THE EFFECT OF SINGLE AND COMPOSITE ADDITIVES ON CD AND PB MOBILITY, SPECIATION AND ACCUMULATION IN LATE RICE (ORYZA SATIVA L.) GROWN ON CONTAMINATED SOIL

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Abstract. This field trial investigated the cadmium (Cd) and lead (Pb) immobilization in contaminated paddy soil. Five stabilizing agents including lime, biochar, phosphatic fertilizer (PF) and two composites (S1, S2) were investigated (1% w/w) for metals immobilization by growing late rice as a test crop. The results revealed the improved pH and decreased metal availability in lime, S1, biochar and S2 treated plots. The Cd and Pb speciation results depicted a decrease in exchangeable Cd content to less mobile forms (29 and 30%) with addition of biochar and S1 in harvest stage samples. The exchangeable fraction of Pb was significantly reduced to 29, 29 and 36% with S1, S2 and biochar, respectively. Moreover, Cd and Pb accumulation in rice grains was lowest in composite stabilizers and biochar treatments. Our results showed a significant increase in leaf photosynthetic rate, transpiration rate, biomass and grains yield in S1 amended plot. These lessening effects are owed to the conversion of available metal contents to the least mobile forms. Our results confirmed the effectiveness of organic additives alone and in mixture with inorganics in reducing metal mobility, improving immobilization, enhancing growth and decreasing accumulation in food parts and showed a great prospect in safer food production.

Keywords: metals immobilization, lime, biochar, phosphatic fertilizer, rice uptake

Introduction

Trace metals especially cadmium (Cd) and lead (Pb) have a strong toxic effect on animals and humans which exerts health concerns worldwide (Chen et al., 2018). Cd and Pb contamination are often reported in agricultural soils due to natural and anthropogenic activities but their entry into the soil environment is mostly associated with agricultural inputs and mining or smelting effluents (Hamid et al., 2018). Cd and Pb are among the most toxic metals due to their high accumulation in edible parts of plants hence posing a high risk of contamination to the food chain (Liang et al., 2016; Igalavithana et al., 2019). Therefore, it is of utmost importance to remediate metals polluted soils without influencing the crop yield (Xiong et al., 2019). Moreover, it is important to unveil the metals availability and related processes for the development of innovative techniques to improve metals stability in soil and reduced accumulation in plants.

In recent years, the sorption of metals with low cost and environment-friendly stabilizers has been emerged as an innovative approach to remove contaminants from polluted sites. Various inorganic or organic stabilizers have been reported for their effectiveness in immobilizing the metals in different soil types (Hamid et al., 2019b). **Stabilizers** reduce metals availability owing to adsorption/stabilization/precipitation/ion exchange attributes. However, many adsorbents show disadvantages due to their less stability, adsorption capacity and success in multi metals contaminated soils (Hu et al., 2017). Moreover, in some cases single additive show effectiveness for one metal and did not present significant difference for other elements. So, composite addition of additives may be more efficient in reducing metal contamination in co-contaminated soil (Lahori et al., 2017). Our earlier reports (Hamid et al., 2019a) also confirmed the effectiveness of composite amendments on metals stability in co-contaminated soils.

Immobilization is a process of remediating the contaminants by transforming their high mobile fraction to less available form. Lime is regarded as one of the most effective immobilizer for metallic compounds (Bade et al., 2012). Liming the polluted region points to reduced metal availability by increasing soil pH and the production of hydroxyl ions due to hydrolysis of CaCO₃ (Wu et al., 2016). Liming induces metals availability by making carbonate-metal precipitates which reduces the exchangeable fraction of metals (Singh and Kalamdhad, 2013). In a field experiment, Rehman et al. (2017a) reported a decrease in available Cd contents with a combined addition of lime and biochar. Recently, in-situ stabilization of metal contaminated soils with phosphatic fertilizer has gained keen attention as an alternative remediation technique. Phosphate induced stabilization comprises of metals precipitation with inorganic phosphate or metals adsorption (Hong et al., 2008, 2010). Phosphatic fertilizers addition can alleviate soil characteristics such as pH, available phosphate contents, soil surface charge or directly interacts with metal ions in the soil. These changes can induce a swing in the metal available form to a more stable form (Zhao et al., 2014; Yan et al., 2015).

Biochar is an organic material pyrolyzed at different temperatures under low oxygenic conditions (Leng et al., 2019) by combustion of wood, plant residues etc. Biochar is a stable compound mainly used in the restoration of metal contaminated sites. Biochar has ability to retain metals owing to complexation, adsorption or ion exchange mechanisms (El-Naggar et al., 2018). Biochar addition improved the plant growth by increasing nutrients availability and reducing leaching in soil (Cornelissen et al., 2018; Li et al., 2019). Meanwhile, metals adsorption on the surface of biochar depends upon raw material, metal concentration and pyrolysis temperature. An improved stability of biochar with high combustion temperature is reported in literature (Song et al., 2012; Bashir et al., 2018).

To date, different inorganic and organic additives have been adopted in stabilization of metal but most of the studies focused on single contaminant and rarely on multimetal contamination. Therefore, there is a shortage of studies focusing on stabilization of multi -metal contamination with composite treatments and the comparison of effectiveness with their commercial competitors. Moreover, this study was established on improved Cd and Pb immobilization with the incorporation of single and composite additives by increasing soil pH and CEC. The objective of our study was (a) to check the efficacy of inorganic and organic amendments alone and their novel mixtures on improved rice biomass and grains yield, (b) to examine the availability of Cd and Pb and subsequent uptake or accumulation by late rice, (c) to gain the insights of immobilization mechanisms by speciation method, (d) to identify the most favorable soil amendment for safer food production on Cd-Pb co-contaminated site.

Materials and methods

Study site, additives collection and application

This field experiment was conducted in a contaminated red paddy field situated in Qujiang County (E 119.01, N 29.05), Zhejiang province, China having annually mean temperature (17 °C) and precipitation (1,723 mm). Cd and Pb contents of studied soil ranged to 0.54 mg kg⁻¹ and 95.42 mg kg⁻¹ respectively (Cao et al., 2019). Late rice cultivar (Yong You 9) was cultured in mid-July (15-07-2017) and three-week (07-08-2017) old seedlings were shifted to the field. This late rice cultivar is been widely used in this specific area due to its high grains production and improved biomass. Lime, biochar, PF (phosphatic fertilizer), organic manure, wood powder, sepiolite, and zeolite were collected from different commercial sources. Biochar used in this experiment was pyrolyzed from coconut shell at 350 °C. Six treatments including control, lime, biochar, PF, S1 and S2 were investigated for their effectiveness as immobilizers. S1 and S2 were cycled by mixing various organic and inorganic additives with clay minerals at different ratios. S1 was derived by mixing biochar with lime, sepiolite and zeolite while S2 was composited by manual mixing of manure with lime and sepiolite. Amendments were applied in triplicates and were arrayed according to RCBD (randomized complete block design) with each plot having area 64 m². Each treatment block was separated with ridges and includes separate inlet and outlet for watering and drainage. All the treatments were applied at 1% (w/w) before 20 days (18-07-2017) of seedling transplantation and were mixed mechanically on the upper layer of soil. Fertilization was completed by adding N (130 kg ha⁻¹), P_2O_5 (50 kg ha⁻¹) and K_2O (150 kg ha⁻¹) in shape of urea, di-ammonium phosphate and muriate of potash before start of experiment. Additional phosphatic fertilizer was not added in the treatment of PF due to its use as a stabilizing agent. Photosynthetic rate (Pn) and transpiration rate (Tr) were measured using an IRGA (infrared gas analyzer) after 70 days of crop growth.

Soil and plant sampling

Soil samples were collected from experimental site for pH, organic carbon, and total metal concentration in soil (*Table 1*). Furthermore, for available metal contents soil sampling was done after the addition of soil additives. Samples were taken 1 day before nursery transplantation (zero month) in the field and at harvest (2017-11-10).

Meanwhile, plants were harvested at maturity and plant height, biomass and grains yield were measured. From each plot, plants were harvested (1 m^2) and weighed for biological yield. Harvested fifteen plants were sampled and brought to the lab for metal analysis. Plants were cut into pieces (roots, shoots and grains), washed with tap water, and then rinsed with ultra-pure water. Plants were oven dried until constant weight, grinded and analyzed for metal accumulation in different parts.

No.	Treatment/soil	рН	Organic carbon (g kg ⁻¹)	Total Pb (mg kg ⁻¹)	Total Cd (mg kg ⁻¹)
1	Soil	5.71	54.76	95.42	0.54
2	Lime	11.12	nd	0.624	0.20
3	Biochar	8.06	387	1.656	0.05
4	PF	7.21	nd	2.57	0.35
5	S 1	11.12	74.68	4.800	0.20
6	S2	11.34	32.11	3.895	0.15

 Table 1. Physico-chemical properties of soil and applied additives

Soil with silty clay texture (Sand 15%, Silt 32.10%, Clay 52.90%) PF: Phosphatic fertilizer, S1 and S2 (stabilizers)

Amendments, soil and plant analysis

Basic physicochemical properties of soil and amendments were measured with standard methods proposed by (Li, 2000; Bao, 2008). Amendments and soil pH were measured by shaking material and water at ratio 1: 2.5 using a PB-10, Sartorius, Germany pH meter (*Table 1*). Total metal concentration in additives and soil was measured by digesting the material with a solution of HNO₃–HF–HClO₄ for 12 h. DTPA extraction was performed to analyze available Cd and Pb concentration in treated soil. Soil (20 g) was shaken with a DTPA-TEA mixture (50 mL) for 2 h at room temperature. Suspension was then filtered from 0.45-µm filter and bio-available Cd and Pb concentration in solution was calculated using ICP-MS (Agilent, 7500a, USA) (Li, 2000; Bao, 2008).

Cd and Pb fractionation in treated and untreated soil at harvest were estimated by the sequential extraction method (*Table 2*) proposed by (Tessier et al., 1979).

Dried and grounded plant samples were digested for accumulated Cd and Pb concentration in different parts. Cd and Pb concentration were measured by digesting 0.20 g of desired plant sample with an acidic solution of $HClO_4$ and HNO_3 at 170 °C for 4 h. Metals concentration in the digested solution was measured with ICP-MS (Bao, 2008).

Fraction	Procedure		
F1: Exchangeable	1 g soil (treated/untreated) shaken with 8 ml of 1 M MgCl ₂ solution $(pH = 7)$		
F2: Carbonate bound	F1 residues extracted with NaOAc (1 M), pH: 5 for 5 h		
F3: Fe-Mn oxide bound	Residues of F2 were mined with 20 mL (0.04 M) NH ₂ OH•HCl in 25% CH ₃ COOH for 6 h at 96 °C		
F4: Organic matter bound	Firstly, F3 residues shaken with 5 mL of H ₂ O ₂ (30%) for 3 h (at 85 °C) and then with 3.2 M ammonium acetate for half an hour and mixture was collected and mixed to make one solution		
F5: Residual	Remaining residues were digested with HNO ₃ HF-HClO ₄ and diluted		

Table 2. The sequential extraction procedure for Cd and Pb

Cadmium and Pb translocation factor (TF) from roots to shoot was calculated with the following formula (Eq. 1):

TF(%) = Cd, Pb concentration in shoots /Cd, Pb concentration in roots * 100 (Eq.1)

Statistical analyses

All the used chemicals and reagents in this experiment were of analytic grade. The digestion tubes were washed with distilled water, put overnight in acidic solution and again washed with distilled water followed by rinsing with ultra-pure water before each use. Data presented are means of three replicates \pm S.E which was estimated with MS Excel 2007. Statistical analysis (LSD test) was performed with SPSS 20.0 and graphical illustration was done with Origin Pro 8.0.

Results

Effect of applied passivators on soil pH and DTPA extractable Cd and Pb at different time intervals

The effect of applied additives on soil pH at zero month and harvest is presented in Figure 1. Zero month samples were taken around 20 days of amendments. There was a quick elevation in soil pH with lime and S2 treatment at zero month. Lime and S2 treatment showed an increase of 1.68 and 0.93 units in soil pH as compared to control. Meanwhile, a significant increase in soil pH with lime, S2, biochar and S1 was also observed at harvest samples. These treatments improved soil pH to a significant level with values of 7.56, 6.84, 6.71 and 6.53 respectively as compared to respective control (5.8). It was noteworthy; that addition of PF did not improv the soil pH at both sampling stages and showed slightly acidic behavior. Cd availability (DTPA extractable) was reduced with biochar and composite mixtures (S1 and S2) (Fig. 2). Composite treatment (S2) showed a significant reduction in Cd availability at zero month while, S1 and biochar were effective in reducing metal availability in harvest stage samples. Maximum Cd was extracted in control treatment at both sampling stages (0.374 and 0.357 mg kg⁻¹). When compared with the control, S1 treatment and biochar significantly (p < 0.05) reduced the DTPA extractable Cd by 63 and 60% respectively in the second sampling stage. Application of treatments reduced the DTPA extractable Cd in following order S1 \approx biochar > S2 \approx lime > PF > control.



Figure 1. Effect of soil amendments on soil pH at different time intervals. Control, lime, biochar, PF, S1 and S2 were employed in triplicates (1% w/w). Error bars with different letters show significance at different sampling time



Figure 2. Effect of soil additives on available Cd and Pb contents of contaminated soil. Control, lime, biochar, PF, S1 and S2 were employed in triplicates (1% w/w). Error bars with different letters show significance at different sampling time

Meanwhile, alone lime and its combination with organic and clay additives (S1) reduced the Pb extractability by 61 and 59% as compared to respective control in harvesting stage soil samples. Biochar treatment also signifies the reduction in Pb extraction in comparison with control by decreasing Pb availability by 56%. Generally, all the applied treatments significantly improved the metals immobilization by decreasing metal mobility and extraction at end of experiment. Pb availability with applied treatments reduced in subsequent order S1 \approx lime > S2 \approx biochar > PF > as compared to control.

Correlation between soil pH and DTPA extractable Cd and Pb at different time intervals

A negative linear correlation was found between soil pH and DTPA extractable Cd and Pb at zero month and harvesting stage samples (*Fig. 3*). Correlation between available Cd and soil pH at both sampling stages showed negative behavior ($R^2 = 0.0027$, p = 0.837 and $R^2 = 0.0182$, p = 0.594). Meanwhile, amendments addition resulted in a significant negative correlation between soil pH and available Pb contents in zero month and harvest samples ($R^2 = 0.2851$, p = 0.022 and $R^2 = 0.3147$, p = 0.015).

Cd and Pb species distribution with applied additives

The chemical speciation of Cd and Pb in zero month and harvest soil samples is summarized in *Figure 4*. The applied treatments showed little difference for Cd portioning at zero month soil samples with maximum exchangeable Cd in control treatments (58%) and lowest in biochar treated blocks (41%). During the experiment, Cd quantities for different species varied with amendments. In general, exchangeable Cd content decreased with an increase in carbonate, oxide, organic bound and residual

fractions. Biochar treatments reduced the Cd exchangeability to 29% followed by S1 (30%), PF (32%), S2 (33%) and lime (37%) as compared to control (50%). The effect of treatments on Pb mobility and partitioning showed the effectiveness of composite treatments and biochar at the experiment end. Pb exchangeable fraction was decreased to 29% each for S2 and S1 followed by biochar (36%), PF (38%) and lime (40%) in comparison with control (63%).



Figure 3. Correlation between soil pH and available Cd and Pb contents of contaminated soil

Effect of applied additives on gaseous exchange

All the applied treatments significantly increased the photosynthetic rate (Pn) of rice (*Fig. 5*). Photosynthetic rate increased in S2 followed by S1 which increased up to 112 and 102% as compared to control. Biochar, PF and lime also showed significant increase in photosynthetic rate by 85, 82 and 75% respectively as compared to the control. Maximum increase in transpiration rate (Tr) was witnessed in S2 and S1 treated plots which was 162% and 152% higher than control (*Fig. 5*). On the other side, biochar, PF and lime also significantly improved the Tr by 124, 118 and 93% respectively, compared to control.

Cd and Pb accumulation in plant parts

Uptake and accumulation of Cd and Pb in rice were significantly affected by treatments (*Table 3*). At maturity all the applied treatments significantly reduced Cd contents in rice roots compared to the control but there was no significant difference among applied additives for Cd accumulation in roots. Adding S2 and biochar

significantly reduced the shoots Cd concentration by reducing uptake to 0.216 and 0.227 mg kg⁻¹ as compared to the control (0.322 mg kg⁻¹). Moreover, Cd content in rice grains was reduced to 0.049 and 0.056 mg kg⁻¹ in S2 and biochar treatments with the highest in control (0.231 mg kg⁻¹). On the other hand, Pb accumulation in rice grains was significantly reduced with S2, S1 and biochar treatment (0.070, 0.101 and 0.126 mg kg⁻¹) respectively which was much less than control (1.818 mg kg⁻¹). Lime and PF treatments also significantly reduced Pb accumulation in rice grains with values of 0.395 and 0.922 mg kg⁻¹.

Translocation of Cd and Pb from roots to shoots is presented in *Figure 6*. There was no significant difference for roots to shoots Cd translocation among the treatments with minimum translocation in S1 (62%) followed by lime (65%). While, Pb translocation in shoots was significantly reduced with biochar and S2 treatments (20% and 29%), as compared to control.

Treatments	Cd concentration (mg kg ⁻¹)			Pb concentration (mg kg ⁻¹)		
	Roots	Shoots	Grains	Roots	Shoots	Grains
Control	$0.462\pm0.038a$	$0.322\pm0.022a$	$0.231\pm0.011a$	$61.10\pm2.61a$	$45.26\pm0.590a$	$1.818\pm0.045a$
Lime	$0.362\pm0.023b$	$0.238\pm0.012bc$	$0.115\pm0.015c$	$45.59\pm0.807bc$	$20.80 \pm 1.732 b$	$0.395\pm0.108c$
Biochar	$0.328\pm0.008b$	$0.227\pm0.007c$	$0.056\pm0.018d$	$40.34\pm1.277c$	$8.20\pm0.508d$	$0.126 \pm 0.019d$
PF	$0.366\pm0.041b$	$0.276\pm0.012b$	$0.177\pm0.011b$	$48.02\pm1.001b$	$21.50\pm0.699b$	$0.922\pm0.026b$
S 1	$0.369\pm0.015b$	$0.231\pm0.016bc$	$0.119\pm0.010c$	$40.97\pm2.21c$	$18.35\pm0.629b$	$0.101 \pm 0.013d$
S2	$0.314\pm0.002b$	$0.216\pm0.007d$	0.049 ± 0.011 d	$45.19 \pm 1.06 bc$	$13.40\pm1.386c$	$0.070\pm0.028d$

Table 3. Effect of soil additives on metal contents of late rice

Control, lime, biochar, PF, S1 and S2 were employed in triplicates (1% w/w). Different letters indicates significantly different values at 5% significance level. All treatments including control receive recommended dose of N, P and K except treatment PF in which phosphorus was not applied. PF: phosphatic fertilizer, S1 and S2 (stabilizers)



Figure 4. Effect of soil additives on Cd and Pb speciation of contaminated soil

Tong et al.: The effect of single and composite additives on Cd and Pb mobility, speciation and accumulation in late rice (*Oryza sativa* L.) grown on contaminated soil



Figure 5. Effect of soil additives on leaf photosynthesis and yield parameters of late rice. Control, lime, biochar, PF, S1 and S2 were employed in triplicates (1% w/w). Error bars with different letters show significance at different sampling time



Figure 6. Effect of soil additives on metals translocation from roots to shoots. Control, lime, biochar, PF, S1 and S2 were employed in triplicates (1% w/w)

Effect of additives on the growth of late rice

Rice biomass was determined at harvest and results revealed that all the additives significantly improved rice biological yield (*Table 4*). But there was no significant difference for biomass yield among the additives. Maximum rice biomass was found in S1 followed by PF, S2, biochar and lime (18342, 18000, 17931, 17903, 17600, 14666 kg ha⁻¹). All the treatments significantly increased grains yield but maximum per hectare grains yield was observed in S1 (7530 kg ha⁻¹) followed by S2 and biochar (7129 and 7026 kg ha⁻¹) which was a significant increase as compared to control. A similar pattern was observed in plant height where all applied treatments significantly improved plant height but with no obvious difference among the additives.

Treatments	Biomass (Kg ha ⁻¹)	Grains yield (Kg ha ⁻¹)	Plant height (cm)
Control	$14666 \pm 240 b$	$6040 \pm 247c$	$93 \pm 1.453b$
Lime	$17600 \pm 351a$	$6741 \pm 274b$	$102 \pm 2.185a$
Biochar	$17903 \pm 250a$	$7026 \pm 241ab$	$104 \pm 2.081a$
PF	$18000\pm568a$	$7022 \pm 98ab$	$106 \pm 2.962a$
S1	$18342 \pm 218a$	$7530 \pm 132a$	$103 \pm 1.763a$
S2	$17931 \pm 470a$	$7129 \pm 82ab$	$104 \pm 1.701a$

Table 4. Effect of soil additives on growth of late rice

Control, lime, biochar, PF, S1 and S2 were employed in triplicates (1% w/w). Different letters indicates significantly different values at 5% significance level. All treatments including control receive recommended dose of N, P and K except treatment PF in which phosphorus was not applied. PF: Phosphatic fertilizer, S1 and S2 (stabilizers)

Discussion

In this field experiment, the effect of several soil additives on metal translocation and accumulation was trialed out by growing paddy rice. Amendments application showed a linear increase in soil pH. Our results found that maximum increase in soil pH was obtained with lime application. Previously, studies have reported an increased soil pH with the lime treatment (Shaha et al., 2012). Liming in the contaminated soil increased soil pH through release of hydroxyl group by hydrolysis mechanism. Our results are in line with Shi et al. (2019) that increase in lime application increased soil pH even under flooded conditions. Effect of lime treatment in increased soil pH was also reported which may cause metals precipitation or adsorption on soil, leading to decreased mobility (Mahar et al., 2015; Yan-bing et al., 2017).

Biochar, lime and their composites reduced the metal availability at both stages. Amending the contaminated soils with biochar can reduce metals availability by mattermetal complex (Shen et al., 2017). Recent experiments have shown the potential of biochar in reducing metal bioavailability because of porous structure, high pH and CEC and dynamic functional groups (Sui et al., 2018; Oiu et al., 2018). Biochar addition alters soil pH resulting in metals precipitation and adsorption on biochar surface. Ability of biochar to bind metals is attributed to different chemical or physical binding or direct sorption on biochar surface (Cornelissen et al., 2018; Schweizer et al., 2018). A lowcost amendment (lime) can retain metals in contaminated soil by reducing their availability. An increase in soil pH with lime treatment may cause an elevation in soil pH further reducing metal extractability due to adsorption or complexing processes (Chen et al., 2016b). Lime addition can improve carbonate and Fe or Mn oxide bound metals that decreased the metal contents in plant tissues (Zhu et al., 2010). The Addition of $CaCO_3$ as liming material considerably reduced the acidic effect and favored the metal precipitation (Simón et al., 2010). Meanwhile, a decrease in metal availability with the addition of Ca(OH)₂ in contaminated soil was also reported in the literature. Composite of lime with organic materials increased soil pH, reduced EC and improved the metals immobilization in contaminated soils (Hamid et al., 2018). Meanwhile, a good correlation was observed between available metals and soil pH at both stages. This correlation may be ascribed to the change in soil pH with applied amendments which is an important factor in regulating the availability of metals (Ma et al., 2010).

Moreover, application of soil amendments reduced the highly exchangeable fraction to least available form. It has been stated that biochar due to high surface area, CEC, high pH and active functional groups can interact with metals in reducing metals bioavailability to field crops. Stable biochar-metal complexes result in reduced metals availability in soil. A decreased Cd bioavailability with biochar application was reported by Li et al. (2009). Moreover, Park et al. (2011) investigated the enhanced Cd immobilization and reduced availability with chicken manure and green waste biochar. Biochar addition tends to improved oxide bound and organic bound metals by formation of precipitates and complexes (Méndez et al., 2013; Wang et al., 2014).

Photosynthetic and transpiration rate were higher in amended blocks as compared to control treatment. Our findings are in line with Tian et al. (2011), who reported that metals toxicity negatively affected plant photosynthesis. The reduced photosynthetic activity in control may be attributed to adverse effects of metals toxicity on plants (Rehman et al., 2017b; Hamid et al., 2019c).

Changes in physical or chemical properties e.g. soil pH and metals availability with additives results in reduced metals accumulation and uptake by plants. In our study organic amendments in composite (S2) or alone (biochar) reduced metals accumulation in rice especially grains. Organic manure changes soil pH and cation exchange capacity of soil which results in improved immobilization (Dourado et al., 2013). Organic wastes have active functional groups that actively form organic-metal complexes. This complexation process might restrict metals mobility in soil and plant (Liu et al., 2015). Combined treatment of additives may increase soil pH and metal accumulation in plant parts. Addition of lime with sepiolite was reported for improved pH and reduced metal contents in the plant (Shirvani et al., 2006). Various studies have confirmed the enriched soil pH with biochar addition (Sun et al., 2014; Chen et al., 2016a). Generally, biochar induced metal stabilization includes metal-phosphate precipitates, metals adsorption, biochar-metal electro static interaction, activated functional groups and chelation (Rodríguez-Vila et al., 2015; Ok et al., 2015; Wiszniewska et al., 2016; Khan et al., 2018). These processes might have favored the reduced accumulation of metals in rice grains. This decreased translocation might be due to improved immobilization with applied additives (Hamid et al., 2018). Biochar and organic source addition helps in improved organic matter contents of soil which further improve nutrients availability and reduces metals activity by enhanced immobilization (Chen et al., 2016a; Khan et al., 2018). It is intended that findings of this field experiment can be used for in-situ metal stabilization but further studies regarding effective dose and long-term stability are still needed.

Conclusion

Amendments application increased the soil pH and reduced metals availability in contaminated soil. Composite treatment S1 and biochar were effective in reducing Cd and Pb extraction while lime application elevated the soil pH to a significant level. This improved metal immobilization with applied treatments is attributed to complexing, adsorption, ion exchange, and/or precipitation processes. Briefly the results of this study indicated the changes in exchangeable Cd fraction to more stable form with biochar application. Meanwhile, composite treatments were effective in reducing exchangeable Pb contents at harvest stage samples. Moreover, composite treatment S2 and biochar not only reduced the metals bio-availability but also assisted in reduced grains Cd and Pb concentration in rice grown on contaminated soil. The results of our experiment pointed out the stability of amendments in improved immobilization of toxic metals in

contaminated soil but their effective dose rate and their effectiveness in different soil types needs to be addressed in later studies. Moreover, future trials regarding amendments' effect on the microbial community, enzymatic activity, long term metals immobilization and their effect under different moisture regimes can be elucidated.

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Conflict of interests. The authors declare that they have no conflict of interests.

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