Synergistic Effect of Zinc and Cadmium for Uptake, Accumulation and Growth Responses in Rice (*Oryza sativa*) Varieties.

Dinum Herath , Aruni Weerasinghe, Deepthi Bandara , and Darshani Wijayawardhana

Abstract**—** Determining the variations among eight rice (*Oryza sativa*) genotypes (R1-R8) with different soil Cd levels respect to the plant growth, accumulation and the effect of Zn on Cd uptake, were studied. Cd - Zn interaction was determined at 25mg/kg of Cd plus 0, 25, 50 mg/kg of Zn addition with two rice varieties (R1 and R6). Results revealed that rice variety, soil Cd level and interactions between them significantly affected ($p<0.05$) to plant height, flag leaf area, root dry weight and Cd accumulation. When increasing the Zn level in soil chlorophyll content and average no. of tillers in R1 and R6 were increased whereas plant height significantly decreased in both. The concentration of Cd in grains, shoot and roots were increased with increasing Zn levels. It concluded that the effect of Zn on Cd uptake and accumulation in plants showed synergistic effect.

Keywords**—** Cadmium, Zinc, Cd-Zn synergistic interaction, Rice.

I. Introduction

CADMIUM (Cd) is one of the most concerned pollutants in terms of food-chain contamination, especially for rice [21]. It is believed that contamination of water sources and agricultural lands with fertilizers and agrochemicals for long periods of time is the main reason for accumulation of Cd [22], [4]. Therefore, Cd contamination in agricultural soils has been reported to be the constraint for food safety. Rice is considered to be a possible source of Cd intake. The maximum permissible level of Cd in polished rice grain according to the Codex committee of the Food and Agriculture Organization of the United Nations (FAO) and by the WHO is 0.4mg/kg [23]. Wu FB and Zhang GP [24] have been reported that plant species and also genotypes within same species differ greatly in their tolerance to Cd stress. Study with a genetically diverse rice collection is a superior source to study on grain Cd accumulation and effect of Cd on growth performances by different rice varieties. Zinc (Zn) is an essential trace element for plants, but is toxic when present at high levels. Cd and Zn are elements having similar geochemical and environmental properties. Their chemical similarity can lead to interaction between Cd and Zn during plant uptake and accumulation in edible plant parts [5]. Most of the experiments are dealing with the interaction between Cd and Zn in plants. But the effect of Zn on Cd uptake and accumulation in plants are not consistent. This shows either synergistic [21] or antagonistic pattern [15]. The aim of this work was to assess the efficiency of Zn on the reduction of Cd accumulation and investigates the variations among rice varieties for Cd tolerance with respect to plant growth parameters.

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II. Procedure

The two pot experiments were carried out in a plant house of the Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka with selected rice (*Oryza sativa* L.) varieties including traditional and new improved. The soil was collected from the faculty field (8o 28’ 40” N 80^o 34’45” E). The basic properties of soil as pH 7.33; total Zn and Cd level in the initial soil was 0.03 and 1.96 mg/kg respectively.

The experiment I was conducted to investigate the effect of Cd on growth parameters and plant accumulation in different rice varieties. Twenty-five kilograms of soil was used in pot experiment with Cd treatment of 50 mg Cd/kg (T2), 100 mg Cd/kg (T3) and the soil receiving no external Cd was used as the untreated soil (T1) as $CdCl_2$.2½ H2O. Eight different rice varieties were used in this experiment. Those were Bg 300 (R1, Oryza sativa L. var. Bg 300), AT 307 (R2, Oryza sativa L. var. AT 307), Bg 352 (R3, Oryza sativa L. var. Bg 352) and Bg 358(R4, Oryza sativa L. var. Bg358) as new improved rice varieties and Suwandel (R5, Oryza sativa L. var. suwandel), Kaluheenati (R6, Oryza sativa L. var. kaluheenati), Pachchaperumal (R7, Oryza sativa L. var. pachchaperumal) and Kuruluthudu (R8, Oryza sativa L. var. kuruluthudu) as traditional rice varieties. Seeds were surface sterilized with 20% clorox and soaked in water for 48 hrs and germinated in parachute trays. After 14 days, plants with two leaf stage were transplanted into the pots. The pot soil was maintained under submerged conditions during the whole growth period. The pots were arranged as a two factor factorial experiment with completely randomized design with three replicates. During the vegetative growth plant height, number of tillers and the flag leaf chlorophyll content were measured. After the ripening stage, rice plants were uprooted and washed with 0.1mM EDTA solution. Then root dry weight was measured and finally Cd accumulation in rice grain, shoot and root were measured. Experiment II was carried out to investigate the effect of Zinc on Cadmium uptake and translocation. Six kilogram of soil were used in this pot experiment and contaminated with 25mg/kg of Cd. Cadmium - Zn interaction was determined at 25mg/kg of Cd plus 0, 25, 50 mg/kg of Zn addition as a Zinc sulfate with two rice varieties namely, R1 and R6 from each plant group. During the vegetative growth plant height, number of tillers and flag leaf chlorophyll content were measured. At the end of the ripening stage plants were uprooted and Cd content in grain, shoot and roots were measured. The sample Cd contents were determining by graphite furnace AAS (Model: GBC GF3000). Samples were authenticated with NIST SRM 164e reference sample. Data were analyzed according to the two factor factorial experiment and interactions between these factors were considered to determine the significance of differences between the pairs of means by analysis of variance (ANOVA). Duncan new multiple range tests were done to determine which means were significantly different from the others by using SAS System

III. RESULTS

A. Effect of Cd on Plant Growth Parameters

Plant height data showed (TABLE I) that R1, R3, R4, R6, R7 and R8 reduced their plant height with increasing Cd level treated. Both R1 and R4 reduced their height significantly ($P<0.05$) with the increasing Cd level treated. Even though R3, R6 and R8 reduced their height with increasing Cd application, the reduction of plant height from no addition of Cd to 50 mg/kg was not significant. Addition of Cd did not significantly affect the plant height of R2. Results showed that rice variety R5 died with 50 and 100 mg/kg Cd addition.

Flag leaf chlorophyll content of different rice cultivars showed different responses with increasing Cd level treated. Flag leaf chlorophyll content was not significant ($P<0.05$) in R1, R2, R4, R6 and R7. R2, R4, R6 and R7 showed higher SPAD value with 50 mg/kg than in both no additions of Cd and 100 mg/kg. But R3 and R8 showed the reduction of flag leaf chlorophyll content with increasing soil Cd levels.

Root dry weight data revealed that, rice plants reduced their root dry weight with increasing Cd application. R4 significantly ($P<0.05$) reduced their root dry weight with increasing Cd application. Similarly, R1 reduced their root dry weight when Cd level was changed from 0 to 50 mg/kg. However, this reduction was not significant, but the increasing 50 to 100 mg/kg caused the significant ($P<0.05$) reduction of root dry weight for R1. Variety R2 responded differently to the Cd level than the other varieties. This variety has significantly ($P<0.05$) reduced their root dry weight when changed from 0 to 50 mg/kg. Although this variety increased the root dry weight from 50 mg/kg to 100 mg/kg treatment numerically, this increase was also not significant. Though R3 significantly reduced the root dry weight when 50mg/kg, R6, R7 and R8 also behaved in similar pattern.

TABLE I
VARIATION IN PLANT GROWTH PARAMETERS IN DIFFERENT RICE VARIETIES WITH INCREASING SOIL CD LEVELS.

Var.	Trt	Plant Growth Parameters A	B	C	D
R1	T1	72 ± 1.41^{a}	42.01 ± 0.74^{a}	18^{a}	5.37 ± 0.48^{a}
	T2	62 ± 2.65^{b}	40.94 ± 0.37^{a}	12^{a}	3.10 ± 1.51^{ab}
	T3	52 ± 3.71^{c}	41.45 ± 0.25^{a}	4.5^{a}	1.13 ± 1.10^{b}
R2	T1	62 ± 9.19^{a}	52.17 ± 0.68^{a}	7.5^{a}	4.05 ± 1.13^{a}
	T2	56 ± 1.59^{a}	52.48 ± 1.11^{a}	7.5^{a}	1.22 ± 0.46^{b}
	T3	62 ± 1.41^{a}	50.80 ± 0.99^{a}	5^{a}	2.65 ± 0.84^{ab}
R3	T1	64 ± 1.51^{a}	37.09 ± 0.69^{a}	23.5^{a}	8.49 ± 1.81^{a}
	T2	61 ± 1.61^{a}	36.89 ± 0.37^{ab}	14.5^{a}	4.35 ± 1.67^{b}
	T3	43 ± 0.53^{b}	35.13 ± 0.67^{b}	3.5^{b}	2.09 ± 0.96^{b}
R4	T1	70 ± 3.00^{a}	45.63 ± 0.05^{a}	26^{a}	4.05 ± 1.13^{a}
	T2	64 ± 2.04^{b}	47.67 ± 1.79^{a}	17.5^{a}	1.22 ± 0.46^{b}
	T3	52 ± 3.06^{c}	46.16 ± 0.43^{a}	4.5^{a}	2.65 ± 0.84^{ab}
R5	T1	81 ± 4.95	44.52 ± 0.53	5	5.23 ± 0.87
	T2-	-	-	-	-
	T3-	-	-	-	-
R6	T1	81 ± 7.50^{a}	43.26 ± 0.62^{a}	9.5^{a}	5.59 ± 1.33^{a}
	T2	77 ± 2.12^{a}	44.18 ± 0.11^{a}	11^{a}	1.49 ± 0.21^{b}
	T3	55 ± 7.94^{b}	43.14 ± 0.39^{a}	5^{a}	1.43 ± 0.37^{b}
R7	T1	87 ± 1.06^{a}	43.52 ± 0.67^{a}	4.5^{a}	4.18 ± 0.83^{a}
	T2	72 ± 10.96^{ab}	46.10 ± 1.56^{a}	1.5^{a}	2.03 ± 0.19^{b}
	T3	61 ± 2.30^{b}	45.20 ± 1.56^{a}	3^{a}	0.98 ± 0.35^{b}
R8	T1	75 ± 1.53^{a}	43.38 ± 0.31^{a}	14.5^{a}	9.83 ± 2.42^{a}
	T2	72 ± 3.54^{a}	39.35 ± 0.21^{b}	10^{a}	4.39 ± 0.43^{b}
	T3	53 ± 1.23^{b}	38.30 ± 0.05^{c}	3^{a}	2.50 ± 1.18^{b}

A : Plant height (cm); B : Flag leaf chlorophyll content; C: Avg. no. of tillers ; D: Dry weight (grams).
Values are mean ± S.D. (n=3); Interaction between rice variety and Cd level is significantly different; Means in rows followed by the different simple letters are significantly different at p=0.05 (Duncan test).

B. Varietal Response to Cd Accumulation and Distribution within Rice Plant Body.

Rice variety, Cd levels and the interaction between rice variety and Cd levels significantly affected grain Cd accumulation. The total grain Cd content in each rice variety of R1, R2, R3, R4, R6, R7 and R8 were statistically ($P<0.05$) different with the Cd levels treated. Generally, the mean grain Cd levels increased with increasing Cd applications except variety in R1. In R1, the grain Cd content in 100 mg/kg was lower (2.26 mg/kg) than the 50 mg/kg (2.719 mg/kg). In R2, R3, R4, R6, R7 and R8 grain Cd content increased with increasing Cd applications. The grain Cd content was ranged from 0.111 to 0.597 mg/kg with no addition of cadmium chloride. Variety R1 was accumulated the lowest amount of Cd (0.111 mg/kg) while R5 accumulated the highest (0.597 mg/kg) under this Cd treatment.

The mean Cd content in all tested rice shoots except in R5 was highly statistically ($P<0.05$) different at all three Cd level treated. For all tested rice varieties, the mean shoot Cd content was increased significantly ($P<0.05$) with increasing Cd application. Cd content in roots of all tested rice varieties except in R5 was significantly different at the tested Cd levels. Results obtained from the study showed that R1, R2 and R6 have increased their Cd accumulation with increasing Cd applications. Other rice varieties except R5 accumulated lower amount of Cd in their roots at 100 mg/kg soil Cd treatment than the 50 mg/kg.

C. Distribution of Cd within plant body.

The results showed, both new improved and traditional rice varieties accumulate and distribute Cd as the sequence of grains< shoots< roots for all tested three soil Cd levels. Therefore, roots accumulate the highest Cd and grains accumulate the lowest for both tested groups.

D. Effect of Zn Treatment on Some Plant Growth Parameters in Cd Treated Soils

The effect of Zn treatment on plant height, flag leaf chlorophyll content and tiller number in Cd treated soil (25mg/kg) are presented in TABLE III. When increasing Zn level, plant height was significantly ($p < 0.05$) reduced in R1. In R6 significant reduction of plant height was observed only in when increasing Zn level from 25 to 50 mg/kg. However, it was not significant ($p < 0.05$) with increasing 0 to 25 mg/kg of Zn level.

TABLE III
THE EFFECT OF ZN TREATMENT ON PLANT HEIGHT, FLAG LEAF CHLOROPHYLL CONTENT AND TILLER NUMBER IN CD TREATED SOIL (25MG/KG).

Var.	Treatments	A	B	C
R1	Control (0 mg/kg)	63 ± 1.50^a	38.00 ± 2.50^a	3 ± 0.58^a
	25 mg/kg	57 ± 0.89^b	39.87 ± 0.57^a	5 ± 0.58^b
	50 mg/kg	54 ± 1.07^c	40.03 ± 2.75^a	4 ± 0.01^a
R6	Control (0 mg/kg)	81 ± 1.50^a	39.57 ± 2.21^a	4 ± 1.16^a
	25 mg/kg	79 ± 1.41^a	41.93 ± 1.53^a	4 ± 1.16^a
	50 mg/kg	75 ± 0.60^b	42.47 ± 1.12^a	5 ± 2.01^a

A: Plant height (cm) ; B : Flag leaf chlorophyll content ; C : Average number of tillers. Values are mean ± S.D. (n=3); Interaction between rice variety and Cd level is significantly different; Means in rows followed by the different simple letters are significantly different at p=0.05 (Duncan test).

Flag leaf chlorophyll content of both rice varieties showed same responses with increasing Zn level treated. Flag leaf chlorophyll content was not significant ($P<0.05$) in both rice varieties. In R1 and R6, showed higher SPAD value with 50 mg/kg of Zn level compared to 0 mg/kg of Zn level in control and 25 mg/kg of Zn level. But the values were not significant. However same pattern were observed in both varieties. Flag leaf chlorophyll content was increased in leaves by increasing concentration of the soil Zn level when compared with control.

The average tiller number results revealed that, rice varieties showed different responses with increasing Zn level treated. In R1 average tiller number was significant ($P<0.05$). Average tiller number was significantly increased with increasing Zn level from 0 to 25 mg/kg. But it was reduced up to 4.00 at 50 mg/kg Zn level. In R6 average tiller number was not significant ($P< 0.05$). However, average tiller number reduced with increasing soil Zn level from 0 to 25 mg/kg and it was increased with increasing from 25 to 50 mg/kg level.

E. Effect of Zn Treatment on Plant Uptake and Accumulation

Cadmium concentration in grains of rice grown under constant Cd level (25 mg/kg) and varied Zn levels are shown in TABLE IV. The study exposed that R6 significantly ($p<0.05$) increased their grain Cd levels with increasing soil Zn level. Similarly R1 increased their grain Cd level when soil Zn level was changed from 0 to 25 mg/kg; however, that was not significant but the increasing soil Zn level from 25 to 50 mg/kg caused the significant ($p< 0.05$) increased of grain Cd concentration in R1.

TABLE IV
CADMIUM CONCENTRATION IN GRAINS OF RICE GROWN UNDER CONSTANT CD LEVEL (25 MG/KG) AND VARIED ZN LEVELS

Var.	Treatment Soil Zn level	Cd Concentrations (mg/kg) Grain	Shoot	Root
R1	Control	0.4139 ± 0.08^a	1.1650 ± 0.14^a	13.7128 ± 1.76^a
	25 mg/kg	0.7750 ± 0.26^a	3.4397 ± 0.12^b	36.6451 ± 7.11^b
	50 mg/kg	1.9659 ± 0.12^b	5.4676 ± 0.67^c	88.5348 ± 6.54^c
R6	Control	0.7259 ± 0.14^a	2.2475 ± 0.16^a	23.498 ± 2.37^a
	25 mg/kg	2.0977 ± 0.17^b	2.9393 ± 0.63^a	56.712 ± 10.93^b
	50 mg/kg	2.6252 ± 0.07^c	5.1604 ± 0.22^b	31.259 ± 7.24^a

Interaction between rice variety and Zn levels are significantly different; Means in rows followed by the different simple letters are significantly different at p=0.05 (Duncan test).

Regression analysis indicated an exponential increase of grain Cd concentration with Zn added. The statistical comparison of grain Cd in fitted curves showed that the Zn effect on Cd concentration was similar in both varieties. Thus Zn had a synergistic effect on the accumulation of Cd in rice grains.

The mean Cd content in both tested rice shoots was statistically ($P<0.05$) different at various Zn level. Both tested rice varieties the mean shoot Cd content was increased significantly ($P<0.05$) with increasing Zn level in soil. It was highly significant in R1; however in R6 the Cd concentration in shoots were not significant ($P<0.05$) at the level of 0 to 25 mg/kg but it was significant when increasing Zn level from 25 to 50 mg/kg.

Cd content in roots of R1 rice variety was significantly ($p<0.05$) different at the tested Zn levels. Results obtained from the study showed that variety R1 root Cd concentration was significantly increased with the increasing Zn level in soil. But different results were showed in variety R6 at the tested Zn levels. Root Cd content in variety R6 was increased with the increasing soil Zn level from 0 to 25 mg/kg. However it was drastically reduced with increasing soil Zn level up to 50 mg/kg. So lower amount of Cd in their roots at 50 mg/kg than the 25 mg/kg of Zn level in soil. Thus Zn had a synergistic effect observed in grains, shoot and roots of R1 and grains shoot of R6 except the roots.

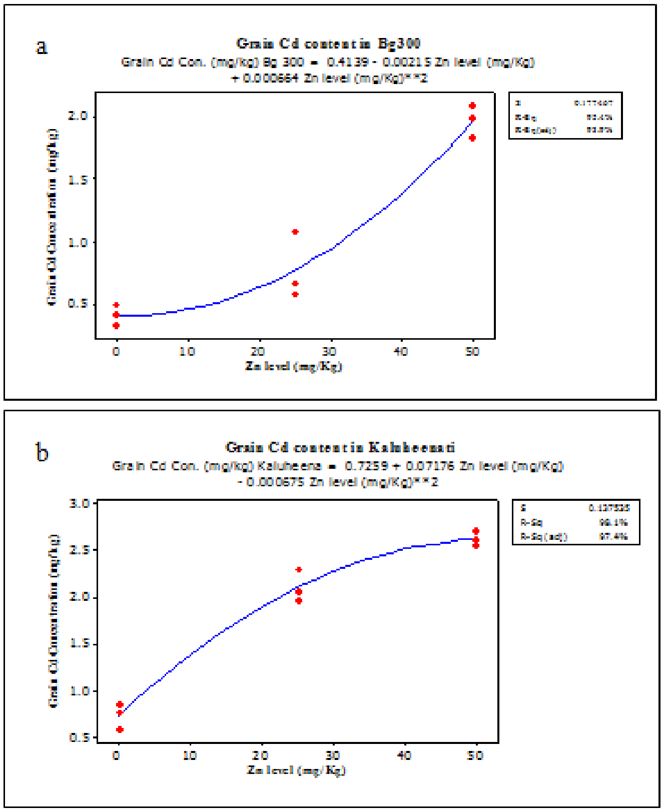

Fig 2 a, b: Cd concentration in rice grains of Bg300(R1) and Kaluheenati (R6) as a function of Zn level in the soil.

IV. Discussions

Cd is strongly phytotoxic and causes growth inhibition and even plant death [20]. In general, Cd in plants reduces the growth in both roots and stems. This effect is partly due to the suppression of the elongation growth rate of cells, especially in the stem, because of an irreversible inhibition exerted by Cd on the proton pump responsible for the process [2]. Liu *et al.*, 2007 [13] reported that toxicity effect of Cd on plant height is varietal dependant; the present study also proved this result. Further, Guo-sheng *et al.*, 2007 did a hydroponic study to investigate Cd accumulation and its toxicity in rice on the Brittle Culm1 (bc1) gene-a fragile rice mutant and its wild type with 0, 0.1, 1.0 and 5.0 µmol/L Cd levels and they concluded that both rice genotypes substantially inhibited the plant growth under high Cd levels (at 1.0 and 5.0 µmol/L Cd levels). Liu *et al.*, 2007 [13] reported that the effect of Cd on dry matter accumulation of rice plants differed with rice cultivar, rice growth stage and plant organ. Results of the present study also revealed significant root dry weight reduction between no addition of cadmium chloride and 50 mg/kg Cd treatment except in R1. The same result was further observed with no addition of cadmium chloride and 100 mg/kg Cd treatment except in R2. Roots responded differently to the soil Cd stress, here some plant genotypes showed comparatively high tolerance (R1, R2 and R6) than others to 100 mg/kg soil Cd treatment while some genotypes showed the slight toxicity (R3, R4, R6 and R7). Yu-jing *et al.*, 2004 [25] also observed significant difference of rice root biomass with 0 mg/kg, 5 mg/kg and 10 mg/kg soil Cd addition. They further reported that the addition of 10 mg/kg Cd to the soil significantly reduced the root bio mass.

When the growing medium Cd concentrations are different, rice plant can accumulate different amounts of Cd in their grains. Different literatures have reported different levels of rice grain Cd with different Cd treatments. As an example, Jing *et al.*, 2009 [10] reported that the mean grain Cd in unpolished rice grains of 110 cultivars as 0.022 mg/kg for paddy soils which contains 0.15 mg/kg soil Cd. Areo and Ae., 2003 [3] have reported that the mean grain Cd content in 31 different rice cultivars as 2.14 mg/kg (for brown rice) with 0.9 mg/kg soil Cd and 3.11 mg/kg with 7.4 mg/kg soil Cd. Further they have reported that the variety Rathal accumulates 2.12 and 3.34 mg/kg Cd in their grains in the same study. In some parts of China, soil Cd content is up to 26 mg/kg and the highest Cd in rice grains was reported as 2.4 mg/kg [23].

Liu *et al.*, 2003 [12] reported that the average Cd accumulation in rice roots were much higher than in stems and leaves and rice grains. Our study too confirmed this phenomenon; it showed that all the tested rice varieties accumulated Cd in the sequence of grain < shoot < root for every soil Cd level treated i.e. rice grains accumulated the lowest and the roots accumulated the highest.

Interactive effects of multiple metal pollution on plant metal accumulations are common but not consistent [14, 16]. Most of the findings may be concluded that Zn reduces the uptake of Cd by both root and foliar system [1]. However, in the present study the interaction effects were synergistic; increasing soil Zn strongly elevating Cd concentration in grain, shoot and roots of rice. These results agreed with the previous reports of Hinsely *et al.*,[8] who reported that repeated sludge applications did result in additional increase of both Cd and Zn contents in corn leaves and grain in calcareous soils. Dudka *et al.*, 1994 [6] concluded that the contents of Cd and Zn in spring wheat parts straw and grain increased with increasing the two metal concentrations in sandy soils. Z. Nan *et al.*, 2001 [17] concluded from their pot experiment that interaction of Cd and Zn resulted in an increase of Cd accumulation and a decrease of Zn uptake in rice plant. Further our findings are in broad agreement with those of the following reports. Smilde *et al.*, 1992 [21] concluded that Zn and Cd were synergistic to some extent, plant Zn uptake increasing with applied Cd on the basis of interaction experiment carried out in loam soil in pots. Moraghan., 1993 [16] reported that the Cd and Zn effects were synergistic to each other in the presence of added Cd and Cd accumulation in flax seed was reduced by added Zn in the absence of added Cd. The present results also suggest that in pots highly contaminated with Cd and Zn, soil Zn should not be expected to decrease Cd significantly in crop parts although there is a strong antagonistic Zn effect on Cd accumulation in plant tissues. Cadmium and Zn might be considered chemically similar elements because they have similar ionic structure and electro negativities. Synergistic interaction strongly suggests that two cations enter in to the plant via a common transport system. Chemical similarity of Cd and Zn result in significant level of Cd affinity for binding sites designed for Zn [19]. Zn transporters ZnT1, ZnT5, MTP1 should transport or at least bind Cd as well [11].so addition of Zn to Cd treated plants facilitate the Cd translocation as a result of competitive interaction between theses metals. The reason for these differences between those results and the work reported here may depend on the Cd and Zn contents and their combinations in soils and also on the soil characters and crop species and tissues [17]. these divergent findings agree with the statement that interaction effects on crop metal uptake in field situations are more difficult to make than in pot or solution experiments.

IV. Conclusions

Cd concentration in different cultivars responded differently to increasing soil Cd levels. Among tested rice varieties R2 was the highly tolerant variety to soil Cd stress. Rice variety R5 is a highly sensitive variety. Plant roots accumulate the highest amount of Cd than shoots and grains and distributed Cd as grains< shoots< roots for all tested soil Cd levels. The concentration of Cd in grains, shoot and roots were increased with increasing Zn supply levels. The effect of Zn on Cd uptake and accumulation in plants showed synergistic effect. It also might depend on plant species and external Cd and Zn concentration levels.

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