Effects of single and combined pollution of Pb, Cd and Cu on growth, metal uptake and translocation in rice

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Abstract. A field experiment was conducted to investigate the single and combined of lead (Pb), cadmium (Cd), and cooper (Cu) on the growth and development of rice plants and the uptake of these heavy metals by rice. The results revealed that the growth and development of rice plants were more affected by combined pollution than by single pollution. The Pb and Cd concentrations in grains under combined pollution are higher than that in single application at same level, indicating that rice grown in soils under combined pollution poses more risk to human health than under single pollution. Biological concentration factors (BCF) of different treatments of Cd and Cu change in the order as single pollution< combined pollution, implying that the upward transporting abilities of Cd and Cu absorbed by rice plants was significantly increased under combined pollution.

1. Introduction

Anthropogenic activities in modern society including mining, smelting, land application of sewage sludge, fertilization, and intensive use of pesticides and herbicides result in heavy metal accumulation in soils [1, 2].Soil pollution by heavy metals is increasingly concerned worldwide because it not only influences soil quality, but also affects crop production ,crop quality ,and food safety and threatens the health and life of animals and humans through food chain [3,4].

Rice is the staple food for two thirds of the Chinese population [5,6], and due to such large consumption, rice is a major heavy metal source to humans in China. it is necessary to perform paddy field experiments under single and combined pollutions. This type of simulation study will help to understand how rice tissues with heavy metal accumulation grow in single and combined pollutions, and will provide growers with scientific evidence on how to predict and avoid their intake of potential harmful elements through rice consumption.

The objective of this study was to investigate the single and combined effects of Pb, Cd and Cu on rice growth. Furthermore, the accumulation and translation of Pb,Cd and Cu in the aerial plants parts under field conditions were also studied.

2. Materials and methods

2.1Experimental Design and Chemical Sampling Protocol

The experiment was performed in a paddy field at a long-term experiment station located in Chenguang Village (44°12′N, 125°33′E), Jilin province of Northeast China during the rice-growing season (from mid-May to late September) in 2014. The physicochemical properties of this paddy soil are shown in Table 1.

In order to investigate the single and combined effects of heavy metals on rice growth, three types (control, single-factor, and three-factor tests) including twelve treatments of rice plantation were designed. The added heavy metals were $Pb(NO_3)_2$, $Cd(NO_3)_2$ and $Cu(SO_4)_2$. According to China Environmental Quality Standard for Soils GB15618-1995 and the results of laboratory-based toxicity tests, we assigned two concentration levels under single pollution: low concentration in accordance with Grade II and high concentration with Grade III. Meanwhile five concentration levels were set under combined pollution. The twelve treatments are (mg/kg^{-1}):

Control : 0) no addition

Single metal: 1) 300 Pb; 2) 500 Pb; 3) 0.3 Cd; 4) 1.0 Cd; 5) 100 Cu; 6) 400 Cu

Three metals: 7) 100 Pb + 0.3 Cd + 50 Cu; 8) 300 Pb + 0.3 Cd + 100 Cu; 9) 500 Pb + 3.0 Cd + 400; 10) 700 Pb + 5.0 Cd + 600 Cu; 11) 900 Pb+ 7.0 Cd + 800 Cu

All treatments were conducted in triplicate and the experiment field was divided into cells of 100×100 cm. The cells were arranged in a randomized complete block design in order to minimize experimental errors. Fertilizers were then applied to all plots according to local farming practices.

Table 1 Soil physical and chemical properties of filed site.

Soil type	pH	Organic matter (%)	CEC (cmol kg−1)	Sand (%)	Silt (%)	Clay (%)	Concentration (mg kg−1 dw soil)		
							Pb	Cd	Cu
clay loam	7.84	2.41	20.5	49.25	22.62	28.13	20.18	0.1022	14.71

* pH was determined in a water suspension of soil using a 1:2.5 ratio (soil: deionized water); CEC (cation exchange capacity) was determined using the unbuffered silver-thiourea method; soil texture was analysed using the sedimentary method; the content of organic matter was determined by oxidizing a soil solution with $K_2Cr_2O_7$ and H_2SO_4 at 170–185 °C, and then titrating the solution with $FeSO_4$

A rice cultivar (hybrid rice: Dongdao 4) widely grown in Jinlin province was selected for this experiment. The germinated seeds were cultivated in the paddy field at the control site for one month. Subsequently, the plants were transplanted into the plots at a density of 28 plants m^{-2}. The plants were cultivated from June to September and irrigated depending on the local weather.

2.2 Sample Collection and Preparation

Sampling campaign was conducted during the growing season at maturity. Two rice plants as well as the corresponding soil from the root zone (0-20cm depth) were collected from each cell during each campaign. The corresponding soil samples were hand-collected by using disposable polyethylene gloves. The plants were divided into root, shoot, grain. Concerning that latter sampling campaigns (at maturity) coinciding with rice ripening, rice grains were, in addition, manually separated using a plastic scissors to prevent rice grain loss.

Samples of rice tissue were cleaned in the field with drinking water, and upon entering the laboratory, were washed through immersion in deionized water in an ultrasonic bath upon to remove deposited particles, and then air-dried in polyethylene bags to avoid cross contamination. The mass of each plant sample was recorded in the field after collection and again in the laboratory after oven-drying at 70 °C to constant weight. The oven-dried samples were ground with a stainless steel grinder to pass through a 150-mesh sieve. The soil samples were thoroughly mixed, and air-dried before chemical analysis. Precautions wad taken to avoid any cross-contamination during sample preparation. The grinder was thoroughly cleaned after processing each sample. The powdered samples were transferred into an open plastic dish and separately enclosed in polyethylene bags and then placed in a desiccator kept at ±4 °C.

2.3 Analytical Methods

For soil samples, 0.20 g of soil was digested with a mix of 5 mL of HNO_3 + 1mL of $HClO_4$ + 1 mL of HF. For plant samples, 0.200 g of root, shoot, or grain was digested with a mix of 5 mL of HNO_3 + 1 mL of $HClO_4$.The resultant solutions were each diluted to 25 mL using 2% HNO_3 and then filtered. The concentrations of heavy metals in the filtrate of soil and plants were respectively determined using an AA-6300C flame atomic adsorption spectrophotometer (AAS) and an AA-6300C graphite furnace AAS (GFAAs) following a standard procedure. Analytical reagent blanks were carried throughout the entire sample-preparation and analytical process. The accuracy of the analytical method (±10%) was estimated by analyzing Standard Reference Material (GBW 07405(GSS-5)) obtained from the Center of National Standard Reference Material of China.

2.4 Calculation of Parameters

Biological concentration factor (BCF) of one element in the plant tissues was calculated as follows (Zayed et al., 1998) :

$$BCF = \frac{Cp}{Cs} \qquad (1)$$

where C_p is the concentration in plant tissues at maturity (mg kg^{-1}), and C_s is the concentration in the soil (mg kg^{-1}).

2.5 Statistical analyses

Statistical analyses were carried out on SPSS 16.0 (SPSS Inc.,USA) and Origin 8.0 (Origin Inc., USA). Analysis of variance (ANOVA) was used to assess the effects of treatments on the measured variables (least significant difference at $P = 0.05$).

3. Results and discussion

3.1 Effects of combined pollution on growth and development of rice

The effects on the growth and development of rice plants are obviously different among the control condition, single pollution and combined pollution. Heavy metal stress shows suppressive effects on the overall growth of rice plants which is manifested as a reduced number of tillers and simultaneous decline in rice yield and dry weight. In terms of yield, the rice yield under single pollution is 8.03-35.43 % lower than under the control condition (Table2). The rice yield under combined treatments is lower than that treated by single treatments except the Pb_{100}+$Cd_{0.3}$+Cu_{50} (higher than single Cu treatments). Compared with the control condition, the rice yield under combined pollution is reduced by 23.44-56.10%. In general, the rice yield in this study decreases in the order as (excluded under Pb_{100}+$Cd_{0.3}$+Cu_{50}): control>single pollution>combined pollution. Similar results were reported by Zhou *et al.* (2003) [7] that the yields of rice plants treated by single elements and double elements were 3–23.2 % lower than under the control conditions.

Table 2 Influences of different treatments on yield of rice plant.

Treatment	Relative yield (%)	Treatment	Relative yield (%)
Control	100	Control	100
Pb 300	82.69	Pb 100 Cd 0.3 Cu 50	76.56
Pb 500	89.60	Pb 300 Cd 1.0 Cu 100	62.47
Cd 0.3	91.97	Pb 500 Cd 3.0 Cu 400	46.12
Cd 1.0	77.96	Pb 700 Cd 5.0 Cu 600	52.29
Cu 100	75.17	Pb 900 Cd 7.0 Cu 800	43.90
Cu 400	64.57		

3.2Variations of heavy metal accumulations among different treatments

The differences among the twelve treatments in heavy metal concentrations in different plant parts at maturity stages are presented in Tables 3.

Concentrations of heavy metals accumulated in tissues are changeable because of the addition of heavy metals in the tested soil. When the metals were applied singly, concentrations of Pb,Cd and Cu in rice roots, shoots, ears and grains all increased with increasing addition of metal treatments (Tables 3). When 500 mg kg^{-1}of Pb was added to soil, the accumulated amount of Pb in plant tissues increased comparison to the addition of 300 mg kg^{-1} Pb under single-element condition. The increment of Pb concentration in roots, shoots, grains was up to 57.14, 21.31 and 0.66 mg kg^{-1}. Cd concentration in plant tissues with high addition (1.0 mg kg^{-1}) increased compared to low Cd addition (0.3 mg kg^{-1}) under single treatment. The increment of Cd concentration in roots, shoots and grains was up to 0.51, 0.16 and 0.16 mg kg^{-1}. With high addition (400 mg kg^{-1}),Cu accumulated in plant tissues increased compared to low addition (100 mg kg^{-1}) under single treatment. The increment of Cu in roots, shoots and grains was up to 26.62, 3.28 and 0.43 mg kg^{-1}.

Table 3 Heavy metal concentrations in different parts of different treatments at maturity (mg kg−1).

Treatment	Root			Shoot			Grain		
	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu
CK	19.07	0.17	17.87	2.52	0.03	2.21	0.13	0.01	0.79
Pb 300	124.71	--	--	13.75	--	--	1.92	--	--
Pb 500	181.85	--	--	35.06	--	--	2.58	--	--
Cd 0.3	--	0.83	--	--	0.14	--	--	0.07	--
Cd 1.0	--	1.34	--	--	0.30	--	--	0.23	--
Cu 100	--	--	73.59	--	--	13.59	--	--	2.46
Cu 400	--	--	100.21	--	--	16.87	--	--	2.89
Pb 100 Cd 0.3 Cu 50	99.80	1.28	64.90	9.96	0.44	8.23	1.67	0.21	1.47
Pb 300 Cd 1.0 Cu 100	210.54	3.29	94.26	25.75	0.71	14.26	2.14	0.30	2.54
Pb 500 Cd 3.0 Cu 400	311.94	3.54	118.76	29.18	1.45	18.76	2.68	0.27	3.57
Pb 700 Cd 5.0 Cu 600	302.00	3.80	130.19	31.82	1.77	16.85	3.00	0.44	4.23
Pb 900 Cd 7.0 Cu 800	316.15	3.95	151.15	36.26	1.69	27.81	3.21	0.47	4.39

Furthermore the variations of heavy metal concentrations were huge among the four plant parts as most of the heavy metals absorbed by rice plants were accumulated in the roots. A lessened amount of heavy metals were transported upward from roots to aboveground parts. Heavy metal concentrations changed generally in the order as root>shoot>grain (at maturity stage), suggesting higher sensitivity of roots to metal stress than aerial parts This might be related to the fact that roots are the first organ to be in contact with heavy metals, so much higher amounts of heavy metals accumulate in the root than in other organs[8,9,10]. In case of residues of heavy metals in the rice roots back to the soil, it is better to remove rice roots in seriously polluted areas.

According to the biological features, Pb and Cd are toxic and nonessential elements whereas Cu is an essential element for the growth and development of plants. Thus, the absorption abilities of heavy metals by different plant parts are obviously different. In combination, the data revealed that the amounts of Pb and Cd accumulated in roots increased mostly compare to single element treatments at same added level (Tables 3). Meanwhile, the distributions of Cd and Cu in shoots and Pb,Cd and Cu in grains were augmented. But the Pb concentrations in shoots and the Cu concentration in roots decreased under combined pollution compared to single element treatment at the same added level. It was intuitively determined that the addition of Pb and Cu could promote the Cd absorption by rice tissues, namely the interaction between Pb,Cu and Cd was synergistic. These results are in agreement with others studies. Liu *et al.* (2003) also found that significant positive correlations between Cd and Cu existed in rice roots and leaves[11].Cd, Pb and Zn accumulation in asparagus bean were positively correlated with each other[12]. Zeng *et al.* (2008) found a synergistic interaction between Cd and Pb in slightly and highly contaminated soils[13].

Generally for single pollution, The Pb concentration in rice grain under Pb$_{300}$ and Pb$_{500}$ Pb concentration in rice grain was above 0.2 mg kg^{-1}, defined as the maximum permissible limit (MPL) recommended by Chinese National Standard Agency (GB2762-2012). The grain Cd concentration exceeded 0.1 mg kg^{-1}, the concentration defined as the MPL recommended by GB2762-2012 when 1.0 mg kg^{-1} Cd was added to the tested soil, but did not exceed MPL when 0.3 mg kg^{-1} Cd was added. The concentration of Cu accumulated in rice grain under single pollution was below the MPL of 10 mg kg^{-1} [14]. These findings show that Cu is more relevant to ecological risk assessments than to food safety assessments for rice grown in Cu-contaminated soils.

3.3Biological concentrations of Pb, Cd and Cu under single and combined pollutions

Biological concentration factor (BCF) is an important parameter in heavy metal uptake studies, and has been widely used in soil nutrient cycling (Wang et al., 2009). BCF is defined as the ratio of element content in plant tissues (i.e., roots, shoots and grains) to that in the rooted soils. BCFs of Pb, Cd and Cu in the four parts of rice plants are listed in Table 4.

Average BCF of Pb, Cd and Cu change in the order as roots >shoots>grains in each treatment (Table 4). Although the elevated soil metal concentrations resulted in higher uptake and accumulation, BCF decreased quickly with the increase of soil heavy metal levels. Noticeably, there was comparatively higher bioavailability to plant with lower content of metal in the contaminated soil. BCF for Cd was remarkably higher than Pb and Cu, indicating that more Cd than Pb and Cu was translocated to rice tissues. Average BCFs of Pb in rice tissues (root, shoot and grain) under single treatment were higher than that under combined treatment at same Pb concentration. There was significant difference ($P>0.05$) in BCFs between low Pb treatment (300 mg kg^{-1}) in rice tissues under single and combined conditions at the same level. Opposite values were observed that average BCFs of Cd and Cu in rice tissues (roots, shoots and grains) under single condition were lower than that under combined condition at same Cd and Cu concentrations. No significant difference in BCF of the rice tissues between single and combined conditions at same level was observed ($P>0.05$, Table 4).

Table 4 Biological concentration factor (BCF) of Pb, Cd and Cu in rice tissues of different treatments.

Treatment	BCF								
	Pb			Cd			Cu		
	Roots	Shoots	Grains	Roots	Shoots	Grains	Roots	Shoots	Grains
CK	0.945a	0.125a	0.006b	1.663c	0.257bc	0.107b	0.907b	0.112b	0.040a
Pb 300	0.416c	0.046c	0.006b	--	--	--	--	--	--
Pb 500	0.364c	0.070c	0.005bc	--	--	--	--	--	--
Cd 0.3	--	--	--	2.768b	0.457b	0.231b	--	--	--
Cd 1.0	--	--	--	1.336bc	0.300c	0.228b	--	--	--
Cu 100	--	--	--	--	--	--	0.736b	0.136b	0.025ab
Cu 400	--	--	--	--	--	--	0.251c	0.042bc	0.007b
Pb 100 Cd 0.3 Cu 50	0.998a	0.100ab	0.017a	4.266a	1.451a	0.701a	1.298a	0.165a	0.029a
Pb 300 Cd 1.0 Cu 100	0.702b	0.086c	0.007b	3.295ab	0.707ab	0.301b	0.943ab	0.143ab	0.023ab
Pb 500 Cd 3.0 Cu 400	0.624bc	0.058c	0.005bc	1.181c	0.484c	0.091b	0.297b	0.047b	0.009b
Pb 700 Cd 5.0 Cu 600	0.431c	0.045c	0.004c	0.759c	0.353c	0.088b	0.217c	0.028c	0.007b
Pb 900 Cd 7.0 Cu 800	0.395c	0.045c	0.004c	0.564c	0.241c	0.066b	0.189c	0.035c	0.005b

*BCF= [element concentration in plant tissue]/ [the concentration of element added to test soil].a, b, c Different letters represent significant differences between different treatments ($P<0.05$).

The translocation of heavy metals in a rice plant is an important process, which affects metal accumulation in rice grains. Reportedly, root-to-shoot translocation and xylem loading capacity may be the crucial processes for Zn density in rice grains [15]. Most of Cd accumulated in rice grains is transported via phloem [16]. The present study demonstrated that BCFs of Cd and Cu from soil to rice tissues (root, shoot and grain) were generally insignificantly smaller under single treatments than under combined treatments ($p>0.05$). But the differences among treatments in BCF of Pb from soil to roots, shoots or grains were comparatively high. BCFs of different treatments change in the order as single > combined and the differences were generally significant ($p>0.05$).

4. Summary

The effects of combined pollution of Pb,Cd and Cu on rice yield were sronger than single pollution. The concentrations of Pb,Cd and Cu accumulated in rice tissues under the combined applications were generally higher than under single applications, especially in grains, which is a serious potential threat to human beings. The addition of Pb and Cu could promote the absorption of Cd by rice tissues. Another important and intriguing result is that under combined pollution, rice even grown in soils that Pb and Cd concentration at the grade II of Standards for Soil Environmental Quality of China (GB15618-1995) poses much risk to human health. But Cu is more relevant to ecological risk assessments than to food safety assessments for rice grown in Cu-polluted soils. The present study also provides evidence that the upward transporting ability of Cd and Cu from soil to roots and aboveground parts under the combined pollution conditions is higher than that under single pollution, which is opposite to that of Pb.

This study throws light on the effects on growth and development of rice in metal combined polluted soils. Moreover, systematic research on the mechanisms involved in metal absorption and transportation in crop is needed.

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