EFFECT OF COMBINED DOSES ON THE UPTAKE OF CADMIUM, CHROMIUM AND LEAD BY THE RICE PLANT (*Oryza sativa* L.)

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Abstract

An experiment was carried out to investigate the uptake of cadmium (Cd), chromium (Cr), and lead (Pb) by nine rice cultivars (*Oryza sativa* L.) grown in nutrient solution treated with different doses of heavy metals: ($Cd_{0.15}$+$Cr_{1.5}$+$Pb_{1.5}$) mg/L; ($Cd_{0.2}$+$Cr_{2.0}$+$Pb_{2.0}$) mg/L and ($Cd_{0.25}$+$Cr_{2.5}$+ $Pb_{2.5}$) mg/L treatment. Significant differences existed in the uptake and translocation of Cd, Cr and Pb among rice plant cultivars. Average Pb uptake was found much higher than that of Cd and Cr in all varieties of rice for all doses. No dose dependent relationship was observed in the uptakes of heavy metals by rice plant. The statistical analysis showed that a significant and positive correlation exists between Cr and Pb uptake of plants by all treatments. Except dose 1, no significant correlation existed between Cd and Cr or Pb at moderate and higher doses (doses 2 and 3). The genotypic differences in Cd, Cr and Pb uptake among the rice plants suggested that it is possible to cultivate low heavy metal accumulating rice cultivars through cultivar selection and breading.

Key words: Rice plant, heavy metal, genotypes, cadmium, chromium, lead.

INTRODUCTION

In recent years there has been increasing awareness and concern over heavy metals contamination of soils and the effects produced on the food chain. Many anthropogenic activities, such as leather processing, electroplating, wood preservation (Shanker *et al.* 2005) and production of re-chargeable nickel-cadmium batteries (Jarup 2003) release large amount of heavy metals like chromium (Cr), cadmium (Cd) and lead (Pb) into the natural environments, causing widespread heavy metal contamination worldwide (Nriagu 1988). These heavy metals may be taken up by crops and then transferred into humans through the food chain, consequently posing a great threat to health (Costa 2000, Jarup *et al.*1998, McLaughlin and Singh 1999). It has been well documented that there is a great difference among crop species and genotypes within a species in regard to heavy metal uptake and accumulation. A high variation in heavy metal accumulation among crops (Bell *et al.* 1997, Kumar *et al.* 1995) and genotypic difference within a crop, such as rice (Liu *et al.* 2003, Cheng *et al.* 2006) has been reported.

Heavy metals contamination in natural environment is often much more complex than that in the artificial environment, due to co-existing and interaction of several heavy metals. Soil pollution by heavy metals is a multi-element problem in many areas of Bangladesh. Khan (2001) reported substantial enrichment of copper (Cu), Cd and Pb in soil, river water and vegetables of Bangladesh. Several studies have been conducted to investigate the combined effect of heavy metals on some plant species. The combined effects of Cd, Cu, nickel (Ni), and zinc (Zn) on *Medicago sativus* (alfafa) (Peralta-Videa *et al.*

2002) and combined effect of Cu, Cd, and Pb on *Cucumis sativus* (cucumber) (An *et al.* 2004) and combined effect of Cd, Cu, Pb and Zn to *Horderum vulgare* (spring barley) (Luo and Rimmer 1995) have previously been reported. An *et al.* (2004) reported that the bioaccumulation of one metal in *Cucumis sativus* was influenced by the presence of other metals in metal mixtures. Combined effects of heavy metals have to be taken into account for ecological risk assessment. Therefore, this study investigated the combined effects of different doses of heavy metals Cd, Cr and Pb uptake and accumulation of these heavy metals in rice plant and find out the relationships of these three heavy metals. We also examined the bioaccumulation pattern to determine if the combination of the heavy metals affects accumulation relationships.

MATERIAL AND METHODS

Plant Material

Nine local rice cultivars (seven high yielding and two local) that are commonly grown and consumed by Bangladeshi people were collected from the Nutrition and Grain Quality Division of Bangladesh Rice Research Institute (BRRI). The collected rice varieties are given below: High yielding- BR7 (BRRI Balam), BRRI dhan28, BRRI dhan29, BRRI dhan33, BRRI dhan41, BRRI dhan42 and BRRI dhan47; Local- Asam Boro and Hashikalmi.

Study Design and Plant Growth

Rice seeds were germinated in a petri dish merged in deionized water for about 72 hr at room temperature using a Whatmann filter paper at the bottom of the petri dish. Three days after starting germination, seedlings of nine rice varieties were transferred to a plastic tray (27 cm × 32 cm) containing Styrofoam-plugged holes sheets fitted with net floating on deionized water and grown for three days. Each Styrofoam contains four evenly spaced holes and containing one rice cultivar. After three days of growth, Yoshida nutrient culture solution **(**Yoshida *et al.* 1976) containing micro and macro nutrient elements in different concentrations was applied to each tray. Seven days after the addition of culture solution, the plants were treated with three different doses of heavy metals mixture. Each treatment had four replicates. On a daily basis, the pH of the solution was monitored. If solution pH was shifted by the root action to 0.5 units above or below the target value, addition of HCl or NaOH was employed to adjust the pH of the solution. Continuous aeration of the solutions was provided and water was added to maintain a constant volume of the solution in each pot. After every seven days, the experimental culture solutions were completely replaced with appropriate combined doses of heavy metals. The process continued till 60 days and plant samples were harvested after 60 days of growth in the nutrient culture solution containing three heavy metals to detect heavy metal uptake in rice plant.

Test Chemicals and Treatment of Heavy Metals

For treatments with heavy metal, nitrate salts of lead [$Pb(NO_3)_2$] and chloride salts of cadmium [$CdCl_2.5H_2O$] and chromium [$CrCl_3.6H_2O$] were used. The experiment was performed with three metals mixture (Cd+Cr+ Pb) of different concentrations. The metals were added to the nutrient culture solution to make mixture of three doses of Cd, Cr and Pb metals, viz. Dose 1: (0.15+1.5+1.5) mg/L; Dose 2: (0.2+2.0+2.0) mg/L; and Dose 3: (0.25+2.5+2.5) mg/L.

Collection and Preparation of Rice Plant for Analysis

Four clumps of paddy plants were harvested as replicated samples of each variety. The whole paddy plant was uprooted and washed thoroughly first with running tap water and then with distilled water for three times. Then plants were dried for four days with a clean paper towel and stored at room temperature for further processing. The samples were then oven-dried at 70°C until obtaining a constant weight. The weights of the plant samples were taken before and after they were dried in an oven. The oven-dried four replicated samples were ground together in a stainless steel mill to fine powder to pass through 80 mesh-net. Then the three heavy metal contents of the samples were determined by the atomic absorption spectrometry (AAS) following microwave acid (HNO_3-HCl, 4:1) digestion of 500 mg plant sample.

Analysis of Plant Sample

The resulting solutions obtained from the microwave digestion were analyzed by flame atomic absorption spectrometry (AAS) model Perkin Elmer Analyst 800. The instrument was calibrated with certified reference materials from the National Institute for Environmental Studies (NIES) of Japan.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version-5 software. Two tailed t-test was performed to analyze the significant difference between uptake and accumulation in rice plants at different doses. Pearson's correlation coefficient was also performed to determine the relations between metal concentrations in plant uptake of metal. p value less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Genotypic and dose variation in plants concentrations of heavy metals

As shown in Table 1, rice plants accumulated a high amount of Cd and remarkable differences existed among different rice cultivars, suggesting that different rice cultivars had different abilities in Cd uptake when treated with Cr, Cd and Pb together in combination. The accumulation of Cd in rice plant was significantly higher ($p<0.05$) at treatment dose 3 compared to the treatment dose 1, whereas there was no significant difference in accumulation of Cd in plant treated with doses 2 and 3. The bioaccumulation pattern in mixtures is not always consistent with the amount of metals supplied. For example, increased amount of each metal in mixture had negligible influence of Cd uptake (Table 1). In the present study, the slight difference between Cd concentrations in doses 2 and 3 of rice plant at higher concentration of metals indicated that increased concentration of Cd with Cr and Pb increased the uptake of Cd to some extent, but not related to the level of metals supplied. Besides the concentration of metals, some other factor(s) like plant metabolism (Kabata-Pendias and Pendias 1992) are responsible for Cd uptake and accumulation in the rice plants.

The results in Table 2 indicated that a great proportion of Cr was accumulated by rice plants when treated with three heavy metals and there was remarkable difference in Cr concentrations among different rice cultivars. The average uptake of Cr in dose 2 was significantly higher ($p<0.05$) than that of dose 1, but no significant difference was found with dose 3 (Table 2). The average concentration of Cr in rice plant increased at treatment dose 2 and then decreased at treatment dose 3, but not statistically significant. There is a clear trend that less uptake of Cr occurred at higher dose of ternary metals mixture.

Table 1. Cadmium contents (μg/g dry weight) of rice plant[a].

Variety (n=9)	Dose 1	Dose 2	Dose 3
BR7 (BRRI Balam)	109.6	116.5	98.4
BRRI dhan28	107.4	209.7	185.8
BRRI dhan29	81.6	220.7	201.4
BRRI dhan33	181.6	149.8	237.8
BRRI dhan41	102.9	169.3	194.7
BRRI dhan42	134.8	207.2	225.9
BRRI dhan47	203.5	158.3	99.5
Asam Boro	170.6	198.2	260.3
Hashikalmi	71.4	72.9	179.3
Average±SEM[b]	**129.3±15.4**	**167.0±16.3**	**187.0±18.8***

[a]Data are the mixture of four replicates of rice plant; [b]Standard error of mean. *Dose 1 vs 3, $p<0.05$.

Table 2. Chromium contents (μg/g dry weight) of rice plant[a].

Variety (n=9)	Dose 1	Dose 2	Dose 3
BR7 (BRRI Balam)	209.4	562.9	292.9
BRRI dhan28	370.4	647.2	415.0
BRRI dhan29	124.0	437.7	425.8
BRRI dhan33	337.4	524.4	544.0
BRRI dhan41	171.6	427.4	392.2
BRRI dhan42	320.5	658.6	508.9
BRRI dhan47	485.9	406.3	178.2
Asam Boro	214.7	222.8	127.2
Hashikalmi	225.8	237.6	448.9
Average±SEM[b]	**273.3±37.9**	**458.3±52.6***	**370.3±47.6**

[a]Data are the mixture of four replicates of rice plant; [b]Standard error of mean. *Dose 1 vs 2, $p<0.05$.

Table 3 demonstrated that the average uptakes of Pb in all treatment doses were quite higher in rice plants than that of Cd and Cr at different doses of combined metals of Cr, Cd and Pb in the nutrient solution. The average accumulation of Pb in rice plant was significantly ($p<0.05$) higher in the treatment of dose 3 compared to dose 1. Although accumulation was also higher in dose 2 compared to dose 1, but not statistically significant. Similarly, uptake of Pb was non-significantly higher in dose 3 compared to dose 2. The result suggested that the uptake of Pb was not dependent on the concentration of heavy metals in the treatment doses. At the same concentration of Cr and Pb (2.0 and 2.5 mg/L) in nutrient solution, uptake of Pb was much higher than that of Cr (Tables 2 and 3) with slightly higher level of Cd treatment in the rice plant. Interestingly, some genotypes showed quite low Pb concentrations in rice plants. These results demonstrate that significant differences existed among the rice cultivars in the absorption and translocation of different metals. The uptake of Cd was the lowest among all elements analyzed in the present study, which is in agreement with the results obtained by Chino and Baba (1981) and Honma and Hirata (1978) for rice grown in nutrient solutions. Bioaccumulation of a single metal was influenced by the presence of other metals, resulting in inhibited or enhanced bioaccumulation of one metal in the mixture. In the present study, the interaction among Cd, Cr and Pb can interfere the uptake and distribution of each metals in rice plants.

Table 3. Lead contents (μg/g dry weight) of rice plant[a].

Variety (n=9)	Dose 1	Dose 2	Dose 3
BR7 (BRRI Balam)	761.5	967.3	724.3
BRRI dhan28	777.3	1348.6	1130.6
BRRI dhan29	207.7	485.4	755.2
BRRI dhan33	890.8	1113.8	1446.8
BRRI dhan41	444.3	895.5	1161.5
BRRI dhan42	772.7	1330.5	1339.5
BRRI dhan47	1130.5	1011.5	629.7
Asam Boro	690.1	689.4	908.8
Hashikalmi	567.6	490.4	1172.0
Average±SEM[b]	**693.6±88.3**	**925.8±107.2**	**1030±95.8***

[a]Data are the mixture of four replicates of rice plant; [b]Standard error of mean. *Dose 1 vs 3, $p<0.05$.

The relationships between the three heavy metals

Pearson correlation coefficient was calculated for the rice plants Cr, Cd and Pb concentrations in three doses. No significant correlation between Cd and Cr or Pb was observed in doses 2 and 3, indicating that Cd accumulation in rice plants would be independent on both Cr and Pb accumulation in higher doses of combined three metals treatment. In contrast, a significant ($p<0.05$) positive correlation between Cd and Cr as well as Pb was observed at lower dose of treatment (dose 1). On the other hand, in all doses of treatment, there was a significant positive correlation between Cr and Pb (Table 4), but the significance was decreased (*$p<0.05$, **$p<0.01$ and ***$p<0.001$) with increased level of Pb concentration (0.15, 0.20, 0.25 mg/L, respectively) in three doses of treatment.

Table 4. Pearson correlation coefficients between heavy metals in different doses.

Dose	Heavy Metal	Plant heavy Metal	
		Cd	Cr
1	Cd	---	0.680*
	Cr	0.783*	0.901***
2	Cd	---	0.363
	Cr	0.304	0.825**
3	Cd	---	0.317
	Cr	0.636	0.757*

Significant at *$p<0.05$, **$p<0.01$ and ***$p<0.001$.

These results indicate that the accumulation of Cr and Pb in rice plants would likely occur simultaneously on dose dependent manner. The statistical analysis showed that the concentrations of the three metals in the rice plant was significantly affected by genotype, concentration of metals and interaction among metals, indicating that accumulation of heavy metals in rice plants was determined not only by the contamination levels, but also by interaction of metals and genetic factors.

Some genotypes with high or low heavy metal concentration in rice plant

In the present study, we examined nine rice genotypes growing in three doses with different heavy metal concentrations. It was found that there was a huge difference among genotypes in rice plants heavy metal accumulation. The genotypic effects on the variation of Cd, Cr and Pb concentrations are much larger that the dose effect. Some genotypes, for instance, BRRI dhan33 and Asam Boro had consistently higher Cd concentrations, BRRI dhan28 and BRRI dhan42 had higher Cr and BRRI dhan33 and BRRI dhan42 had higher Pb concentrations in plants, respectively. On the other hand, BR7 and Hashikalmi had low Cd, Asam Boro had low Cr, and BRRI dhan29 had low Pb concentrations in three doses of heavy metals in plants.

The present study demonstrated that great differences existed among the rice cultivars in the uptake of heavy metals. Other researches also reported significant differences among rice cultivars in the uptake of heavy metals (Wang and Gong 1996, Wu *et al.* 1999). In the present study, we found that the genotypic effect in the variation of Cr, Cd and Pb concentrations were much larger than the environmental (dose) effect. Meanwhile we found that some genotypes showed consistently higher or lower plant Cr, Cd and Pb concentrations than others under the three doses with different concentrations of heavy metals. The results confirmed the possibility of controlling and reducing Cr, Cd and Pb concentrations in rice grains through developing and using the cultivars with low accumulation. In the present study, Cd concentration in rice plants showed no correlation with Cr and Pb concentration in the higher doses. In contrast, a synergistic interaction between Cr and Pb was detected in three different doses of heavy metal concentrations, indicating that plant uptake of Cr and Pb tends to change with the growth conditions (doses of heavy metals). In the high concentration of combined heavy metals (dose 3), there was less significant correlation of Cr and Pb uptake in rice plant. It indicates that there are interactions among the metals to be involved in biochemical processes within plants. Several studies reported that the presence of one metal influenced uptake of the other metal (Luo and Rimmer 1995, Peralta-Videa *et al.* 2002). The results obtained in the present investigation advances us to do more researches in the field of complexity of heavy metals.

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REFERENCES

An, Y. J., Y. M. Kim, T. I. Kwon and S. W. Jeong. 2004. Combined effect of copper, cadmium, and lead upon *Cucumis sativus* growth and bioaccumulation. *Sci. Total Environ*. **326**: 85-93.

Bell, M. J., M. J. McLaughlin, G. C. Wright and J. Cruickshank. 1997. Inter and intra-specific variation in accumulation of cadmium by peanut, soybean, and navy bean. *Aust. J. Agric. Res*. **48**: 1151-1160.

Cheng, W., G. Zhang, H. Yao, W. Wu and M. Xu. 2006. Genotypic and environmental variation in cadmium, chromium, arsenic, nickel, and lead concentrations in rice grains. *J. Zhejiang Uni. Sci. B.* **7**: 565-571.

Chino, M and A. Baba. 1981. The effects of some environmental factors on the partitioning of zinc and cadmium between roots and tops of rice plants. *J. Plant Nutr*. **3**: 203-214.

Costa, M. 2000. Chromium and Nickel. In: R. K. Zalups and J. Koropatnick (eds.). *Molecular Biology and Toxicology of Metals*. Taylor and Francis, Great Britain., pp. 113-114.

Honma, Y and H. Hirata. 1978. Noticeable increase in cadmium absorption by zinc deficient rice plants. *Soil Sci. Plant Nutr*. **24**: 295-297.

Jarup, L. 2003. Hazards of heavy metal contamination. *Br. Med. Bull*. **68**: 167-182.

Jarup, L., M. Berglund, C. G. Elinder, G. Nordberg and M. Vahter. 1998. Health effects of cadmium exposure – a review of the literature and a risk estimate. *Scand. J. Work Environ. Health*. **24**: 1-51.

Kabata-Pendias, A and H. Pendias. 1992. *Trace Elements in Soil and Plants*. 2nd ed. CRC press, Inc. Boca Raton, Florida, USA. 365 pp.

Khan, A. H. 2001. *Heavy metals profile in Bangladesh Environment: Soil and Irrigation Water Components.* Final Completion Report. Bangladesh Agricultural Research Council, Dhaka.

Kumar, P. B. A., V. Dushenkov, H. Motto and I. Raskin. 1995. Phytoextraction: The use of plants to remove heavy metals from soils. *Environ. Sci. Tech*. **29**: 1232-1238.

Liu, J. G., J. S. Liang, K. Q. Li, Z. J. Zhang, B. Y. Yu, X. L. Lu, J. C. Yang and Q. S. Zhu. 2003. Correlations between cadmium and mineral nutrients in absorption and accumulation in various genotypes of rice under cadmium stress. *Chemosphere*. **52**(9): 1467–1473.

Luo, Y. and D. L. Rimmer. 1995. Zinc-copper interaction affecting plant growth on a metal-contaminated soil. *Environ*. *Pollut*. **88**: 79-83.

McLaughlin, M. J. and B. R. Singh. 1999. Cadmium in soils and plants: a global perspective. In: M. J. McLaughlin and B. R. Singh (eds.). *Cadmium in soils and plants*. Kluwer Academic Publishing, Netherlands., pp. 13-21.

Nriagu, J. O. 1988. Production and Uses of Chromium. In: J. O. Nriagu and E. Nicboer (eds.). *Chromium in the Natural and Human Environments*. John Wiley and Sons, New York, USA., pp. 81-105.

Peralta-Videa, J. R., J. L. Gardea-Torresdey, E. Gomez, K. J. Tiermann, J. G. Parsons and G. Carrillo. 2002. Effect of mixed cadmium, copper, nickel and zinc at different P^H upon alfafa growth and heavy metal uptake. *Environ. Pollut*. **119**: 291-301.

Shanker, A. K., C. Cervantes, H. Loza-Tavera and S. Avudainayagam. 2005. Chromium toxicity in plants. *Environ*. *Int*. **31**: 739-753.

Wang, K. R and H. Q. Gong. 1996. Compared study on the cadmium absorption and distribution of two genotypes rice. *Agro. Environ. Protect*. **15**: 145-149.

Wu, Q.T., L. Chen and G. S. Wang. 1999. Differences on Cd uptake and accumulation among rice cultivars and its mechanism. *Acta. Ecologica Sinica*. **19**: 104-107.

Yoshida, S. I., D. A. Forno, J. H. Cock and A. Gomez. 1976. *Laboratory Manual for Physiological Studies of Rice*. International Rice Research Institute, Manila (Philippines). 83 pp.