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REGULAR ARTICLE

Influence of charcoal-based soil amendments on growth and nutrient uptake of rice (*Oryza sativa*) in Cadmium contaminated soil

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ABSTRACT

Cadmium contamination in paddy soils contributes to Cd toxicity and health risk to humans through consumption of Cd-contaminated rice. The potential of charcoal-based soil amendment in alleviating cadmium (Cd) toxicity in rice grown in paddy soil with low nitrogen availability was assessed in order to identify strategies to curve the problems of Cd contamination in rice. A pot culture experiment was conducted using a Cd-spiked soil with treatment combinations involving two types of charcoal amendments: carbonized rice hull (CRH) and wood charcoal (WC); and three levels of Cd concentration (0, 10 and 20 mg kg^{-1}). CRH and WC amendments reduced the dry matter yield, plant height, tiller number and chlorophyll-SPAD value of rice in the Cd-contaminated soil. CRH and WC amendments reduced the soluble N but enhanced the residual N, water soluble and exchangeable K, Mg, Ca and Si in soil. The Cd^{2+} in soil solution was significantly ($p<0.01$) reduced by CRH and WC which resulted to significantly lower ($p<0.01$) Cd uptake by rice plants compared to soil without amendments. CRH was more effective than WC in lowering Cd availability and uptake by rice plants which could be attributed to the higher surface area of CRH compared to WC. Overall, CRH and WC decreased Cd availability by increasing the pH and providing additional sorption sites for Cd which resulted in lower Cd concentration and uptake of rice plants.

Keywords: Carbonized rice hull; Cd availability; Nutrient dynamics; Soil solution; Wood charcoal

INTRODUCTION

Exposure to cadmium (Cd) can cause kidney diseases in humans and was reported to be the reason for widespread epidemics ("*itai-itai*") in Japan during the 1970s (US-EPA, 2007). Deposition of Cd in agricultural soils occurred mostly through atmospheric sources (Nabulo et al., 2006) followed by application of livestock manure, sewage sludge, phosphate fertilizers and industrial or agricultural wastes (Nicholson et al., 2003; Nziguheba and Smolders, 2008). There is a high incidence of Cd deposition in agricultural lands near mining areas as well (Jung and Thornton,1996; Ko et al., 2010). An average of 0.4 mg kg^{-1} Cd was found in soils of Korea, with 54 and 7 out of 120 sampling sites exceeding the threshold (1.5 mg/kg) and corrective action limits (4 mg/kg), respectively (Ko et al., 2010). The high contents of Cd in agricultural soils, especially in rice fields warrants special consideration because of the unique agronomic and nutritional/bioavailability characteristics of Cd in paddy soils and with rice plants. Compared to other food crops, rice has the ability to accumulate soil Cd in grain while excluding Fe, Zn and Ca even though the soil contains 100-times more Zn than Cd (Chaney et al., 2004). Such characteristic response of rice plants to Cd in soil increased the potential entry points of Cd into the human diet, placing the majority of Asian countries on a high risk of Cd toxicity.

There are numerous techniques for remediation of Cd contaminated farm land which either reduces the soil total metal load, or the mobility and bioavailability (Tang et al., 2016). Examples include dig and fill method, electro-kinetic remediation, chemical elution, stabilization and solidification, phytoremediation, field management and combined remediation. As pointed out by Tang et al. (2016), the existing methods for Cd remediation in soil

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have problems related to sustainability and economic profitability, environmental intrusion, and practical applicability in farm lands without disrupting the production systems. In Cd contaminated paddy soils, soil washing with calcium chloride (Makino et al., 2007) and ferric chloride (Makino et al., 2008) were found to have no negative effects on rice straw and grain yields but affected soil fertility properties by decreasing exchangeable cations (Mg and K). The use of solar cells to drive electrokinetic migration of Cd in contaminated soil was demonstrated by Yuan et al. (2009) but its effectiveness greatly depended on weather conditions and financial capabilities of poor farmers to adopt the technology. Soil dressing was effective to prevent Cd uptake of rice but very costly, requires a thick volume of uncontaminated soil to cover contaminated soils, and a possibility of rice grain from deeply rooted cultivars (Arao et al., 2010). Li et al. (2012) used laboratory-synthesized aromatic hydrocarbons for extracting Cd in a spiked-soil although they are yet to recommend it for use in soil remediation purposes due to lack of idea about its biodegradation and adsorption in soil particles. It may also incur high costs for application on a large scale. Therefore, there is an urgent need to develop a remediation technique of Cd-enriched paddy soil which are low-cost, easy-to-operate, non-disturbing to the soil structure and nutrients, highly efficient in Cd soil load reduction, and associated with no interference of normal crop planting (Tang et al., 2016).

Utilization agricultural waste to curve some unfavorable environmental problems associated with waste disposal and pollution remediation are being promoted. Biochar for example which was initially developed for carbon sequestration in soils is sought as soil amendments for pollution remediation because of its low cost and high adsorbing potential of heavy metals and other organic pollutants (Cao et al., 2011; Inyang et al., 2011; Inyang et al., 2012). In paddy soils, field application of biochar from rice hulls is popular as a means to dispose rice hulls from rice mills, and as a secondary source of nutrients. Benefits of using biochar in rice field include an increase in yield and N use efficiency by reducing NO_2 emission (Zhang et al., 2012), improvement of soil quality and reduction of methane emission (Xie et al., 2013). Some studies emphasized the capacity of biochar generated from carbonized rice hulls, wheat straw (Cui et al., 2011; Bian et al., 2014), sewage sludge (Khan et al., 2013), and wood (Lucchini et al., 2014), to immobilize Cd and other heavy metals like Cu, Zn, Cr, and Ni in paddy soils. Although the benefits of biochar application in paddy soils have been documented, there is a need to verify the impact of CRH and WC not only on Cd availability and uptake of rice plant but also its effect on nutrient dynamics and availability affecting the growth and development of rice. Therefore, this study dealt with the effect of carbonized rice hull (CHR) and wood charcoal (WC) on Cd availability and uptake of rice in a Cd-spiked paddy soil with limiting N content. We have selected CHR and WB because these are commonly available in rice producing areas and important soil amendments for sustainable nutrient management in paddy soils. The results of this study would be useful in identifying appropriate strategies of Cd remediation in paddy soils with special consideration on soil nutrient management. Therefore, the specific objectives of our study were (i) to determine the growth and physiological response of rice in a Cd-spiked soil as influenced by CRH and WC amendments, and (ii) to monitor the concentration of Cd and available N in the soil solution and nutrient availability in soil, and (iii) to quantify the Cd uptake of rice in a Cd spiked-soil applied with CHR and WC.

MATERIALS AND METHODS

The soil used in the experiment was obtained from the Agricultural Experiment Station and Research Facility, Kyungpook National University, Daegu, South Korea. The soil was mixed and sieved to pass through a 2-mm sieve prior to use. Sub-samples were collected for chemical characterization. Biochar materials which are carbonized rice hull (CRH) and wood charcoal (WC) were obtained commercially. Based on the information from manufacturers, CRH was prepared at a pyrolysis temperature of 350 °C while WC was prepared at 450°C.

The chemical properties of soil and biochar materials are presented in Table 1. The soil is near neutral (pH = 6.9) and contained considerable amounts of exchangeable calcium and magnesium, and high amounts of acid-extractable silicon. It has very low organic matter (0.04%) and N content (0.02%). Between the two biochar used in the study, WC has higher pH, EC, total C and C/N ratio than CRH while CRH has higher total N than WC. Also, WC contains higher amounts of water soluble, exchangeable, and acid-extractable calcium and magnesium. A significant amount of silicon is present in CRH, plus a high amount of exchangeable and 0.1 N HCl-extractable potassium. Based on the analyses, CRH is inferior to WC in terms of Ca and Mg contents but a potential source of silicon and potassium, which was found to be beneficial for rice.

Treatments, experimental set up and design

The experiment was laid down in a two-factorial design with three levels of Cd concentration (0 ppm, 10 ppm, and 20 ppm) and three soil amendment treatments (0 (no amendments), 2% (w/w) CRH, and 2% (w/w) WC) in a Completely Randomized Design (CRD) with 3 replications. The soil was spiked with cadmium chloride hemi-

Table 1: Chemical and physical properties of soil and biochar materials and before experiment[1]

	Soil	CRH	WC
pH	6.92±0.14	7.38±0.08	9.67±0.05
EC (uS cm^{-1})	59.2±8.22	229.37±12.52	425.67±18.12
Total C (%)	0.04±0.002	44.62±1.05	79.14±6.22
Total N (%)	0.02±0.0012	0.24±0.002	0.06±0.001
C/N ratio	2±0.0016	186±24	1319±126
Water soluble (mg kg^{-1})			
K	0.62±0.01	189.1±2.12	206.0±16.24
Ca	1.05±0.01	1.97±0.03	5.91±0.04
Mg	0.98±0.02	5.05±0.04	12.45±0.02
Si	34.34±0.18	64.41±1.02	46.1±0.84
Exchangeable (mg kg^{-1})			
K	6.10±0.15	684.0±12.36	232.9±10.14
Ca	211.7±10.4	47.51±2.15	230.6±12.84
Mg	102.3±8.2	24.15±0.65	38.91±2.46
Si	32.43±4.32	115.7±12.48	51.86±3.28
Acid extractable (mg kg^{-1})			
K	9.14±0.16	689.4±15.24	310.1±14.25
Ca	239.3±12.6	137.9±10.18	977.7±20.14
Mg	168.3±10.3	36.96±2.58	55.24±5.15
Si	298.9±21.32	41.73±3.12	42.65±3.14
Surface area (m^2 g^{-1})*		32.8	26.3
Pore volume (cm^3 g^{-1})*		0.052	0.124

[1]Data are means and standard deviation of triplicate sample analysis.
*Data supplied by the manufacturer of biochar

pentahydrate ($CdCl_2 \cdot 2.5\ H_2O$; Sigma-Aldrich, Korea) in deionized H_20. The amount of Cd was computed based on the oven dried weight of soil samples and dissolved in 500 ml of deionized water before thoroughly mixing. After applying the Cd solution and mixing, the soil was covered with black polyethylene sheets and aged for 30 days under glasshouse condition. After the 30-day soil aging, the soils were transferred into 5L plastic pots at 5kg soil per pot and submerged for another 30 days prior to application of CHR and WC. Submergence was maintained at 3- cm standing water and mixed every 2 days. The CRH and WC were sieved to pass through a wire mesh screen No. 10 before it was mixed with the soil at 2.0% (w/w). The soil and biochar mixtures were allowed to stand and remain in submerged condition for another 8 days before transplanting of rice seedlings (14-day old *Ilpum* cultivar). Plants were grown until maximum vegetative stage (62 days after transplanting, DAT), maintaining a 3-cm standing water during the whole experimental period. The plants were fertilized at the rate of 180-110-150 kg N, P_2O_5 and K_2O ha^{-1} using urea, superphosphate and muriate of potash. 70 % of N and K, and 100% of P were applied basally while the rest of N and K were supplied 45 days after transplanting (RDA, 1999).

Soil solution sampling and analysis

Porous fiber tube (Rhizon Flex-10 cm, Rhizosphere Research Products, Wageningen, NL) samplers were installed/inserted in the middle of pots 2 days before initial soil solution sampling (Ultra and Han, 2015). Solution samples were extracted 5-times from 0 DAT in 14-day intervals using a 10-mL disposable plastic syringe connected to the porous fiber tube. The soil solution was withdrawn by applying suction using 10 ml plastic syringe at an average rate of 2ml hour^{-1}. A total of 27 soil solution samples were taken at each sampling time. Soil solution pH and electrical conductivity (EC) were immediately measured directly at the day of extraction using a Benchtop pH/Conductivity Meter (Orion Star A215, Thermo Fisher Scientific Inc.). After measurement, the solutions were filtered to remove impurities using a filter paper (Whatman #42) suspended in a glass funnel, acidified (0.1% of final acid concentration) using concentrated HCl and checked for the concentration of Cd using a flame-type Atomic Absorption Spectrophotometer (AAnalyst 700, Perkin Elmer) based on Pansu and Gautheyrou (2007). Total soluble-N in soil solution was analyzed from the same soil solution samples by a CHNS-elemental analyzer (FlashEA 1112 Series, Thermo Electron Corp., Rodano, Italy) for liquid samples (Jeong et al., 2015).

Plant growth measurements and uptake of cadmium

Plant height (cm), tiller count, and chlorophyll-SPAD (Chlorophyll Meter SPAD-502, Konica Minolta Sensing Inc.) values were gathered from 30~60 DAT. The experiment was terminated at maximum vegetative stage (62 DAT). Plants were uprooted from the pots and separated roots and shoots. Roots were thoroughly washed to remove soil particles and air-dried. Dry matter weight was measured after oven-drying the samples at 70°C for 5 days. Oven-dried samples were then finely ground using a blender (WSG30E, Waring Commercial, Torrington, CT06790). Approximately 1.00 g sample of root and shoot were subjected to wet ashing (Campbell and Plank, 1998) and the concentration of Cd in solutions was determined using a flame-type Atomic Absorption Spectrophotometer (AAnalyst 700, Perkin Elmer).

Soil and biochar analysis

Soil samples collected before and after the experiment as well as the CRH and WC samples before the experiment were air dried, sieved to pass through 2mm, and stored in -20°X prior to analysis. Total C and N in this samples were analyzed using a CHNS-elemental analyzer (FlashEA 1112 Series, Thermo Electron Corp., Rodano, Italy). Samples were powdered and analyzed in five replicates. The C/N ratio was calculated based on the C and N contents of the samples (Fujine, 2014; Ultra and Han, 2015). The available N was analyzed by 1N KCl extraction (1:5, w/v) followed by steam distillation in a close system (Bremner and Keeney, 1965). Water extractable and acid extractable bases were determined by extracting 10 g of soil sample with deionized water or 0.1N HCl solution, respectively at 1:5 soil-extractant ratio for 1 hour in a rotary shaker and filtered. The concentration of K, Ca, Mg and Si were determined by ICP (ICPE-9800, Shimadzu, JAPAN; Pansu and Gautheyrou, 2007). Duplicate analysis of each sample and inclusion of laboratory standard samples were done to ensure accuracy.

Data analyses

Analysis of Variance (ANOVA) was performed through the Statistical Analysis System ver. 9.1 (SAS Institute, Cary, North Carolina, USA). The mean separation method used was Tukey's HSD at $P< 0.05$.

RESULTS

Effect of cadmium levels and amendments on growth and physiology of rice

The influence of charcoal-based amendments on the growth of rice plants in Cd-contaminated soil was manifested in terms of plant height, tiller count and leaf SPAD readings (Table 2). At 60 DAT, differences in terms of plant height were significant (P<0.01). Plants from CRH treatments was 7 - 17 percent shorter than those plants from No Amd and WC treatments. Across amendment types, rice grown in 20 ppm Cd-spiked soil (56.17 cm) appeared inferior to those grown in 0ppm (61.5 cm) and 10 ppm Cd (60.33). Tallest plants (66.25 cm) was observed from 0 ppm Cd x No Amd while the shortest (52.25 cm) was observed from treatment with 20 ppm Cd x CRH treatment (p<0.000, Table 2). Plants grown in soil with No Amd (5.67) have significantly higher number of tillers compared to those applied with CRH (4.42) or WC (5.5). Specifically, an average of 6.75 tillers per plant was produced in 0 ppm Cd-No Amd treatment while the 10 ppm Cd-CRH treatment has 3.75 tillers/plant only. Soils with 0 ppm Cd-No Amd treatment resulted in rice plants with the highest shoot, root, and total biomass (P<0.000) compared with other treatments (Table 2). The average shoot biomass (6.61 g) and total biomass (8.24 g) were lowest with 0 ppm-CRH treatment while the lowest root biomass was obtained in 10 ppm Cd-CRH treatment (2.45 g). Across different levels of Cd contamination, CRH (3.53) was observed to have a significantly higher shoot/root biomass ratio compared to No Amd (2.6) and WC (2.68). Plants from 0ppm Cd-CRH (4.27) had the highest shoot/root biomass ratio while those from 20 ppm-WC (2.55) treatment had the lowest shoot/root biomass ratio.

The chlorophyll formation (reflected by SPAD values) was also significantly (P<0.001) affected by amendment application (P<0.01), Cd application (P<0.05) and the date of sampling (P<0.01). Regardless of biochar amendments and Cd treatments, SPAD values were significantly higher (P<0.05; Fig. 1a) at 30 DAT (36.89) compared to SPAD value obtained at 45 DAT (32.04) and 60 DAT (34.74). Regardless of the biochar amendments and date of sampling, plants in 20 ppm Cd (35.22) had significantly higher (P<0.01) SPAD value compared to 0ppm Cd (33.97) and 10 ppm Cd treatments (64.48; Fig. 1b). On the other hand, significantly higher SPAD (P<0.01) values was observed from No Amd (35.53) treatments compared to those plants from CRH (34.69) and WC (33.44) regardless of the Cd treatment and time of sampling (Fig. 1c).

Cadmium uptake and distribution in the shoots and roots of rice

Table 3 showed the Cd concentration and uptake of rice from 10 ppm and 20ppm Cd treatments were presented while the 0 ppm C treatment was excluded because Cd was not detected in plant tissues. As expected, an increasing the Cd concentration in soil significantly increased the Cd concentration in shoots or roots of rice (P<0.01). However, the presence of WC and CRH resulted in differential effects on Cd concentrations in rice plants. At

Table 2: Plant height, tiller number, and biomass yield of rice grown in Cd-contaminated soil as affected by different Cd concentrations and soil amendments at 60 days after transplanting[1]

Treatments				Biomass yield (g)			
Cd	Soil amendments	Plant height	Tiller number	Shoot	Root	Total	Shoot/root ratio
0 ppm Cd	No Amd	66.25±3.9a	6.75±0.0a	15.25±0.84a	6.12±1.19a	21.37±1.52a	2.56±0.49c
	CRH	56.50±6.6b	4.75±0.5c	6.61±0.53d	1.63±0.42e	8.24±0.75d	4.27±1.19a
	WC	62.00±2.4a	6.25±0.9a	12.08±0.57b	4.33±0.84b	16.41±0.67b	2.88±0.61c
10 ppm Cd	No Amd	63.50±1.7a	5.25±0.6bc	14.27±0.90a	5.79±0.38a	20.06±1.11a	2.47±0.18c
	CRH	57.00±2.9b	3.75±0.5c	7.28±0.88cd	2.45±0.44d	9.73±1.28c	3.00±0.27b
	WC	60.50±2.1ab	5.75±0.9b	10.67±1.13b	4.11±0.40b	14.77±1.06b	2.62±0.42c
20 ppm Cd	No Amd	60.00±2.3ab	5.00±0.5c	12.43±0.45b	4.63±0.86b	17.05±0.46b	2.76±0.56c
	CRH	52.25±1.7c	4.75±0.09c	7.59±0.48c	2.30±0.17d	9.89±0.56c	3.31±0.27b
	WC	56.25±1.5b	4.50±0.05c	8.18±1.00c	3.24±0.24c	11.42±0.76c	2.55±0.49c
Amendments		**	**	**	**	**	*
Cd treatment		**	ns	**	**	**	*
Amd x Cd		**	**	**	**	**	*

[1]Means within the same column, followed by the same letter (s) are not significantly different to each other based on Tukey's test at 5% level of significance

10 ppm Cd in soil, the shoot Cd concentration was higher in WC (21.72 mg/kg) treatment compared to No Amd (9.85 mg/kg); while those grown in CRH treatments had zero Cd concentration. The root Cd concentration was lowest in CRH amended soil (15.04 mg/kg) while those in the WC treatments (96.41 mg/kg) have higher than those in CRH amended soil but comparable to the those in No Amd treatments (100.97 mg/kg). At 20 ppm Cd in soil, the shoot Cd concentration was significantly lower (P<0.01) in CRH treatment (8.08 mg/kg) than the WC (24.81 mg/kg) and the No Amd (20.19 mg/kg). The root Cd concentration in CRH (160.09 mg/kg) and WC treatments (161.40 mg/kg) were about 36% lower than the No Amd treatment (248.77 mg.kg). The shoot/root ratio of Cd concentration was highest with 10 ppm Cd-WC treatment (0.23) and lowest with 10 ppm Cd-CRH treatment (0.00).

Significant interaction effect of soil amendment and Cd treatment was observed on the Cd uptake of rice plants (P<0.01). The No Amd x 20ppm Cd (0.25 mg/plant) treatment and 10ppm Cd-WC treatment (0.23 mg/plant) had significantly higher shoot Cd uptake than other treatments while the lowest shoot Cd uptake was observed in 10 ppm Cd-CRH (0.00 mg/kg) and 20 ppm Cd-CRH (0.06 mg/plant) treatments. The root Cd uptake was highest in 20ppm Cd-No Amd treatment (1.12 mg/plant) while the lowest was observed in 10 ppm Cd-CRH treatment (0.04 mg/plant). Similar to root Cd uptake, the total Cd uptake of rice plants was highest in 20ppm Cd-No Amd treatment (1.37 mg/plant) while the lowest was observed in 10 ppm Cd-CRH treatment (0.04 mg/plant). The shoot/root ratio of Cd uptake was highest with 10 ppm Cd-WC treatment (0.59) and lowest with 10 ppm Cd-CRH treatment (0.00).

Soil solution pH, EC, and nutrient concentrations

The pH of soil solutions at 0 DAT ranged from 5.5 - 6.0 and increased gradually towards neutrality with increasing days of continues submergence. From 0 to 28 DAT, the soil solution pH of CRH and WC amended soil were higher compared to soil without amendments regardless of the Cd treatment. At 42 DAT, pH variations among treatments have narrowed. It appears that the soil solution pH of WC amended soil was relatively higher (pH = 6.85) compared to the soils amended with CRH (pH = 6.55) and the control (pH = 6.48). This is regardless of the Cd concentration, especially at the early stage of growth (Fig. 2). Similarly,

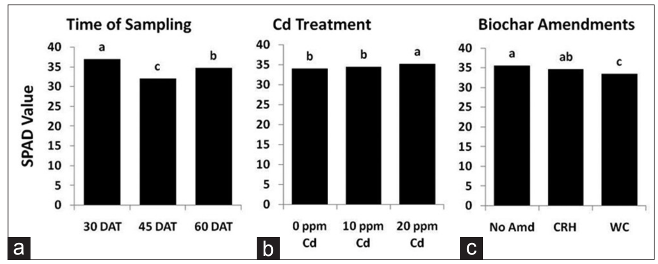

Fig 1. Main effects of sampling date (a), Cd treatment (b) and biochar amendments (c) on SPAD value of rice plants grown in Cd-contaminated soil. ANOVA results: Amendments**, Cd treatment*, Amendment x Cd treatment^{NS}, Date*; Tukey's LSD value: Amendments = 1.212, Cd treatment = 1.2125, Date = 1.3796.

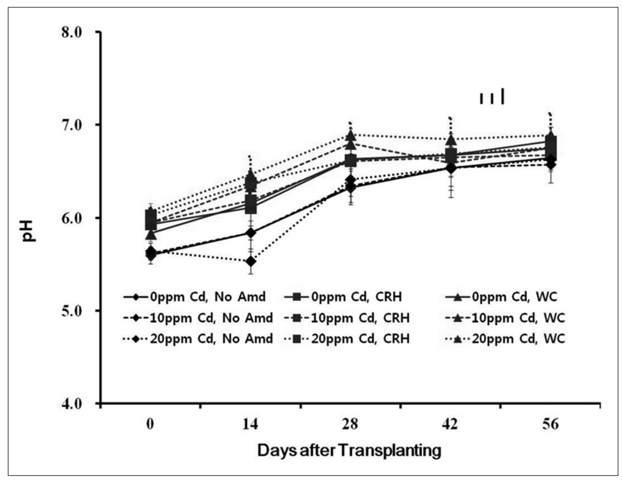

Fig 2. Soil solution pH during the growing period of rice grown in Cd-contaminated soil as affected by charcoal-based amendments. Data are means and standard deviation. Bars indicate least significant differences when comparing treatment means within sampling period (left), between sampling period (middle), and between any two data (right).

Table 3: Cadmium concentration and uptake of rice grown in Cd-contaminated soil as affected by different Cd concentration and soil amendments 60 at days after transplanting[1].

Treatments		Cd concentration (mg kg^{-1})			Plant uptake (mg pot^{-1})			
Cd	Amendment	Shoot	Root	Shoot/root ratio	Shoot	Root	Total	Shoot/root ratio
10 ppm Cd	No Amd	9.85±0.53b	100.97±4.73c	0.10±0.01c	0.14±0.01c	0.59±0.07b	0.73±0.06b	0.24±0.04c
	CRH	0.00±0.00c	15.04±21.27d	0.00±0.00e	0.00±0.00e	0.04±0.05d	0.04±0.05e	0.00±0.00e
	WC	21.72±0.25a	96.41±3.94c	0.23±0.01a	0.23±0.02a	0.40±0.05c	0.63±0.04c	0.59±0.11a
20 ppm Cd	No Amd	20.19±1.94a	248.77±62.41a	0.08±0.01c	0.25±0.03a	1.12±0.13a	1.37±0.15a	0.23±0.03c
	CRH	8.08±0.81b	160.09±27.21b	0.05±0.01d	0.06±0.00d	0.37±0.06c	0.43±0.06d	0.17±0.03d
	WC	24.81±8.87a	161.40±40.14b	0.15±0.03b	0.20±0.05b	0.52±0.13b	0.72±b0.18b	0.38±0.01b
Amendments		**	**	**	**	**	**	**
Cd treatment		**	**	**	**	**	**	**
amd*cd		**	**	**	**	**	**	**

[1]Means within the same column, followed by the same letter (s) are not significantly different to each other based on Tukey's test at 5% level of significance

the electrical conductivity (EC) was significantly lowered ($P<0.01$) by WC and CRH amendments compared to the control (No Amd) especially at later sampling (Fig. 3).

Fig. 4 showed available N concentration in soil solution (NH_4^+ -N and NO_3^--N,) during the growing period of rice as affected by different treatments. Application of CRH and WC significantly reduced the available N concentration during the 14, 28, 42 and 56 DAT ($P<0.001$). CRH amendments resulted in a higher reduction of available N in soil solution than those soils amended with WC and the No Amd treatments during the entire sampling period. The reduction from the initial levels of available N in CRH and WC treatments were in the range of 65-80% and 30-70%, respectively. Lowest available N concentration in CRH and WC amended soils was 0.8 mg/L and 1.4 mg/L, respectively. The Cd treatment has no significant effect on available N concentration in soil solution.

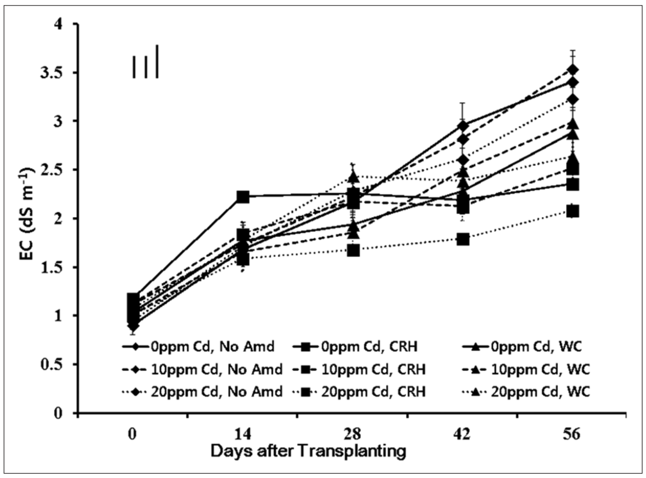

Fig 3. Soil solution electrical conductivity during the growing period of rice grown in Cd-contaminated soil as affected by charcoal-based amendments. Data are means and standard deviation. Bars indicate least significant differences when comparing treatment means within sampling period (left), between sampling period (middle), and between any two data (right).

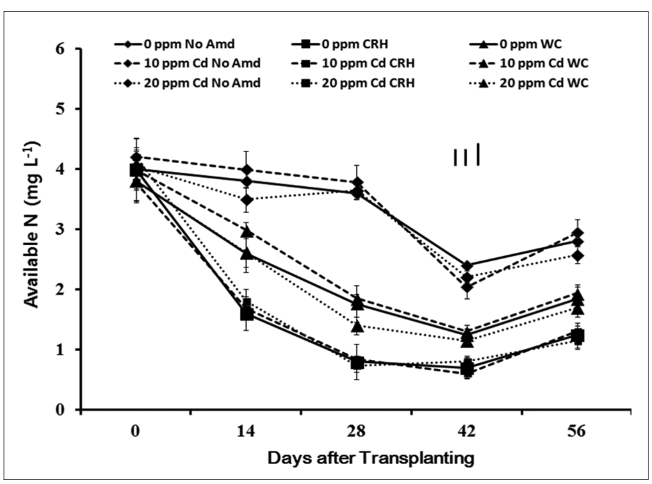

Fig 4. The concentration of available N (NH_4^+ and NO_3^-) in soil solution during growing period of rice grown in Cd-contaminated soil as influenced by different charcoal-based soil amendments. Data are means and error bars represent the standard deviation. Bars indicate least significant differences when comparing treatment means within sampling period (left), between sampling period (middle), and between any two data (right).

The concentration of total C, total N; available N, and the water soluble and 0.1N HCl acid extractable bases in soil after the experiment were significantly affected by different treatments (Table 4). Total C, total N and available N significantly increased with the application of charcoal-based amendments ($P<0.01$). On the average, total C content was increased to 4.7% and 7.15% due to CRH and WC amendments, respectively. The total N content was increased to 0.14% and 0.12% due to CRH and WC amendments, respectively; while the available N was increased to 19.9 mg/kg soil and 17.1 mg N/kg soil due to CRH and WC amendments respectively. WC application resulted in a greater increase of total C in soil whereas CRH application resulted in higher total N and available N, compared to the No Amd treatment. The water soluble K, Ca, Mg and Si in soil were significantly increased by CRH and WC ($P<0.01$). Regardless of Cd treatment, the average water soluble K content in soil was increased to 19.9 mg/kg and 17.13 mg/kg due to CRH and WC amendments, respectively. Water soluble Si increased up to 42.27 mg/kg and 32.35 mg/kg due to CRH and WC amendments, respectively. In the exchangeable fractions in soil, only the exchangeable K was increased by the application of soil amendments.

Cadmium concentration in soil solution

The Cd concentration in the soil solution was significantly decreased ($P<0.001$) by the application of soil amendments and the effects was dependent on the Cd treatment (Fig. 5). In 10 ppm Cd-spiked soils, the No Amd treatment had the highest Cd concentration from 0 DAT (1.8 mg/L) but slightly declines to 1.48 mg/L at 56 DAT. In contrast, soils amended with CRH and WC had 0 mg L^{-1} Cd concentrations at 14 DAT and remained the same until the last sampling in (Fig. 5). In 20 ppm Cd-spiked soils (Fig. 5),the No Amd treatment has the highest Cd concentration (0.44-0.28 mg/L) in soil solution all throughout the experiment. After the 14 DAT, CRH and WC application gradually decreased the Cd concentration in soil solution towards later sampling periods. CRH amendment resulted to 0 mg L^{-1} at 42 DAT onwards while the WC application reduced the Cd concentration within 0.10 to 0.20 mg L^{-1} particularly during the later period of the experiment.

DISCUSSIONS

In this study, biochar at 2% by weight was applied based on the recommended application rate of 1-4% in most areas (Dong et al., 2015; Pratiwi and Shinogi, 2016) that

Table 4: Total C and N, available N and water soluble and exchangeable bases of soils collected from different treatments after rice cultivation[1]

Treatments	Total C (%)	Total N (%)	Avail N (mg kg^{-1})	Water Soluble (mg kg^{-1})				Exchangeable (mg kg^{-1})			
				Ca	Mg	Si	K	Ca	Mg	Si	
0 Cd											
No Amd	0.05c	0.080c	6.84c	9.2c	1.02c	0.82c	28.2c	73.6c	218.6	98.8	32.2
CRH	4.42b	0.124b	12.24a	19.2a	1.32b	1.12b	40.21a	153.6a	208.3	102.2	38.2
WC	6.91a	0.116b	10.48ab	18.2b	1.48a	1.82a	32.41b	145.6b	216.4	104.3	33.2
10 Cd											
No Amd	0.01c	0.065c	7.4c	8.6c	1.04a	0.78c	26.8c	68.8c	216.4	96.2	30.2
CRH	4.12b	0.144a	10.87a	21.2a	1.44a	1.34b	44.24a	169.6a	206.8	101.2	36.24
WC	7.21a	0.118b	9.34b	16.4b	1.38b	1.74a	34.26b	131.2b	214.2	103.21	34.2
20 Cd											
No Amd	0.06c	0.076c	6.26c	14.2c	1.04c	0.88c	28.4c	113.6c	212.4	94.34	31.12
CRH	4.32b	0.152a	11.88a	19.3a	1.36b	1.28b	42.36a	154.4a	204.6	103.62	37.65
WC	7.34a	0.124b	8.24b	16.8b	1.54a	1.62a	30.58b	134.4b	213.2	104.4	34.2
Amendments	**	**	**	**	**	**	**	**	ns	ns	ns
Cd treatment	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Amd x Cd	ns	*	*	ns	ns	ns	ns	ns	ns	ns	ns

[1]Means within the same column, followed by the same letter(s) are not significantly different

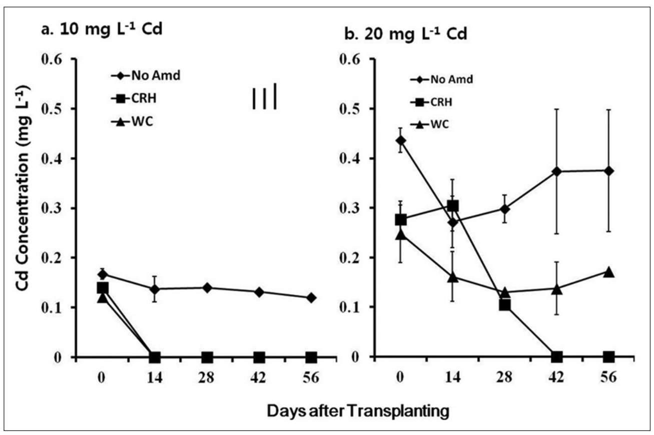

Fig 5. Cd concentration in soil solution at different sampling period. Data are means and error bars represent the standard deviation. Bars indicate least significant differences when comparing treatment means within sampling period (left), between sampling period (middle), and between any two data (right).

have an abundant supply of rice hulls as farm by-products. The results demonstrated how charcoal-based amendments would potentially affect the growth and physiology of rice grown in a cadmium-contaminated soil with very limiting nitrogen. Growth differences were visible due to amendments applied to the soil as manifested in terms of plant height, the number of tillers, chlorophyll-SPAD values, and dry matter yield. The increase in soil Cd concentration from 0 to 20 ppm decreased the plant growth, dry matter yield, number of tillers, and leaf chlorophyll contents of rice. In addition, soils amended with CRH produced shorter plants, fewer tillers, lower SPAD values, and lower biomass yield compared to No Amd and the WC. These findings showed that the negative impact of Cd in rice plants could be reduced by the application of CRH and WC but rice growth could be seriously reduced also by charred-materials by possibly absorbing essential elements such as N and some other nutrients. The growth reduction of rice as a result of decreased N availability is evident in the decrease in total available N in the soil solution due to soil application of CRH and WC (Fig. 4). This result corroborates with those reported by Deenik et al. (2010), indicating a substantial reduction of plant growth in biochar-amended soil. Aside from available N reduction, there is a high possibility that the availability of exchangeable bases could be reduced by CRH and WC amendments because of it high and nonselective affinity to cations (Zhang et al., 2012). Although we did not determine the concentration of these cations in soil solution during the course of the experiment, our data showed an increase of K, Ca, Mg and Si concentration of water soluble and exchangeable K in the soil after plant harvest indicated high nutrient retention. Excessively high nutrient retention in soil could be detrimental to the soil nutrient availability (Available N and exchangeable K) particularly because the soil used in this study has low N concentrations. Our data showed that CRH had a higher capacity to reduce the available N and the residual, exchangeable and water soluble cations WC which could be attributed to the differences in the quality of charcoal and adsorption capacity of the materials used. CRH has higher surface area than WC as it were obtained from different sources and produced by different process conditions (Table 1).

The ability of CRH and WC to reduce the effect of Cd on rice plant could be attributed mainly to its ability to reduce the Cd availability in soil. Our data have shown that the availability of cadmium in Cd-spiked paddy soil was greatly affected by CRH and WC as evident in the reduced Cd concentration and uptake of rice (Table 3). The reduction of Cd availability in CRH and WC amended soil could be the consequence of additional sorption sites provided by the high surface area of these amendments (Kitamura et al., 2002; Chun et al., 2004; Inyang et al., 2010; Inyang et al., 2012), and the effect of these amendments on soil pH which in turn affected Cd solubility (Kashem and Singh, 2001). However, the difference in Cd concentration and uptake, and Cd translocation into shoots as indicated by shoot/root ratio of Cd concentration and uptake of rice between CRH and WC amended soil (Table 2) could be attributed to the difference in the magnitude of the binding site generated because the surface area of CRH is higher than those of WC. In addition, studies have shown that the adsorption capacity of biochar depends partly on the type of the raw materials and the pyrolysis process influencing the quality and quantity of the surface functional groups (Ahmendna et al., 2000; Kitamura et al., 2002; Chun et al., 2004) which are true to this experiment. It appeared that CRH and WC have different nature of surface functional groups influencing their metal absorption capacity.

Aside from increased adsorption capacity due to high surface area of CRH and WC, increased of the soil solution pH due to CRH and WC amendments (Fig. 2) resulted in the generation of negative charges in soil and biochar per see, thus increasing additional sorption sites for Cd (Jiang et al. 2012). Although continued submergence of paddy soils will result in a pH adjustment to neutrality, our data have shown that the magnitude of pH increase was higher due to CRH and WC amendments compared to No Amd treatments which would imply that CRH and WC amendments will enhance the generation of additional negative charges in soil. Aside from this, Sartape et al. (2013) and Houben et al. (2013) demonstrated that the increase in pH to neutral levels promoted metal hydroxide precipitation thus lowering the dissolved Cd^{2+} concentration which could have occurred also in this experiment. Therefore, the reduction of available Cd and reduced plant uptake is a consequence of increased sorption sites brought about by the physical increase of surface area, generation of negative charges due to pH increase due to CRH and WC amendments in soil, and promoted cadmium hydroxide precipitation. It should also be noted that CRH and WC contained different amounts of cations that may have interacted differently with Cd in the soil solution. Our results indicated that CRH is more effective than WC in reducing Cd availability in soil and could be attributed to the inherent differences in the raw material and its interaction with pH changes in CRH and WC-amended soils.

CONCLUSIONS

Overall, the results obtained in the present study have demonstrated the potential short-term effect(s) of CRH and WC on nutrients and Cd dynamics in paddy soil with very limiting nutrients especially N. In contrast to previous claims that charcoal-based amendments can improve soil fertility and crop productivity, our results showed otherwise but were effective in lowering Cd concentration in the soil solution and prevented Cd uptake by the rice plant. Some previous studies attributed this ability of biochar to improve soil fertility and productivity through enhanced retention of nutrients in soil against leaching (Singh et al., 2010; Beesley and Marmiroli, 2011; Beesley et al., 2011; Lehmann et al., 2011), but in this particular soil, such functions had limited the nutrients availability for plant uptake. Therefore, CRH and WC could be used to decrease the potential risk of Cd contamination in rice but careful consideration should be made to improve nutrient availability to sustain growth and yield. Long-term field trials should be conducted to determine the effectiveness of CRH and WC in remediating Cd contamination in paddy soils across different soil environments with varying soil fertility status, nutrient management and cadmium concentrations.

Author's contribution

VUUltra - Designed and conducted the experiments, review data analysis and interpreted the results. JPNunez - Conducted the experiment and performed analyses and interpretation of data. SCLee - Supervised the project implementation, secure funding and check final output.

REFERENCES

Ahmendna, M., W. E. Marshall and R. M. Rao. 2000. Production of granular activated carbon from select and agricultural by-products and evaluation of their physical chemical and adsorption properties. Biores. Technol. 71: 113-123.

Arao, T., S. Ishikawa, M. Murakami, K. Abe, Y. Maejima and T. Makino. 2010. Heavy metal contamination of agricultural soil and countermeasures in Japan. Paddy Water Environ. 8(3): 247-257.

Beesley, L. and M. Marmiroli. 2011. The immobilization and retention of soluble arsenic, cadmium and zinc by biochar. Environ. Pollut. 159(2): 474-480.

Beesley, L., E. Moreno-Jiménez, J. L. Gomez-Eyles, E. Harris, B. Robinson and T. Sizmur. 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environ. Pollut. 159(12): 3269-3282.

Bian, R., S. Joseph, L. Cui, G. Pan, L. Li, X. Liu, A. Zhang, H. Rutlidge, S. Wong, C. Chia and C. Marjo. 2014. A three-year experiment confirms continuous immobilization of cadmium and lead in contaminated paddy field with biochar amendment. J. Hazard. Mater. 272: 121-128.

Bremner, J. M. and D. R. Keeney. 1965. Steam distillation methods for determination of ammonium, nitrate and nitrite. Anal. Chim. Acta. 32: 485-495.

Cao, X., L. Ma, Y. Liang, B. Gao and W. Harris. 2011. Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environ. Sci. Technol. 45(11): 4884-4889.

Chaney, R. L., P. G. Reeves, J. A. Ryan, R. W. Simmons, R. M. Welch, and J. S. Angle. 2004. An improved understanding of soil Cd risk to humans and low cost methods to phytoextract Cd from contaminated soils to prevent soil Cd risks. Biometals. 17(5): 549-553.

Chun, Y., G. Y. Sheng, C. T. Chiou and B. S. Xing. 2004. Compositions and sorptive properties of crop residue-derived chars. Environ. Sci. Technol. 38: 4649-4655.

Cui, L., L. Li, A. Zhang, G. Pan, D. Bao and A. Chang. 2011. Biochar amendment greatly reduces rice Cd uptake in a contaminated paddy soil: a two-year field experiment. Bioresources. 6(3): 2605-2618.

Deenik, J. L, T. McClellan, G. Uehara, M. J. Antal and S. Campbell. 2010. Charcoal volatile matter content influences plant growth and soil nitrogen transformations. Soil Sci. Soc. Am. J. 74(4): 1259-1270.

Dong, D., Q. Feng, K. McGrouther, M. Yang, H. Wang and W. Wu. 2015. Effects of biochar amendment on rice growth and nitrogen retention in a waterlogged paddy field. J. Soils Sediments. 15(1): 153-162.

Fujine, K. 2014. FlashEA 1112 elemental analyzer (CHNS): User guide. Int. Ocean Discov. Program. 1: 1-12.

Houben, D., L. Evrard and P. Sonnet. 2013. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. Chemosphere. 92(11): 1450-1457.

Inyang, M., B. Gao, P. Pullammanappallil, W. Ding and A. R. Zimmerman. 2010. Biochar from anaerobically digested sugarcane bagasse. Biores. Technol. 101(22): 8868-8872.

Inyang, M., B. Gao, W. Ding, P. Pullammanappallil, A. R. Zimmerman and X. Cao. 2011. Enhanced lead sorption by biochar derived from anaerobically digested sugarcane bagasse. Sep. Sci. Technol. 46(12): 1950-1956.

Inyang, M., B. Gao, Y. Yao, Y. Xue, A. R. Zimmerman, P. Pullammanappallil and X. Cao. 2012. Removal of heavy metals from aqueous solution by biochars derived from anaerobically digested biomass. Biores. Technol. 110: 50-56.

Jeong, K. W., B. S. Kim, V. U. Ultra Jr and S. C. Lee. 2015. Effects of rhizosphere microorganisms and wood vinegar mixtures on rice growth and soil properties. Korean J. Crop. Sci. 60(3): 355-365.

Jiang, T. Y., J. Jiang, R. K. Xu and Z. Li. 2012. Adsorption of Pb (II) on variable charge soils amended with rice-straw derived biochar. Chemosphere. 89(3): 249-256.

Jung, M. C. and I. Thornton. 1996. Heavy metal contamination of soils and plants in the vicinity of a lead-zinc mine, Korea. Appl. Geochem. 11(1): 53-59.

Kashem, M. A. and B. R. Singh. 2001. Metal availability in contaminated soils: I. Effects of flooding and organic matter on changes in Eh, pH and solubility of Cd, Ni and Zn. Nutr. Cyclic. Agroecosyst. 61(3): 247-255.

Khan, S., C. Chao, M. Waqas, H. P. H. Arp and Y. G. Zhu. 2013. Sewage sludge biochar influence upon rice (*Oryza sativa* L) yield, metal bioaccumulation and greenhouse gas emissions from acidic paddy soil. Environ. Technol. 47(15): 8624-8632.

Kitamura, T., T. Taguchi and H. Katayama. 2002. The effects of carbonizing conditions on the specific surface area of charcoal from sugi (*Cryptomeria japonica*) wood II: the effects of carbonizing temperature and carbon dioxide concentration in carbonizing atmospheres. Mokuzai Gakkaishi. 48(4): 272-279.

Ko, B. G., S. J. Park, D. B. Jung, M. K. Kim, G. Y. Kim, S. Y. Hong and D. B. Lee. 2010. Characteristics of soil heavy metal contents in the agricultural areas near closed mine in Korea**.** 19th World Congress of Soil Science, Soil Solutions for a Changing World. 1-6, August 201. Published on DVD, Brisbane, Australia.

Lehmann, J., M. C. Rillig, J. Thies, C. A., Masiello, W. C. Hockaday and D. Crowley. 2011. Biochar effects on soil biota – A review. Soil Biol. Biochem. 43(9): 1812-1836.

Li, Y., X. Hu, X. Song and T. Sun. 2012. Remediation of cadmium-contaminated soil by extraction withpara-sulphonato-thiacalix-4-arene, a novel supramolecular receptor. Environ. Pollut. 167: 93-100.

Lucchini, P., R. S. Quilliam, T. H. DeLuca, T. Vamerali and D. L. Jones. 2014. Does biochar application alter heavy metal dynamics in agricultural soil? Agric. Ecosyst. Environ. 184: 149-157.

Makino, T., H. Takano, T. Kamiya, T. Itou, N. Sekiya, M. Inahara and Y. Sakurai. 2008. Restoration of cadmium-contaminated paddy soils by washing with ferric chloride: Cd extraction mechanism and bench-scale verification. Chemosphere. 70(6): 1035-1043.

Makino, T., T. Kamiya, H. Takano, T. Itou, N. Sekiya, K. Sasaki and K. Sugahara. 2007. Remediation of cadmium-contaminated paddy soils by washing with calcium chloride: Verification of on-site washing. Environ. Pollut. 147(1): 112-119.

Nabulo, G., H. Oryem-Origa and M. Diamond. 2006. Assessment of lead, cadmium, and zinc contamination of roadside soils, surface films, and vegetables in Kampala City, Uganda. Environ. Res. 101(1): 42-52.

Nicholson, F. A., S. R. Smith, B. J. Alloway, C. Carlton-Smith and B. J. Chambers. 2003. An inventory of heavy metals inputs to agricultural soils in England and Wales. Sci. Total Environ. 311: 205-219.

Nziguheba, G. and E. Smolders. 2008. Inputs of trace elements in agricultural soils via phosphate fertilizers in European countries. Sci. Total Environ. 390: 53-53.

Pratiwi, E. P. A. and Y. Shinogi. 2016. Rice husk biochar application to paddy soil and its effects on soil physical properties, plant growth, and methane emission. Paddy Water Environ. 1: 1-12.

Pansu, M. and J. Gautheyrou. 2007. Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods, Springer Science & Business Media, New York.

RDA. 1999. (Rural Development Administration, Korea). Fertilization Standard of Crop Plants, National Institute of Agricultural Science and Technology, Suwon, p. 148.

Sartape, A. S., A. M. Mandhare, P. P. Salve, D. K. Pawar and S. S. Kolekar. 2013. Kinetic and equilibrium studies of the adsorption of Cd (II) from aqueous solutions by wood apple shell activated carbon. Desalination Water Treat. 51(22-24):4638-4650.

Singh, B. P., B. J. Hatton, B. Singh, A. L. Cowie and A. Kathuria. 2010. Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. J. Environ. Qual. 39(4): 1224-1235.

Tang, X., Q. Li, M. Wu, L. Lin and M. Scholz. 2016. Review of remediation practices regarding cadmium-enriched farmland soil with particular reference to China. J. Environ. Manage. 181: 646-662.

Ultra, V. U. Jr. and S. H. Han. 2015. Elevated atmospheric temperature and CO_2 altered the growth, carbon, and nitrogen distribution and the rhizosphere properties of *Platanus occidentalis* L. seedlings. Turk. J. Agric. Forest. 39(5): 679-691.

US-EPA. 2007. U. S. Environmental Protection Agency, Distributed Structure-Searchable Toxicity Database. Available from: http://www.epa.gov/ncct/dsstox/. [Last accessed on 2013 Feb 16].

Xie, Z., Y. Xu, G. Liu, Q. Liu, J. Zhu, C. Tu, J. E. Amonette, G. Cadisch, J. W. Yong and S. Hu. 2013. Impact of biochar application on nitrogen nutrition of rice, greenhouse-gas emissions and soil organic carbon dynamics in two paddy soils of China. Plant Soil. 370(1-2): 527-540.

Yuan, S., Z. Zheng, J. Chen and X. Lu. 2009. Use of solar cell in electrokinetic remediation of cadmium-contaminated soil. J. Hazard. Mater. 162(2): 1583-1587.

Campbell, C. R. and C. O. Plank. 1998. Preparation of plant tissue for laboratory analysis. Methods for Plant Analysis, CRC Press, Boca Raton, FL, p. 37.

Zhang, A., R. Bian, G. Pan, L. Cui, Q. Hussain, L. Li, J. Zheng, J. Zheng, X. Zhang, X. Han and X. Yu. 2012. Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: A field study of 2 consecutive rice growing cycles. Field Crops Res. 127: 153-160.