

Effect of Biochar on Chemical Forms of Cd in Rice

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ABSTRACT: A pot experiment of ‘Shennong 9816’ treated by 0, 2 mg kg⁻¹ cadmium(Cd) and 0, 1.4, 14 g kg⁻¹ biochar were conducted to investigate the effect of biochar on the chemical forms of Cd in rice. The sequence chemical extraction method was used to study chemical forms of Cd in roots, stems and leaves of rice. Results indicated that the highest Cd concentration in vegetative tissues was F3 (extracted by 1 M NaCl), followed by F4 and F5 (extracted by 2% HAC and 0.6 M HCl, respectively). Biochar reduced Cd in F1 and F2 (extracted by 80% ethanol and d-H₂O, respectively). In conclusion, biochar could reduce the pollution risk of Cd in the food chain, however, the effect of biochar detoxification had not worked from the total Cd contents of rice organs at tillering stage.

KEYWORD: Cd; biochar; chemical forms; rice.

1 INSTRUCTION

Cadmium (Cd), as a widespread heavy metal, is released into the environment due to the industrial and agricultural activities. Meanwhile, Cd can be absorbed by plants and highly accumulated in tissues. For humans, due to its high soil-plant mobility, Cd can be transferred to the food chain, posing a serious problem on safe food production to threaten human health [1].

There is some evidence that Cd chemical forms may be associated with tolerance and detoxification in plants. This is also proven by the study of Cd chemical forms in *Phytolacca americana* L. [2], *Kandelia obovata* (S., L.) Yong [3] and *Porphyra yezoensis*[4].

As one of the most important crops globally, especially in Asia, rice provides staple food for over 60% population in China. The Cd of rice is a important source adding the Cd to human body.

Previous studies were mainly concentrated on the accumulation of heavy metals, effect of heavy metals on plant growth, and effect of root exudates on heavy metal toxicity [5, 6]. However, to our knowledge, few articles had reported how the biochar affects the chemical forms of Cd in rice.

Biochar is a stable carbon-rich product obtained from pyrolysis of biomass with little oxygen. It is a newly developed environmental remediation material and has garnered research attention in recent years. Biochar has multi-functional properties that make it a promising and effective environmental

adsorbent to remove organic and inorganic pollutions in soil and water.

The purpose of this study was to estimate both the concentrations of Cd chemical forms in rice at different growth stages under different biochar and Cd levels and to explore the potential mechanisms behind these dynamics.

2 MATERIALS AND METHODS

2.1 Soil and plant preparation

Pot experiments were conducted at Shenyang Agriculture University, Liaoning province, China in 2014. The original brown soil used in this study was selected from farmland. The soil were air-dried, passed through a 5-mm sieve, weighted, and allowed 15.0 kg to be placed in plastic pots. The soil pH was 5.9; its organic matter content, alkali-hydrolyzable N, available P and available K were 1.18%, 107.33 mg kg⁻¹, 34.52 mg kg⁻¹ and 98.41 mg kg⁻¹, respectively. The concentrations of total Cd and extractable Cd in soil were 0.15 mg kg⁻¹ and 0.09 mg kg⁻¹, respectively. Besides, the concentrations of total cadmium in biochar was 0.6 mg kg⁻¹.

Shengnong 9816, a kind of rice seed, was selected in this experiment. When seedlings grew onto 5-leaf stage, the uniform plants were selected and transplanted to pot on 23th May, 4 points per pot, 2 seedlings per point. Fertilizer was applied per pot after transplanting as follows: Urea 0.428 g kg⁻¹ soil, CaHPO₄ 2H₂O 0.523 g kg⁻¹ soil, K₂SO₄ 0.247 g kg⁻¹ soil, and the urea 1.5g was added to per pot at

tillering stage. The plant was taken care of according to cultivation system. Sampling period: tillering stage, filling stage and dough stage.

2.2 Laboratory processing and sampling strategy

Two Cd treatments, Cd0 (Cd = 0 mg kg⁻¹ DW soil) and Cd2 (Cd = 2 mg kg⁻¹ DW soil), were jointly applied with three biochar (C) treatments, C0 (C = 0 g kg⁻¹ DW soil), C21 (C = 1.4 g kg⁻¹ DW soil, 21 gram per pot) and C210 (C = 14 g kg⁻¹ DW soil, 210 gram per pot). The Cd0 and Cd2 treatments were obtained by mixing the original soil with Cd. Cd as cadmium chloride was added to each pot. There are six treatments containing the controls (no biochar and Cd added) with 12 replicates. At harvest, the whole plants were separated into roots, stems and leaves. The roots were soaked in 20 mmol L⁻¹ Na₂-EDTA for 15 min to remove metal ions adhering to the root surface, and roots, stems and leaves were washed with distilled water. All plant samples were dried in an oven for 30 min at 105 °C, and then at 70 °C to a constant weight through 2 mm sieve for further experiment. Cd concentrations of roots, stems and leaves were determined by mixed-acid (HNO₃-HClO₄).

2.3 Extraction of chemical forms of Cd

Tessier's sequential extraction procedure was used. According to reference [7], roots, stems and leaves were chosen by dry materials. Six Cd chemical forms were extracted in the order of the extraction solutions by 80% ethanol in F1, distilled water (d-H₂O) in F2, 1 M NaCl in F3, 2% HAC in F4, 0.6 M HCl in F5 and Cd in the residue was F6.

Each of the pooled supernatant solution and the residue were then evaporated on an electric-plate at 75 °C to constant weight, then digested at 200 °C with an acid oxidative mixture of HNO₃:HClO₄ (4:1, v:v). Root, stem, leaves and extractable concentrations of Cd were tested by atomic absorption spectrophotometry (AAS, Hitachi Z-5000, Japan),

2.4 Statistical analysis

All experimental data were analysis by the SPSS statistical software package (version 17.0) and Excel2010. All data reported in this experiment were presented as means ± standard deviation (SD). Duncan's tests was used to determine the significant differences between means (p<0.05).

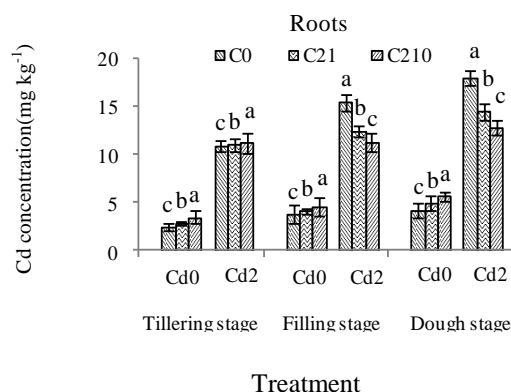
3 RESULTS AND DISCUSSION

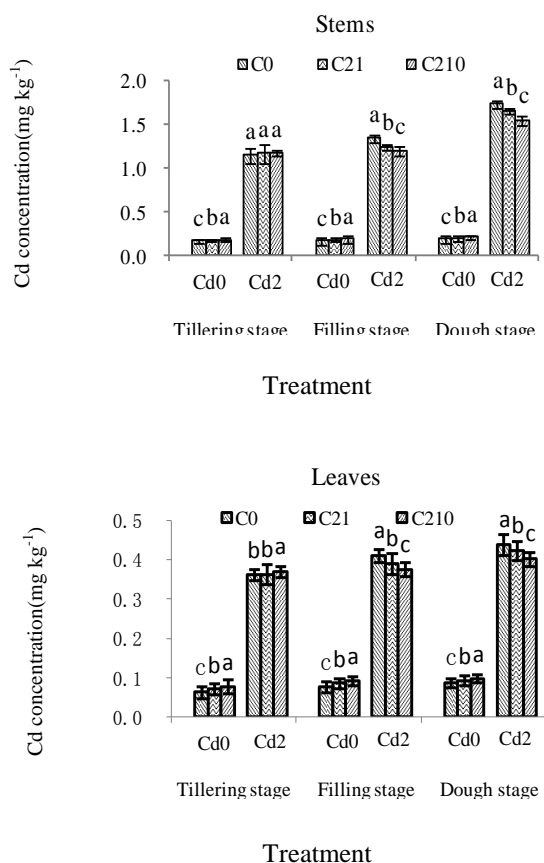
3.1 Cd accumulation and transformation

Fig. 1 showed that the total Cd concentrations in different organs decreased following the order of

dough stage > filling stage > tillering stage. Besides, the Cd concentrations in rice at three growth stages were highest in the roots, followed by the stems and leaves.

In addition, adding biochar to soil had significant effects on Cd concentrations in the roots and leaves (P<0.05). In the whole rice growth period, the Cd concentrations of Cd2 treatments in roots, stems and leaves were higher than those of Cd0 treatments. In Cd0 treatments, Cd concentrations were increased significantly (P<0.05) with the increasing of biochar at every growth stage. This might because the biochar used in this experiment itself contained a certain amount of Cd. When biochar applied to the soil, the Cd released slowly over time, and to be absorbed by the rice finally. In Cd2 treatments, at tillering stage, Cd concentrations in roots and leaves increased with the increasing of biochar content. This showed the effect of biochar detoxification had not worked from the total Cd contents of rice organs. The reason needed to be further analyzed. At the other two stages, the change tendency of Cd concentrations in roots, stems and leaves were similar, all decreasing with increasing the amount of biochar, and achieved significant level (p<0.05). The reasons were probably multifactorial. Firstly, biochar could increase soil pH, reduce the concentrations of exchangeable Cd. Only when the effective Cd concentrations in soil reduced, could the Cd absorbed by plants be decreased. Secondly, the heavy metal could be fixed by biochar, which could reduce the heavy metals exposure in rhizosphere and migration to the plants. Namgay et al. (2010) [8] found biochar could significantly reduce the effectiveness of corn biological Cd. In this experiment, the heavy metal Cd in soil was fixed by biochar and meanwhile the effectiveness of Cd was reduced in this way. Thirdly, biochar could increase plant biomass and then would be diluting the Cd concentrations. Reference [9] reported that high biomass enabled peanut plants to retain larger amount of Cd within their vegetative tissues while maintaining a lower Cd concentrations due to dilution effect. But overall, in the whole growth period of rice, the Cd concentrations in roots, stems and leaves showed the tendency of roots > stems > leaves.





3.2 Chemical forms of Cd

Chemical forms of Cd in rice organs were explored under different biochar content (Table 1).

As showed in Table 1, Cd concentrations of different chemical forms were Cd2 > Cd0. With the passage of growing period, the tendency of Cd concentrations of the same treatment was dough stage > filling stage > tillering stage. Besides, the concentrations of different chemical forms differed significantly among roots, stems and leaves. The study showed that both concentrations and percentage of Cd were clearly more in roots than in stems and leaves. We concluded that for the same treatment, the highest Cd concentration was F3 (extracted by 1 M NaCl), followed by F4 and F5 (extracted by 2% HAC and 0.6 M HCl, respectively), the rest forms were lower with the increasing biochar. In roots, the proportion of F3, F4 and F5 were respectively 36.54%-49.04%, 16.41%-31.00%, 9.34%-14.46%. In stems, 29.74%-43.37%, 17.70%-26.93%, 8.86%-13.27%, respectively. In leaves, 35.49%-49.06%, 18.50%-26.79%, 8.23%-12.88%, respectively. The six kinds of chemical forms were in the same plant, but the allocation proportion in roots, stems and leaves were different.

The chemical forms of heavy metals were closely related to their biological activities and detoxification in plant. The different chemical forms of Cd which could be extracted by different extracting agents related to the toxicity degree and migratory ability of Cd. For instance, inorganic Cd (F1, extracted by 80% ethanol) and water-soluble Cd

(F2, extracted by d-H₂O) exhibited remarkably stronger migratory capacity and were more deleterious to plant cells in comparison with undissolved Cd phosphate (F4) and Cd oxalic (F5) [10]. Previous studied related to Cd tolerance in *Kandelia obovate* (S., L.) Yong [3] also showed that a majority of Cd both in roots and leaves was associated with pectates and proteins (F3), followed by Cd phosphate complexes (F4). This conclusion was the same as ours. Of course, there were different results. For example, in *Phytolacca americana* L. F3 was the dominating form of Cd in leaves and stems, but the subdominant traction was F1 [2]. These differences might due to the varied Cd and biochar levels applied or the diverse plant species tested. F1 and F2 were mainly combined with nitrate ions, chlorides, organic acids and dihydric phosphates [11], which contributed most of Cd stress in plants. However, F3 was found to bind to proteins and pectic acids, while the pectic acids could fix the Cd. As Cd had a strong affinity to proteins or sulfhydryl compounds (-SH) and other side chains, it could easily combine with proteins [12] and disturb the enzyme activity, so NaCl extractant might be responsible for the adaptation of plants to Cd stress. This was also the reason why F3 was the most in the body. Besides, F4, F5 and F6 showed the weakest migration activity and were less harmful to the plants.

There were significant differences in the Cd2 treatments with the different applying biochar content ($p < 0.05$), and the remediation effect of C210 was more obvious than C21. Compared with the treatment added no biochar but Cd, in roots, each chemical extractant were reduced respectively by 42.57%, 48.41%, 30.63%, 20.71%, 10.67%, 14.97%. In stems, 11.95%, 16.25%, 5.14%, 2.46%, 10.00%, 3.33%, respectively. In leaves, 16.18%, 18.46%, 7.30%, 5.44%, 4.28%, 2.96%, respectively. As could be seen in the six kinds of chemical speciation, F1 and F2 were fallen the most, which might because F1 and F2 had the strongest capacity of transformation, and it was the biochar that makd the Cd transformation from easily forms into difficult forms. To some extent, the biochar reduced the toxicity of Cd in rice.

From Table 1, we found that after adding biochar and Cd, the Cd concentrations of different chemical forms among roots, stems and leaves existed significant differences. In the Cd0 treatments, the Cd concentrations of different chemical forms among roots, stems and leaves at three different growth stages were increased with the increasing biochar content. The percentage of F1, F2 increase and F4, F5 decreased (Table 1). In the Cd2 treatments, at tillering stage, the Cd concentrations and percentage of F1, F2 and F3 in roots, stems and leaves decreased with the increasing biochar content, while F4, F5 and F6 increased. At the other two stages, the Cd concentrations of different chemical forms in

roots, stems and leaves decreased with the increasing biochar concentrations. Besides, the percentage of F1 and F2 decreased, F4 and F5 increased. Two group comparison results showed that biochar significantly enhanced both the concentrations and

proportion of F1 and F2 for the Cd0 treatments, while it decreased in the Cd2 treatments. So the adsorption and detoxification effects of biochar were more obvious in the treatment added Cd.

TABLE I Concentrations of different chemical forms of Cd in rice.

Stages	Organs	Treatment	Cd content (mg kg ⁻¹)					
			F1	F2	F3	F4	F5	F6
Tillering	Roots	C0Cd0	0.130±0.002c	0.198±0.006c	1.202±0.028c	0.538±0.016c	0.392±0.016c	0.267±0.014c
		C21Cd0	0.150±0.005b	0.257±0.012b	1.373±0.018b	0.608±0.013b	0.419±0.010b	0.282±0.017b
		C210Cd0	0.281±0.014a	0.333±0.016a	1.660±0.038a	0.718±0.018a	0.488±0.021a	0.296±0.009a
		C0Cd2	0.728±0.221a	1.260±0.321a	5.079±1.406a	2.020±0.688c	1.401±0.421c	0.436±0.205c
		C21Cd2	0.560±0.115b	1.191±0.201b	4.816±0.553b	2.445±0.178b	1.478±0.218b	0.518±0.010b
		C210Cd2	0.532±0.110c	0.824±0.151c	4.658±0.418c	2.998±0.640a	1.535±0.090a	0.639±0.035a
	Stems	C0Cd0	0.016±0.002b	0.013±0.001b	0.065±0.005b	0.031±0.002b	0.017±0.002a	0.013±0.001b
		C21Cd0	0.017±0.001ab	0.014±0.002b	0.068±0.003a	0.033±0.002ab	0.017±0.002a	0.013±0.006b
		C210Cd0	0.010±0.004a	0.016±0.006a	0.070±0.012a	0.033±0.009a	0.018±0.004a	0.015±0.001a
		C0Cd2	0.139±0.036a	0.140±0.035a	0.376±0.084a	0.244±0.061c	0.143±0.043c	0.094±0.016c
		C21Cd2	0.122±0.016b	0.127±0.022b	0.364±0.076b	0.297±0.012b	0.144±0.051b	0.105±0.013b
		C210Cd2	0.110±0.024c	0.105±0.013c	0.346±0.109c	0.313±0.041a	0.154±0.014a	0.135±0.009a
	Leaves	C0Cd0	0.004±0.001a	0.005±0.001a	0.029±0.002a	0.013±0.001a	0.006±0.001a	0.006±0.001b
		C21Cd0	0.005±0.001a	0.005±0.001a	0.031±0.003a	0.014±0.001a	0.007±0.001a	0.008±0.002a
		C210Cd0	0.006±0.002a	0.007±0.001a	0.033±0.002a	0.015±0.002a	0.007±0.001a	0.009±0.001a
		C0Cd2	0.038±0.022a	0.038±0.026a	0.139±0.063a	0.075±0.033c	0.045±0.021b	0.026±0.020a
		C21Cd2	0.036±0.011b	0.034±0.015b	0.133±0.042b	0.086±0.041b	0.047±0.032ab	0.027±0.012a
		C210Cd2	0.030±0.026c	0.033±0.021b	0.131±0.039b	0.099±0.022a	0.048±0.016a	0.029±0.024a
Filling	Roots	C0Cd0	0.173±0.003c	0.241±0.004c	1.873±0.027c	0.714±0.019c	0.511±0.014c	0.316±0.008c
		C21Cd0	0.235±0.008b	0.274±0.011b	1.947±0.041b	0.768±0.011b	0.554±0.011b	0.353±0.002b
		C210Cd0	0.294±0.019a	0.368±0.003a	2.121±0.014a	0.864±0.008a	0.591±0.005a	0.371±0.016a
		C0Cd2	0.832±0.209a	2.067±0.237a	6.400±0.940a	4.045±0.583c	1.514±0.129c	0.614±0.066c
		C21Cd2	0.678±0.194b	1.593±0.113b	4.636±1.099b	3.588±0.656b	1.488±0.162b	0.497±0.336b
		C210Cd2	0.567±0.144c	1.224±0.089c	4.149±0.698c	3.517±0.838a	1.463±0.126a	0.424±0.199a
	Stems	C0Cd0	0.011±0.002b	0.016±0.001b	0.068±0.006c	0.031±0.002b	0.015±0.004a	0.016±0.001a
		C21Cd0	0.015±0.001a	0.017±0.001ab	0.071±0.014b	0.033±0.003a	0.016±0.002a	0.017±0.002a
		C210Cd0	0.017±0.001a	0.019±0.002a	0.073±0.009a	0.034±0.007a	0.015±0.002a	0.018±0.001a
		C0Cd2	0.125±0.043a	0.164±0.032a	0.476±0.014a	0.290±0.065a	0.146±0.053a	0.128±0.018a
		C21Cd2	0.125±0.021b	0.137±0.043b	0.433±0.076b	0.286±0.041b	0.136±0.026a	0.121±0.008b
		C210Cd2	0.123±0.032c	0.125±0.016b	0.423±0.095c	0.282±0.074c	0.121±0.043b	0.120±0.021b
	Leaves	C0Cd0	0.004±0.004a	0.006±0.003b	0.037±0.012b	0.014±0.004b	0.007±0.002a	0.007±0.001b
		C21Cd0	0.005±0.002a	0.008±0.002ab	0.041±0.009a	0.016±0.001a	0.007±0.001a	0.009±0.003ab
		C210Cd0	0.006±0.001a	0.009±0.002a	0.041±0.021a	0.017±0.003a	0.008±0.001a	0.010±0.001a
		C0Cd2	0.044±0.021a	0.042±0.041a	0.158±0.073a	0.089±0.041a	0.049±0.021a	0.029±0.008a
		C21Cd2	0.040±0.012b	0.037±0.011b	0.151±0.055b	0.085±0.011b	0.048±0.011ab	0.029±0.021a
		C210Cd2	0.036±0.039c	0.034±0.019c	0.150±0.014b	0.082±0.009c	0.047±0.009b	0.027±0.014a
Dough	Roots	C0Cd0	0.213±0.001c	0.258±0.004c	2.027±0.082c	0.745±0.109c	0.619±0.022c	0.418±0.054c
		C21Cd0	0.275±0.018b	0.324±0.012b	2.386±0.054b	0.805±0.016b	0.651±0.012b	0.463±0.010b
		C210Cd0	0.313±0.009b	0.399±0.004a	2.764±0.071a	0.938±0.044a	0.705±0.020a	0.516±0.058a
		C0Cd2	1.012±0.171a	2.660±0.374a	6.826±1.219a	4.944±1.134a	1.676±0.231a	0.822±0.015a
		C21Cd2	0.755±0.212b	1.818±0.158b	5.374±0.761b	4.245±1.867b	1.526±0.148b	0.761±0.010b
		C210Cd2	0.581±0.106c	1.372±0.257c	4.735±1.056c	3.920±0.992c	1.497±0.314c	0.699±0.084c
	Stems	C0Cd0	0.016±0.001b	0.016±0.003b	0.075±0.012c	0.033±0.003b	0.019±0.002a	0.019±0.001a
		C21Cd0	0.017±0.002b	0.019±0.001a	0.078±0.024b	0.034±0.002ab	0.020±0.002a	0.020±0.001a
		C210Cd0	0.020±0.004a	0.021±0.006a	0.082±0.005a	0.035±0.001a	0.020±0.001a	0.021±0.002a
		C0Cd2	0.202±0.041a	0.235±0.032a	0.549±0.043a	0.378±0.014a	0.205±0.015a	0.150±0.008a
		C21Cd2	0.188±0.024b	0.208±0.021b	0.535±0.086b	0.370±0.052b	0.192±0.018b	0.146±0.011b
		C210Cd2	0.178±0.013c	0.196±0.005c	0.521±0.021c	0.369±0.017b	0.185±0.005c	0.145±0.029b
	Leaves	C0Cd0	0.004±0.001b	0.007±0.002b	0.040±0.021b	0.017±0.003a	0.009±0.001a	0.010±0.001a
		C21Cd0	0.005±0.004ab	0.008±0.001ab	0.042±0.009ab	0.018±0.002a	0.009±0.001a	0.010±0.002a
		C210Cd0	0.007±0.001a	0.009±0.005a	0.043±0.014a	0.018±0.005a	0.010±0.002a	0.011±0.001a
		C0Cd2	0.045±0.008a	0.046±0.012a	0.171±0.079a	0.094±0.035a	0.049±0.023a	0.034±0.016a
		C21Cd2	0.042±0.032b	0.041±0.041b	0.165±0.021b	0.093±0.071a	0.049±0.015ab	0.033±0.019a
		C210Cd2	0.037±0.019c	0.037±0.023c	0.159±0.047c	0.089±0.019b	0.047±0.042b	0.033±0.006a

4 CONCLUSION

In summary, plants cultivars differed in accumulation and translocation of Cd, as well as its chemical forms. In rice, Cd analysis in different plant organs demonstrated that large proportion of Cd was stored in roots. This could be considered as the first barrier to defend itself against Cd toxicity. This study also concluded that Cd was accumulated high concentrations in the rice at dough stage than the other stages. Chemical forms research identified most of Cd was extracted by 1M NaCl, which was integrated with pectates and protein, for Cd being fixed by pectic acids. Compared with Cd0 treatments, biochar deficiency could decrease Cd uptake and accumulation in rice. With increasing biochar content, F1 and F2, which had greater toxicity and stronger migration ability than the other forms of Cd decrease. However the effect of biochar detoxification had not worked from the total Cd contents of rice organs at tillering stage.

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