



Full Length Article

Organic and Inorganic Amendments Immobilized Cadmium and Improved Maize Growth and Yield in Cd-contaminated Soil

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Abstract

In situ immobilization of contaminants by application of organic and inorganic amendments is one of the effective techniques to reduce availability of heavy metals to plant. A pot trial was carried out to evaluate the effects of inorganic (rock phosphate) and organic amendments (press mud compost) for Cd immobilization to improve maize growth and yield in Cd-contaminated soil. Cadmium chloride salt was used for spiking (0, 10 and 30 mg Cd kg⁻¹ soil). Rock phosphate was applied at the rate of 1% and 2% alone and in combination of press mud compost 2%. These amendments were applied alone and in all possible combinations. Results indicated that Cd contamination negatively affected and the effect was more pronounced under 30 mg Cd kg⁻¹ compared to lower levels of Cd stress. But the immobilization of Cd due to the combined application of rock phosphate and press mud compost improved the growth and yield of maize both in uncontaminated and Cd contaminated soil. Application of 1 and 2% rock phosphate and press mud compost alone and in combination improved the plant growth at all Cd stress levels. However, combined application of rock phosphate (2%) and press mud compost (2%) promoted plant height (1.21 times), root length (3.2 times), shoot dry mass (1.57 times) and root dry mass (5.3 times) at 30 mg kg⁻¹ Cd stress level as compared to respective untreated stressed control plant. In conclusion, Cd stress was highly detrimental to maize plants under higher level of contamination. Moreover, combined application of rock phosphate and press mud compost immobilized Cd and ultimately improved the growth and yield of maize in Cd contaminated soil. © 2019 Friends Science Publishers

Keywords: Immobilization; Cadmium stress; Rock phosphate; Press mud compost; Maize

Introduction

Heavy metal contamination of soil has become the emerging issue worldwide and the sources of these metals include both natural and anthropogenic. Anthropogenic activities are major cause of contamination of agricultural lands and this problem needs special attention. Irrigation of cultivated area with sewage water is the main reason for accumulation of heavy metals in soil (Alghobar and Suresha, 2017; Chaoua *et al.*, 2018). Heavy metals contamination of soil occurs through industrial activities, mining, waste production and by the application of chemical pesticides (Zhang *et al.*, 2015). Heavy metals remain in soil and destroy soil microorganisms and reduce plant growth. These metals are also transferred to the plants and affect human health by entering the food chain (Srivastava *et al.*, 2017). Both essential and non-essential heavy metals higher than their permissible limits in plants and animals

cause various morphological, genetic and physiological abnormalities such as mutagenic effects, growth reduction and increase mortality (Hassan *et al.*, 2016; Rehman *et al.*, 2018).

Remediation of heavy metal contaminated soils can be carried out by various physical, chemical and biological techniques. Biological remediation technique is gradually being accepted as the standard practice for the restoration of heavy-metal-contaminated soils. However, it is more eco-friendly and cost effective compared to the conventional chemical and physical methods, which are often very expensive and ineffective when metal concentrations are low. Moreover, conventional methods also produce significant amounts of toxic sludge (Ayangbenro and Babalola, 2017). The most feasible biological remediation technique is further divided into strategies such as stabilization and extraction. Metal stabilization technique was found effective because it is less destructive for environment,

economical and effective in reducing heavy metals bioavailability (Khalid *et al.*, 2017; Sharma *et al.*, 2018). In stabilization technique, various amendments (composed with bentonite, phosphate, humic acid, biochar, sepiolite powder, etc.) for immobilization are applied that reduce the bioavailability and soil leaching of heavy metals (Hossain *et al.*, 2017; Hussain *et al.*, 2017). Bioavailability of heavy metals is reduced by the processes such as ion exchange, stable complex formation with organic chelates and surface precipitation (Olaniran *et al.*, 2013; Khalid *et al.*, 2017). The application of organic amendments like compost and agro industrial waste to the contaminated soils, influences different soil properties such as improvement in fertility status, soil physical and chemical properties and alter the distribution of heavy metals in soil (Park *et al.*, 2011; Hossain *et al.*, 2017). Compost is formed when microorganisms convert paper, sludge, grass, food wastes, leaves and manure into a material that looks like soil.

Compost produced from manure and agricultural waste has been found effective in reducing the bioavailability of heavy metals in soil (Beesley *et al.*, 2011; Xie *et al.*, 2018). Press mud that is an organic fertilizer is considered as good soil conditioner (Kumar *et al.*, 2017). Rock phosphate with smaller grain size is suitable to increase the stability and decrease the availability of heavy metals to plants (Zhao *et al.*, 2014). Mechanisms used by rock phosphate for the stabilization of heavy metals include heavy metal exchange with calcium present in rock phosphate, ion exchange and surface complexation (Fernane *et al.*, 2013).

Cadmium is ranked as number seven noxious heavy metal among 20 most toxic heavy metals (Jaishankar *et al.*, 2014). One of the major sources of Cd contamination in Pakistani agricultural soils is irrigation with waste water (Ahmad *et al.*, 2015). Cadmium enters in plant cells through ion carrier channels and through proteins that transport ions (Mojiri, 2011; Song *et al.*, 2017). Metabolic processes such as respiration, closing and opening of stomata, water relations, photosynthesis, mineral nutrition and water relations are adversely affected due to the Cd contamination (Gallego *et al.*, 2012; Ali and Nas, 2018). Cadmium contamination destroys the enzymes used in carbohydrate metabolism and Calvin cycle and causes reduction in crop yield (Shi *et al.*, 2010; Ali and Nas, 2018). Heavy metal stress leads towards production of reactive oxygen species (ROS) which react with macromolecules and other metabolites such as lipids, proteins, DNA, photosynthetic pigments and proteins and hence reduce the growth and development of the plants (Swarbreck *et al.*, 2014). Plants have antioxidant mechanisms against these ROS that include both enzymatic (dismutase, peroxidase, catalase and glutathione reductase) and non-enzymatic (ascorbic acid, phenolics, flavonoids and glutathione) (Ali and Ashraf, 2011; Ali and Nas, 2018).

Maize (*Zea mays* L.) is one of the profitable crops in Pakistan and third important crop worldwide (Naqvi *et al.*, 2011) with high heavy metal accumulation potential.

This attribute of maize crop can be used as an indicator of the level of soil contamination (Wong, 1996) and also offers phytoremediation potential. Maize is annual cereal crop with rapid growth and large biomass of fibrous root system and can grow under adverse soil and climatic conditions (Zhang *et al.*, 2009; Sattar *et al.*, 2016). Moreover, it is not listed as hyperaccumulator plant, so it can be used for immobilization of heavy metals in soil. Furthermore, compost and rock phosphate, previously, have been used to enhance the growth and biomass of maize (Amanullah and Khan, 2017). Therefore, combination of compost and rock phosphate not only can improve the growth of maize but also be helpful in Cd immobilization in the contaminated soil. Keeping in view the above facts, present study was designed and conducted to observe the potential of rock phosphate and press mud compost to enhance immobilization of Cd and growth and yield of maize under Cd stressed soil.

Materials and Methods

A pot trial was carried out to determine the role of rock phosphate (RP) and press mud compost (CP) on maize growth and yield under Cd contaminated soil in the wire house of the Institute of Soil and Environmental Sciences (ISES), University of Agriculture Faisalabad, Pakistan. Soil was taken from ISES research farm, University of Agriculture, Faisalabad which was dried, ground and passed through 2 mm sieve, mixed and homogenized before filling the pots. Soil was spiked with Cd using cadmium chloride salt to maintain three contamination levels *i.e.*, 0, 10 and 30 mg Cd kg⁻¹ soil. For this purpose, soil was dispersed over the polythene sheet, mixed and homogenized to get equal contamination of soil. Then water was applied to keep the soil wet up to field capacity level and incubated for 3 weeks.

Determination of Physicochemical Characteristics of the Soil

For physicochemical characteristics of the soil (Table 1), standard procedures were adopted as described by U.S. Salinity Lab. Staff (1954) and Page (1965). The complete methodology to determine the soil properties are presented by Rehman *et al.* (2015). In short, hydrometer method was used to determine the particle size of soil. Soil pH was measured with pH meter (Lovibond, model Sensodirect 100). Soil saturated paste extract was prepared and soluble ions and cation exchange capacity (CEC) were measured by standard methods. Electrical conductivity (EC_e) of the extract obtained was measured with conductivity meter (Jenway model-4070). To determine the saturation percentage, soil saturated paste in known weight was taken and oven dried at 105°C until constant weight was obtained. After that saturation percentage was calculated by subtracting the mass of dry soil from the wet soil weight, divided by oven dry weight of soil and to get percentage,

the value multiplied with 100. To determine the bioavailable cadmium concentration in soil, 10 g soil was extracted with ammonium-bicarbonate diethylene-triamine penta-acetic acid (AB-DTPA, pH 7.6) solution (Soltanpour, 1985).

Pot Experiment

The experiment was laid out following the completely randomized design with three replications. Maize was grown under three levels of Cd *i.e.*, 0, 10 and 30 mg kg⁻¹ Cd. Rock phosphate at the rate of 1 and 2%, and 2% press mud compost containing organic carbon (28.9%), pH (6.85), available phosphorus (2%), total magnesium (1.01%), total calcium (1.04%) and total nitrogen (1.70%) were applied alone and in all possible combinations. Five hybrid maize seeds (genotype DK-6103) were sown into each pot having 10 kg of soil. After the germination, thinning was done to two plants in each pot. Irrigation was done according to the plant requirement of water and moisture content present in the soil using tap water. Pots were supplied with NPK at the rate of 75-37.5-37.5 mg kg⁻¹ using urea, diammonium phosphate and potassium sulphate fertilizers, respectively. The whole PK was applied as basal amount whereas N was added in three splits. Chlorophyll spade value was determined using chlorophyll meter (SPAD-502) during the 10 to 11 a.m. While physiological parameters were measured using portable photosynthetic system (CIRAS-3, USA). At harvest, growth and yield parameters were recorded following Mehboob *et al.* (2018) and Cd concentration was determined in root, shoot and grains of maize plants.

Determination of Organic Matter

Walkley-Black method was used to determine the organic matter (FAO, 1974; Walkley and Black, 1934). In this method, 1 g of soil was taken in 500 mL beaker. Then, 10 mL of 1N potassium dichromate solution and 20 mL concentrated H₂SO₄ were added to the beaker and the beaker was swirled to mix the suspension. Then, this suspension was allowed to stand for 30 min. After that 200 mL of distilled water and 10 mL of concentrated H₃PO₄ were added and this mixture was allowed to cool. In this suspension, 15 drops of indicator diphenylamine were added and titrated with ferrous ammonium sulphate solution, then titration was stopped when the color changed from violet to bluish green and two blanks alongside were also prepared. Oxidizable organic carbon (%) was calculated by subtracting volume of sample from blank volume and multiplied it with 0.3 and molarity of ferrous ammonium sulphate solution and by dividing with weight of soil. Total organic carbon (%) was calculated by multiplying oxidizable organic carbon with 1.334. Formula used for calculation of organic matter was as:

$$\text{Organic matter (\%)} = 1.724 \times \text{total organic carbon (\%)}$$

Determination of Cd Content in Plants

Cadmium content in plant samples was determined by digestion of plant samples (root, shoot and grain). The plant samples (1 g each) were taken in 25 mL conical flask and kept overnight. After adding 5 mL HNO₃, it was digested in 10 mL of HNO₃-HClO₄ (3:1, v-v) on hot plate (Ryan *et al.*, 2001). Concentration of Cd in plant digest was determined using Atomic absorption spectrophotometer (Thermo Electron S series). Formula used for bioavailable Cd determination was as below.

$$\text{Cadmium (mg kg}^{-1}\text{)} = \frac{\text{Cd in extract (mg L}^{-1}\text{)} - \text{Cd in blank}}{\text{Weight of soil (g)}}$$

Bioconcentration Factor of Roots and Shoots

Bioconcentration factor (BCF) was calculated as the ratio of metal concentration in plant (roots and shoots) to that in soil using the following formulae:

$$\text{BCF of root} = \frac{\text{Metal concentration in root (mg kg}^{-1}\text{)}}{\text{Metal concentration in soil (mg kg}^{-1}\text{)}}$$

$$\text{BCF of shoot} = \frac{\text{Metal concentration in shoot (mg kg}^{-1}\text{)}}{\text{Metal concentration in soil (mg kg}^{-1}\text{)}}$$

Translocation Factor (TF)

Translocation factor indicates the transfer of metal from shoot to root and also indicator of whether the plant is hyperaccumulator or excluder under the given conditions. TF was calculated as ratio of Cd concentration in shoot to that in root as:

$$\text{TF} = \frac{\text{Cd concentration in shoot (mg kg}^{-1}\text{)}}{\text{Cd concentration in root (mg kg}^{-1}\text{)}}$$

The Root/shoot Ratio

The root/shoot ratio is a very important indicator of metal stress that is confronted by plants (Chiu *et al.*, 2005). It was calculated as follows:

$$\text{Root/Shoot ratio} = \frac{\text{Root dry weight}}{\text{Shoot dry weight}}$$

Relative Production Index

The relative production index (RPI), related to the influence of the metal on the variation of dry matter production, is obtained by following equation (Paiva *et al.*, 2005):

$$\text{RPI} = \frac{\text{Dry matter produced under Cd stress condition}}{\text{Dry matter produced with Cd absent}} \times 100$$

Statistical Analysis

Impact of treatments on all growth, physiological and yield parameters was studied by Statistic 8.1. Linear models were applied and ANOVA (analysis of variance) was used to compare the treatments. Honestly significant difference (HSD) test was applied to observe the difference in treatments. Graphs were prepared by using the software MS Excel against respective parameter means along with \pm SE (standard error).

Results

Cadmium stress negatively affected the growth, yield, and chlorophyll content and physiological parameters of maize plants under Cd spiked soil (Table 2 and 3). Higher concentration of Cd (30 mg kg^{-1} soil) resulted in more reduction in all parameters of maize plants as compared to its lower concentration (10 mg kg^{-1} soil). However, the application of rock phosphate and press mud compost significantly improved all the attributes at all Cd stress levels. Moreover, the combination of rock phosphate along with press mud compost performed better than their sole application. Application of higher dose of rock phosphate (2%) and press mud gave better results than the lower dose (1%).

Data (Table 2) showed that Cd stress decreased the plant height, root length and, dry mass of shoot and root; and the effect was more at higher Cd concentration (30 mg kg^{-1} soil) than the lower levels of Cd stress. Application of 1 and 2% rock phosphate and press mud compost alone and in combination improved the plant growth at all Cd stress levels. However, combined application of rock phosphate and press mud compost (2% both) resulted in more improvement of maize growth as compared to the rest of the treatments under Cd spiked soil. Combination of RP (2%) and CP (2%) under higher level of Cd stress promoted the plant height (1.21 times), root length (3.2 times), shoot dry mass (1.57 times) and root dry mass (5.3 times) at 30 mg kg^{-1} Cd stress level as compared to respective untreated stressed control plant.

The results revealed that Cd stress significantly decreased the chlorophyll content, photosynthetic rate, transpiration rate, stomatal conductance, 100 grain weight and grain yield of maize plants when compared with respective untreated stressed control plants (Table 3). Application of different treatments of RP and CP significantly improved all the above attributes under Cd stressed conditions compared to untreated control. The application of 2% RP along with 2% CP improved the chlorophyll content (1.44 times), photosynthetic rate (3.06 times), transpiration rate (1.84 times), stomatal conductance (1.76 times), 100 grain weight (1.47 times) and grain yield (48%) of maize as compared to untreated stressed control plant at stress level of 30 mg kg^{-1} of soil.

Fig. 2 shows the relative production index (RPI) of treated and untreated plants under different Cd stress levels.

Table 1: Physical and Chemical properties of soil

Parameters	Unit	Value
Sand	%	48.97
Silt	%	27.53
Clay	%	23.5
Textural class	-----	Sandy clay loam
Saturation percentage	%	37
ECe	dS m ⁻¹	4.07
pH	-----	7.78
Organic matter	%	0.71
Total nitrogen	%	0.06
Available phosphorus	mg kg ⁻¹	3.50
Extractable potassium	mg kg ⁻¹	69
CEC	cmol. kg ⁻¹	6.8
Cadmium	mg kg ⁻¹	ND*

ND*: Not Detectable; ECe: Electrical Conductivity; CEC: Cation Exchange Capacity

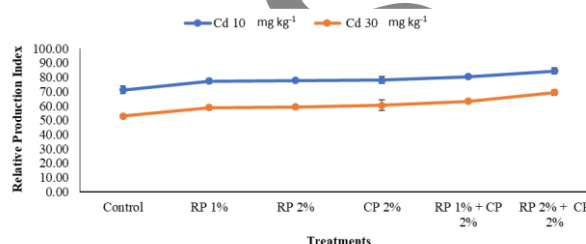


Fig. 1: Effect of rock phosphate and press mud compost on relative production index of maize in Cd (0, 10, 30 mg kg⁻¹) contaminated soil

Bars presented S.E and columns displayed the mean. All the means displayed by changed letters significantly differ with respect to HSD test at probability level of 5%. Cd: Cadmium; RP: Rock Phosphate; CP: Press Mud Compost

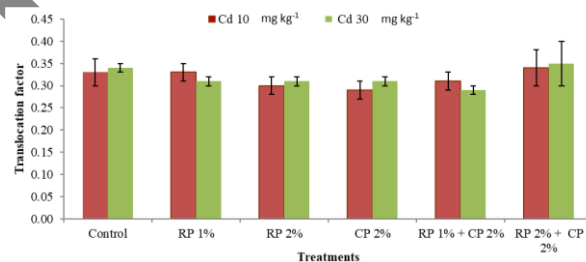


Fig. 2: Effect of rock phosphate and press mud compost on translocation factor of maize in Cd (0, 10, 30 mg kg⁻¹) contaminated soil

Bars presented S.E and columns displayed the mean. All the means displayed by changed letters significantly differ with respect to HSD test at probability level of 5%. Cd: Cadmium; RP: Rock Phosphate; CP: Press Mud Compost

Application of different treatments of rock phosphate and press mud compost showed higher RPI when compared with respective untreated stressed control. Higher relative production indices were observed in plants under 10 mg kg^{-1} Cd spiked soil compared to their respective treatments under 30 mg kg^{-1} Cd spiked soil. Combined application of RP (2%) and CP (2%) to 30 mg kg^{-1} Cd spiked soil resulted in maximum increase in RPI (31%) as compared to respective untreated stressed control.

Increasing Cd stress level significantly resulted in decreased root/shoot ratio of maize plants (Table 4). However, application of different treatments of rock phosphate and press mud compost, significantly,

Table 2: Effect of inorganic and organic soil amendments on plant height, root length, and dry masses of maize grown in cadmium contaminated soil

Treatments	Plant height (cm)			Root length (cm)		
	0 mg Cd kg ⁻¹	10 mg Cd kg ⁻¹	30 mg Cd kg ⁻¹	0 mg Cd kg ⁻¹	10 mg Cd kg ⁻¹	30 mg Cd kg ⁻¹
Control	89.2 hi	68.0 j	49.2 k	31.0 ij	22.7 l	11.3 m
RP 1%	98.3 fg	92.3 gh	69.3 j	36.3 gh	28.1 jk	23.5 kl
RP 2%	113.7 e	99.8 f	83.1 i	44.9 ef	37.1 gh	28.5 j
CP 2%	121.8 d	112.3 e	84.8 i	50.8 d	42.2 f	35.8 hi
RP 1% + CP 2%	133.3 b	124.3 cd	95.6 f-h	61.7 b	48.6 de	40.9 fg
RP 2% + CP 2%	142.3 a	129.3 bc	108.8 e	70.0 a	56.7 c	48.1 de
HSD	7.3464			4.9906		
	Shoot Dry Mass (g)			Root Dry Mass (g)		
Control	15.8 e-h	11.8 ij	9.1 j	3.8 f-h	2.2 gh	1.3 h
RP 1%	18.2 de	14 g-i	11.2 ij	4.5 ef	3.6 f-h	2.1 f-h
RP 2%	21 cd	16.8 e-g	12.8 hi	5.9 de	4.2 ef	3.2 fg
CP 2%	22.7 c	17.6 d-f	14.5 f-i	7.7 bc	6.2 de	3.9 ef
RP 1% + CP 2%	28.3 b	22.6 c	17.9 d-f	8.9 ab	7.3 b-d	5.5 c-e
RP 2% + CP 2%	35.4 a	29.6 b	23.4 c	10.2 a	8.7 ab	8.1 ab
HSD	3.447			1.7948		

†Means sharing similar letters are statistically similar to each other at p=0.05. HSD shows honestly significant difference. Cd: Cadmium; RP: Rock Phosphate; CP: Press Mud Compost

Table 3: Effect of rock phosphate and press mud compost on chlorophyll content, photosynthetic rate, transpiration rate, 100 grain weight and grain yield of maize in Cd (0, 10, 30 mg kg⁻¹) contaminated soil

Treatments	Chlorophyll Content (SPAD)			Photosynthetic rate (μmol m ⁻² s ⁻¹)			Transpiration rate (μmol m ⁻² s ⁻¹)		
	Cd 0 mg kg ⁻¹	Cd 10 mg kg ⁻¹	Cd 30 mg kg ⁻¹	Cd 0 mg kg ⁻¹	Cd 10 mg kg ⁻¹	Cd 30 mg kg ⁻¹	Cd 0 mg kg ⁻¹	Cd 10 mg kg ⁻¹	Cd 30 mg kg ⁻¹
Control	29.33 e	19.67 gh	18.33 h	10.33 g	8.33 h	5.33 i	2.70 hi	2.20 i	1.93 i
RP 1%	34.00 d	23.17 fg	21.67 f-h	15.33 f	10.33 g	8.33 h	3.67 fg	3.50 gh	2.00 i
RP 2%	37.33 cd	27.50 e	25.33 ef	20.33 d	15.33 f	11.33 g	4.80 de	4.47 ef	2.47 i
CP 2%	41.67 b	34.17 d	28.67 e	25.33 c	20.33 d	14.33 f	5.67 c	5.37 cd	3.70 fg
RP 1% + CP 2%	48.17 a	41.00 bc	35.00 d	30.33 b	25.33 c	17.33 e	6.93 b	6.10 c	4.47 ef
RP 2% + CP 2%	51.33 a	48.17 a	39.33 bc	35.33 a	30.33 b	20.33 d	8.10 a	7.00 b	5.50 cd
HSD value at 1%	4.2137			1.5841			0.803		
	Stomatal Conductance (mmol m ⁻² s ⁻¹)			100-grain weight (g)			Grain yield (g plant ⁻¹)		
Control	140.6 j	120.6 k	70.6 m	12.83 hi	10.67 ij	8.83 j	98.0 fg	93.3 gh	67.6 k
RP 1%	160.6 h	140.6 j	96.0 l	14.17 g-i	12.67 h-j	10.83 ij	102.3 d-f	97.6 fg	74.3 jk
RP 2%	180.6 f	160.6 h	120.6 k	17.17 e-g	15.83 f-h	12.83 hi	106.6 c-e	100.3 e-g	80.3 ij
CP 2%	200.0 d	180.6 f	145.6 i	20.17 c-e	18.17 d-f	15.8 f-h	110.3 bc	104.6 c-f	87.6 hi
RP 1% + CP 2%	230.6 b	200.6 d	170.6 g	23.17 a-c	21.83 b-d	18.8 d-f	115.0 ab	108.6 b-d	94.0 gh
RP 2% + CP 2%	250.6 a	220.6 c	195.6 e	27.00 a	24.67 ab	21.8 b-d	120.3 a	114.3 ab	100 e-g
HSD value at 1%	0.924			3.8722			7.5677		

†Means sharing similar letters are statistically similar to each other at p=0.05. HSD shows honestly significant difference. Cd: Cadmium; RP: Rock Phosphate; CP: Press Mud Compost

Table 4: Effect of rock phosphate and press mud compost on bioconcentration factors and root/shoot ratio of maize in Cd (0, 10, 30 mg kg⁻¹) contaminated soil

Treatments	Bioconcentration factor of shoots			Bioconcentration factor of roots			Root/shoot ratio		
	Cd 0 mg kg ⁻¹	Cd 10 mg kg ⁻¹	Cd 30 mg kg ⁻¹	Cd 0 mg kg ⁻¹	Cd 10 mg kg ⁻¹	Cd 30 mg kg ⁻¹	Cd 0 mg kg ⁻¹	Cd 10 mg kg ⁻¹	Cd 30 mg kg ⁻¹
Control	-	0.3 a	0.28 ab	-	0.94 a	0.84 a-c	0.24 b-f	0.18 ef	0.14 f
RP 1%	-	0.28 ab	0.26 a-c	-	0.87 ab	0.83 a-c	0.25 a-f	0.26 c-f	0.19 d-f
RP 2%	-	0.25 b-d	0.24 b-d	-	0.82 a-c	0.79 a-c	0.28 a-e	0.25 a-e	0.26 b-f
CP 2%	-	0.21 c-e	0.23 b-d	-	0.72 b-d	0.75 b-d	0.34 ab	0.35 a	0.27 a-d
RP 1% + CP 2%	-	0.18 e	0.19 de	-	0.57 de	0.67 c-e	0.31 a-d	0.32 a-d	0.31 a-d
RP 2% + CP 2%	-	0.17 e	0.19 de	-	0.51 e	0.56 de	0.29 a-e	0.29 a-d	0.35 a-c
HSD value at 1%	0.055			0.18			0.11		

†Means sharing similar letters are statistically similar to each other at p=0.05. HSD shows honestly significant difference. Cd: Cadmium; RP: Rock Phosphate; CP: Press Mud Compost

improved root/shoot ratio of plants under Cd stressed soil. Combined application of RP (2%) and CP (2%) to 30 mg kg⁻¹ Cd spiked soil resulted in maximum increase (147%) in root/shoot ratio when compared with respective untreated stress control followed by combined application of RP (1%) and CP (2%) that showed 121% increase in root/shoot ratio compared to respective untreated stress control.

Concentration of Cd in the roots, shoots and grains of maize plants was observed by applying rock phosphate and press mud compost in different combinations to reduce its

uptake by the plant and to immobilize in soil. Data with respect to Cd concentration in roots, shoots and grains are presented in Fig. 3-5. In soil without contamination, no Cd was detected in roots, shoots and grains of maize. However, Cd concentration in plant parts increased by increasing its content in soil. In 10 mg kg⁻¹ Cd spiked soil, minimum Cd concentration (non-detectable in grain, 83 and 82% less than the untreated control) was recorded in roots and shoots, respectively, compared to respective untreated stressed control followed by combination of RP (1%) and

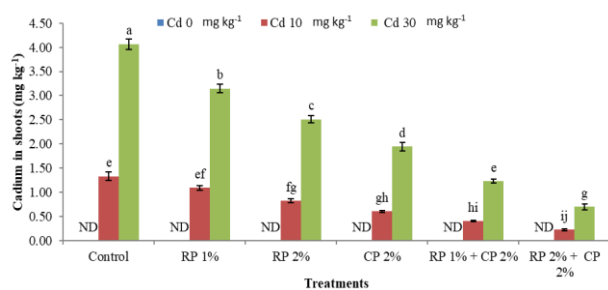


Fig. 3: Effect of rock phosphate and press mud compost on Cd concentration in shoots of maize in Cd (0, 10, 30 mg kg⁻¹) contaminated soil

Bars presented S.E and columns displayed the mean. All the means displayed by changed letters significantly differ with respect to HSD test at probability level of 5%. Cd: Cadmium; RP: Rock Phosphate; CP: Press Mud Compost

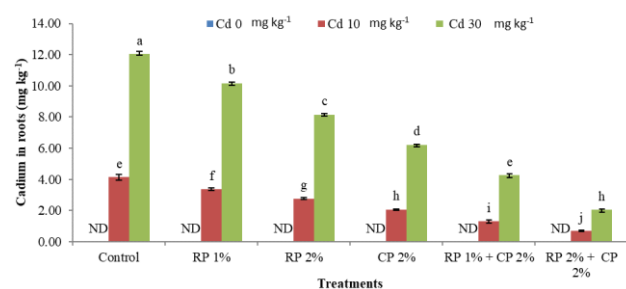


Fig. 4: Effect of rock phosphate and press mud compost on Cd concentration in roots of maize in Cd (0, 10, 30 mg kg⁻¹) contaminated soil

Bars presented S.E and columns displayed the mean. All the means displayed by changed letters significantly differ with respect to HSD test at probability level of 5%. Cd: Cadmium; RP: Rock Phosphate; CP: Press Mud Compost

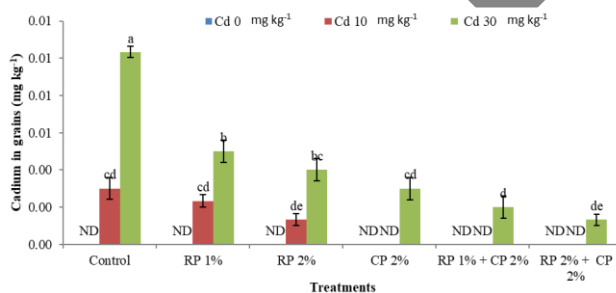


Fig. 5: Effect of rock phosphate and press mud compost on Cd concentration in grains of maize in Cd (0, 10, 30 mg kg⁻¹) contaminated soil

Bars presented S.E and columns displayed the mean. All the means displayed by changed letters significantly differ with respect to HSD test at probability level of 5%

CP (2%) that resulted in 64% and 69% decrease in the Cd concentration in roots and shoots compared to respective untreated unstressed control while Cd in grains of maize was non-detectible.

Similarly, combined application of **RP** (2%) and **CP** (2%) caused maximum reduction (83, 83 and 87%) in the uptake of Cd in roots, shoots and grains of maize, respectively, compared to respective untreated control under 30 mg kg⁻¹ Cd spiked soil followed by combined application of **RP** (1%)

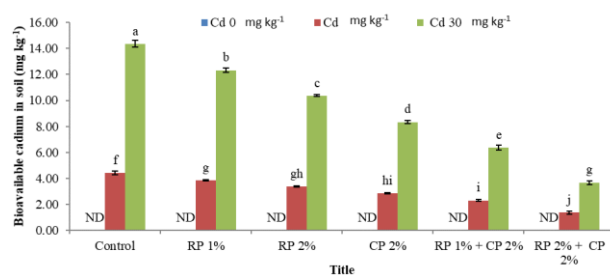


Fig. 6: Effect of rock phosphate and press mud compost on bioavailable fraction of Cd in soil after maize harvest in Cd (0, 10, 30 mg kg⁻¹) contaminated soil

Bars presented S.E and columns displayed the mean. All the means displayed by changed letters significantly differ with respect to HSD test at probability level of 5%. Cd: Cadmium; RP: Rock Phosphate; CP: Press Mud Compost

and **CP** (2%) that resulted in 64.5, 69.6 and 80.6% decrease of Cd concentration in roots, shoots, and grains of maize, respectively, compared to respective untreated stressed control.

Data regarding bioconcentration factor (BCF) are summarized in Table 4. Highest bioconcentration factor of shoot and root was observed in control plant under 10 as well as 30 mg kg⁻¹ Cd spiked soil. Application of different treatments of rock phosphate and press mud compost significantly decreased the bioconcentration factor of both shoot and root at all Cd stress levels. Maximum reduction in BCF of shoot and root was observed in plants treated with combined application of **RP** (2%) and **CP** (2%) under 10 mg kg⁻¹ Cd spiked soil that was 33% and 46%, respectively, as compared to respective untreated stressed control plants. Translocation factor showing the transfer of Cd from root to shoot of maize plants was depicted in Fig. 1. All the treatments remained statistically at par in transferring the Cd from root to shoot.

Data (Fig. 6) regarding bioavailable fraction of Cd in soil after harvest showed that maximum reduction (74 and 69%) in bioavailable Cd concentration was recorded in pots treated with combination of **RP** (2%) and **CP** (2%) followed by combination of **RP** (1%) and **CP** (2%) that caused 56 and 48% reduction in bioavailable Cd concentration under 30 and 10 mg kg⁻¹ Cd spiked soil compared to respective untreated pots, respectively.

Discussion

Under normal conditions, uptake of Cd at higher concentrations caused severe damage to growth and yield of the maize plants. However, application of rock phosphate and press mud compost successfully immobilized the soil Cd that significantly improved the all physiological attributes of the maize plants resulting in improved growth and yield of maize. It is an established fact that Cd is a toxic metal having no physiological function in plants. Retarded growth is a very common symptom that can be seen in plants grown under Cd stressed soil. It might be because of

involvement of toxic Cd in plant metabolism, or inhibition of other essential nutrient due to high concentration of antagonistic toxic metal. It is quite possible that stunted growth of Cd exposed plant can be due to deficiency of essential elements such as phosphorus, which has been observed to make insoluble complexes with Cd (Gonçalves *et al.*, 2009). In reference to the results of present study, improved growth of maize plants treated with rock phosphate and press mud compost can be attributed to less uptake of Cd due to the formation of insoluble phosphorus-Cd complexes that resulted in increased immobilization of Cd in the soil. It might also be due to the enhanced uptake of mineral phosphorus that resulted in improved growth and ultimately the yield of maize. Similar results were reported by Zhao *et al.* (2014) who narrated that the application of rock phosphate improved the immobilization of Cd in the soil and reduced its transport to the shoot of plant. Thawornchaisit and Polprasertb (Thawornchaisit and Polprasert, 2009) also observed that rock phosphate reduced the Cd leaching and mobile Cd forms by immobilizing the Cd in contaminated soil. Many inorganic amendments found effective to reduce accumulation and uptake of Cd in plants (Rehman *et al.*, 2015; Arshad *et al.*, 2016). Use of P containing compounds decreased uptake of Cd and transport in agriculture crops (Rizwan *et al.*, 2016). Applying phosphorus reduced the carbonic and exchangeable Cd fraction compared to treatment where both were absent. However application of phosphorus enhanced residual and organic Cd fraction in soil (Dalir *et al.*, 2013).

In this study, parameters related to growth and yield of maize reduced in Cd contaminated soil as compared to control (non-spiked soil). However, an increase in growth and yield was found by combined application of rock phosphate and press mud compost in Cd contaminated soil. These findings were supported by Salt *et al.* (2000) who reported that root exudates act as chelates for metals and hamper the uptake of metal into the plant cells and increased the plant growth.

Cadmium toxicity reduced the photosynthetic rate, stomatal conductance as well as chlorophyll content of plants. The chlorophyll content is commonly used to evaluate the effect of environmental stresses in plants. The reason is that alteration in pigment contents are directly related to visual symptoms of plant illness and photosynthetic productivity (Anjum *et al.*, 2016). Heavy metals commonly retard metabolic processes by hindering the reactions of enzymes. Reduced chlorophyll content linked with heavy metal stress can be the consequence of the denaturation of enzymes responsible for chlorophyll biosynthesis (Vijendra *et al.*, 2016). This reduction in the chlorophyll content can slow down the stomatal conductance and decrease the photosynthetic rate. In our study, chlorophyll content of maize leaves decreased gradually with increasing Cd contamination levels. This decrease in chlorophyll content might have caused reduction in stomatal conductance and photosynthetic rate. However,

combined application of rock phosphate (2%) and press mud compost (2%) was found effective to improve photosynthetic rate, stomatal conductance and chlorophyll content in Cd stressed soil. These findings were supported by many reports that explain that Cd toxicity, transport and uptake by plant can be reduced by the application of various inorganic and organic amendments such as manures, biochar, compost and silicon and as a result photosynthetic rate, stomatal conductance and chlorophyll content enhanced (Adrees *et al.*, 2015; Rehman *et al.*, 2015).

Relative production of dry matter decreased with increasing level of Cd stress which is similar to results observed by Marcele *et al.* (2013) who reported the decreased relative production of dry matter in response to increased level of Cd contamination. Increased RPI due to the application of rock phosphate and press mud compost is attributed to increased stress tolerance and decreased uptake of Cd by the maize plants. Root/shoot ratio is used as indicator of environmental stress. Increased root/shoot ratio in response to application of rock phosphate and press mud compost can be linked to increase tolerance of plants to Cd stress (Chiu *et al.*, 2005).

In the present study, parallel increase in the Cd concentration to the increase in applied Cd in the soil was observed irrespective of the plant parts of the maize. These findings are supported by the Ling *et al.* (2017) who reported the linear enhancement in the uptake of Cd by different plant parts. They also observed the higher uptake of Cd by the maize roots as compared to the shoots. Similar results regarding the increasing concentration of Cd were observed in the cotton plants (Cheema *et al.*, 2009). However, they also reported much higher accumulation of Cd in roots compared to the shoots. In our study, much less Cd concentration in grain compared to vegetative part of maize was observed that suggests less translocation of Cd from vegetative parts to the grains of maize. Another possible reason might be the dilution effect of cadmium concentration in grain, which was achieved by increased grain yield of maize where combination of rock phosphate (2%) and press mud compost (2%) was applied under 10 and 30 mg Cd kg⁻¹ of soil, respectively.

As organic matter in soil can decrease the Cd uptake by plant through the formation of organic complexes (Kim *et al.*, 2015), therefore, press mud compost might have decreased the accumulation of heavy metals to above ground parts or edible parts of plant by organic-metal complexes due to the increased organic matter in soil. According to Bolan *et al.* (2003) precipitation is the main mechanism for the immobilization of Cd as metal-phosphate by phosphate containing amendments. This mechanism supports the present study where rock phosphate was used as source of phosphate and to immobilize Cd in soil.

Bioconcentration factors decreased by increasing the level of rock phosphate or press mud compost or both. Similar results were observed by many researchers (Rezvani and Zaefarian, 2011; Arifin *et al.*, 2012). The decreased

translocation of Cd from soil to plant parts might be due to restriction in soil-root transfer at higher Cd concentration and due to increase immobilization of metal interfering with rock phosphate and organic matter. Dilution factor due to added organic matter may also be one of the reasons (Chiu et al., 2005; Lehto et al., 2016).

Translocation factor was observed slightly higher under increased level of Cd. However, all the treatments showed the TFs values less than one. Baker (Baker, 1981) reported that plants are classified as metal excluder if translocation factor (shoot to root ratio) is less than one. Data suggested that Cd poorly translocate from roots to shoots. The translocation of Cd is often reduced due to the characteristics of this metal to produce Cd-phytochelatin complex by confiscation in the vacuole (Lux et al., 2010). Cadmium translocation from roots to shoots possibly happens within the xylem. The concentration of free cadmium in the symplast can be affected highly by cellular confiscation of Cd and consequently, it can influence the movement of cadmium all over the plants (Zhi-xin et al., 2007). Accordingly, these data suggest that Cd translocated very poorly throughout the plant, given the important difference of contaminant content among its three compartments. The Cd content in root, stem and leaves were superior to the range 5-30 mg kg⁻¹, which is potentially toxic to plants (Pendias and Kabata-Pendias, 2010).

It can be concluded that combined application of rock phosphate and press mud compost improved growth of maize and reduced the bioavailable Cd in the soil. So, maize along with combined application of rock phosphate and press mud compost can be successfully grown in Cd contaminated soil.

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