biochar. In previous studies, heavy metal sorption/adsorption by green waste (Beesley et al., 2010; Venegas et al., 2016), and biochar (Cui et al., 2016; Ramzani et al., 2016a) has been reported. The effect of chitosan on adsorption of heavy metals from waste water is well known (Nghah et al., 2011; Dalida et al., 2011; Padilla-Rodríguez et al., 2015; Li et al., 2016), but to the best of our knowledge, only a few studies are available for chitosan's effect on heavy metal immobilization in soil (Kamari et al., 2011a; Padilla-Rodríguez and

Codling, 2016).

The novelty of this study is to 1) evaluate and compare the potential of biochar (BC), chitosan (CH) and green waste (GW) to immobilize Cd in soil, 2) improvement in plant antioxidant defense mechanism and growth of mung bean seedling. These novel aspects of the present study illustrated the positive effect of the examined amendments (CH, BC and GW) and whether which one was best for all indicators.

2. Materials and methods

2.1. Soil physico-chemical properties

Test soil was collected from an uncultivated field of unknown history. The soil was cleaned to remove plant and stone pieces. After shade drying, a sub sample was analyzed for physico-chemical properties. The hydrometer method was used for soil texture determination (Gee and Bauder, 1986). Soil pH and electrical conductivity (EC) were measured in saturated soil paste. Walkley-Black method was used for soil organic matter determination (Nelson and Sommers, 1982). A simple acid dissolution method was used to determine soil calcium carbonate (CaCO_3) content (Allison and Moodie, 1965). Plant available phosphorus (P), nitrogen (N), and potassium (K) were determined by methodology proposed by Watanabe and Olsen (1965), Bremner and Mulvaney (1982), and Richards (1954), respectively. All examined experimental soil physico-chemical properties were noted in Table 1.

2.2. Immobilizing amendments properties and treatment plan

Chitosan (CH), with 90% deacetylation and average molecular weight of 161 kDa was purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Biochar (BC) was prepared at 450 °C with wheat straw as a feed stock. Green waste (GW) was in the form of compost originating from vegetable and plant residues.

Table 1
Physico-chemical properties of soil and organic amendments.

Properties	Soil		Units		
Texture	Loamy sand				
Sand	83.0 ± 2.9		%		
Silt	11.0 ± 0.9		%		
Clay	6.0 ± 0.4		%		
CaCO_3	2.5 ± 0.09		%		
pH	8.1 ± 0.6		—		
Organic matter	0.49 ± 0.02		%		
EC_e	1.2 ± 0.05		dS m^{-1}		
Available N	88 ± 3.4		mg kg^{-1}		
Available P	6.2 ± 0.3		mg kg^{-1}		
Available K	74 ± 2.8		mg kg^{-1}		
DTPA-Cd	0.09 ± 0.003		mg kg^{-1}		
Organic amendment properties					
Properties	Biochar	Green waste	Units	Chitosan	Values and units
Organic carbon	53 ± 2.1	49 ± 2	%	Molecular formula	$(\text{C}_6\text{H}_{11}\text{NO}_4)_n$
pH	7.89 ± 0.1	7.72 ± 0.1	—	Deacetyl degree	90%
EC_e	2.1 ± 0.1	1.99 ± 0.1	dS m^{-1}	pH	7.4
CEC	18.9 ± 1.6	11.9 ± 1.1	$\text{Cmole}_c \text{ kg}^{-1}$	Insoluble substances	≤1.0%
C/N ratio	48 ± 2.5	41 ± 2.2	—	Loss on drying	≤8.0%
Total N	43 ± 2.2	52 ± 2.4	g kg^{-1}	Arsenic	≤0.00006%
Total P	32 ± 2.1	27 ± 1.8	g kg^{-1}	Viscosity	200
Total K	38 ± 2.3	31 ± 2.3	g kg^{-1}	Heavy metals	≤0.0015%
Cd	5.6 ± 0.3	7.2 ± 0.4	mg kg^{-1}	Ignition residue	≤1.0%

Data of soil, biochar and green waste are the average of 3 replications + SE.

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