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Cytogenetic Effects of Individual and Combined Treatment of Cd^{2+}, Cu^{2+} and Zn^{2+} in *Vigna radiata* (L.) Wilczeck

Manoj Kumari[1*], Vijay Kumar Sinhal[2], Alok Srivastava[1] and V.P. Singh[1]

[1]*Department of Plant Science, M.J.P. Rohilkhand University, Bareilly -243006, India*
[2]*Department of Botany Maharaja Agrasen Mahavidyalaya, Bareilly, UP , India*

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*Corresponding Author

Tel : +91-9219894002

Email: mrs.manojsingh@rediffmail.com

Summary

The present investigation has been designed to evaluate the cytogenetic effects of individual treatment of Cd^{2+} (0.2mg L^{-1}), Cu^{2+} (6.5, 7.5 and 8.5mg L^{-1}),and Zn^{2+} (6.3, 7.3 and 8.3mg L^{-1}) and combined treatments of Cd^{2+} (0.2mg L^{-1}) with Cu^{2+} (6.5, 7.5 and 8.5mg L^{-1}) and Zn^{2+} (6.3, 7.3 and 8.3mg L^{-1}) in two cultivars i.e.PDM-139 and K-851 of *Vigna radiata* (L.) Wilczek. The impacts of these doses were measured in terms of germination percentage, plant height, fresh and dry weight of plants, number and weight of root nodules, pollen and seed fertility, mitotic index and mitotic anomalies. The results showed that germination percentage, plant height, fresh and dry weight of plant, number and weight of root nodules, pollen and seed fertility, and mitotic index in both the cultivars were significantly reduced under the influence of all the individual treatment of heavy metals however, mitotic anomalies were enhanced. Cd^{2+} induced reductions in above mentioned growth parameters were significantly enhanced due to Cu^{2+} supplementation but such reductions were significantly recovered with Zn^{2+} supplementation. On the other hand, supplementation of Cu^{2+} increased the Cd^{2+} induced mitotic anomalies however Zn^{2+} supplementation was able to decrease the mitotic anomalies. It is clear from the present study that the individual treatments of Cd^{2+}, Cu^{2+} and Zn^{2+} are toxic to both the cultivars of *Vigna radiata* (L.) and the toxicity caused by Cd^{2+} is enhanced by Cu^{2+} supplementation but recovered by Zn^{2+} supplementation.

Key Words: Cadmium, Copper, Zinc, Combination, Cytogenetic, *Vigna radiata*

Introduction

Heavy metals at extremely micro concentrations affect different cellular components, thereby interfering with the normal metabolic functions [1]. Important sources of heavy metals include metalliferous mining, smelting processes, industrial emissions, effluents, vehicle emission, dumped waste material, sewage sludge, pig slurry, composted town refuse, fertilizers, oil emeliorants, pesticides, contaminated dusts and rainfall etc. [2]. Metalliferous environments are often contaminated by more than one metal in potentially toxic concentrations that may have synergistic, additive or antagonistic effects on plants [3].

Cadmium is highly toxic and persistent environmental poison for plants and animals [17]. Cadmium has been classified as group I human carcinogen by the International Agency for Research on Cancer [4]. Cd^{2+} released into the environment tends to concentrate in soils and sediments where it is potentially available to rooted plants. The available Cd^{2+} thereby enters biogeochemical cycle, becomes bioconcentrated [5] and even affects human health e.g. the itai - itai disease caused by cadmium-contaminated rice in Japan. Cd^{2+} induces biochemical [6,7] and genetic [8] changes in plant metabolism and causes a number of toxic symptoms in plants such as inhibition in seed germination [9], growth [10], and nitrogen assimilation [11, 12].

Zn^{2+} is needed as a micronutrient and is an important component of many vital enzymes having a catalytic, cocatalytic and structural role as well as being a structural stabilizer for proteins, membranes and DNA binding proteins (Zn-fingers) [13]. Higher concentrations of heavy metals cause reduction in plant growth and development [14, 15, 16, 17]. Cadmium and zinc (IIB transition elements) have a similar electronic configuration and valence state, possessing equal affinities for sulphur, nitrogen and oxygen ligands and hence similar geochemical and environmental properties [18]. In the recent years, various workers have documented the responses of plants to combinations of Zn^{2+} and Cd^{2+} in soil as well as in solution culture [19, 20].

Copper is also known as essential nutrient for plant growth and development. It is a component of Cu/Zn superoxide dismutase. Despite its role in cellular metabolism the range of Cu^{2+} concentration suitable to an optimum growth of plant is very narrow. Beyond the upper limit the Cu^{2+} is strongly toxic for photosynthetic organisms [21, 22] by affecting the morphology and anatomy of plant organs, photosynthetic and respiratory process, structure and function of membranes, nutrients uptake [23], and nitrogen metabolism [24]. Cu^{2+} is a potent inducer of DNA damage (Hartwing & Schwerdtle, 2002). Various workers reported that combination of Cu^{2+} and Cd^{2+}

induced more pronounced toxicity to plant growth and nitrogen assimilation as compared to their isolated treatments [25, 26].

In Bareilly region, *Vigna radiata* (L.) Wilczek (mungbean) is a main cultivated pulse crop. Bareilly city has a number of industries which are releasing heavy metals into the waste water. These heavy metals contaminate the soil of agricultural field and affect the yield and quality of crop plants growing in that soil. It has been analyzed that waste water of Bareilly city contains non-essential heavy metal Cd^{2+} (0.2mg L^{-1}) and essential heavy metals Cu^{2+} (7.5mg L^{-1}) and Zn^{2+} (7.3mg L^{-1}). Therefore, present investigation has been designed to evaluate the cytogenetic effects of individual treatments of Cd^{2+}, Cu^{2+},and Zn^{2+} and combined treatment of Cd^{2+} with Cu^{2+} and Zn^{2+} in *Vigna radiata* (L.) Wilczek.

Materials and Methods

The seeds of two cultivars i,e. PDM-139 and K-851 of *Vigna radiata* (L.) Wilczek were presoaked in distilled water for 8 hours and then soaked for 12 hour with individual doses of Cd^{2+} (0.2mg L^{-1}), Cu^{2+} (6.5, 7.5 and 8.5mg L^{-1}),and Zn^{2+} (6.3, 7.3 and 8.3mg L^{-1}) and combined doses of Cd^{2+} (0.2mg L^{-1}) with Cu^{2+} (6.5, 7.5 and 8.5mg L^{-1}) and Zn^{2+} (6.3, 7.3 and 8.3mg L^{-1}). The heavy metal soaked seeds were thoroughly washed with distilled water. Some of the heavy metal treated seeds were transferred on moist filter paper in Petri dishes to examine the germination percentage, mitotic index and mitotic anomalies. The mitotic index and mitotic anomalies were studied by squash preparation of root tip cells in 2% acetotocarmine stain. To examine the cytogenetic effects of these metals on plant height, fresh and dry weight, number of nodules, fresh and dry weight of nodules, pollen fertility and seed fertility, some of the heavy metal treated seeds were also sown in the field.

Results and Discussion

Germination Percentage

The seeds of both the cultivars of *Vigna radiata* (L.) treated with individual doses of Cd^{2+}, Cu^{2+} and Zn^{2+} showed a marked reduction in germination percentage. Treatments of Cu^{2+} were more toxic than Cd^{2+} and Zn^{2+}. In both the cultivars, combination of Cu^{2+} with Cd^{2+} significantly enhanced the Cd^{2+} induced reduction in germination percentage however combined doses of Zn^{2+} and Cd^{2+} significantly reduced the inhibition in germination percentage as compared to Cd^{2+} alone (Table 1). Such inhibition in seed germination is probably due to the result of interference of heavy metals with the respiratory activity and mobilization of reserves [27] the changes in cellular permeability, inhibition of protease activity and / or direct toxicity to the embryo [9].

Plant Height, Fresh Weight and Dry Weight

Plant height, fresh weight and dry weight of both the cultivars were significantly decreased with the treatment of individual doses of Cd^{2+}, Cu^{2+} and Zn^{2+}. Both the cultivars were more susceptible to Cu^{2+} than Cd^{2+} and Zn^{2+} treatments. Cd^{2+} generated reductions in plant height, fresh weight and dry weight were significantly enhanced due to Cu^{2+} supplementation but significantly reduced with Zn^{2+} supplementation. The inhibition in plant growth by metal supply may be a consequence of inhibition of some primary physiological processes i.e. photosynthesis, nitrogen assimilation and other metabolic processes [25]. The higher concentrations of heavy metals have been reported to retard cell division, cell differentiation and their elongation affecting plant growth and development [28]. Heavy metals also interfere with nutrients uptake responsible for better plant growth [29].

Number, Fresh Weight and Dry Weight of Root Nodules

Treatments of individual doses of Cd^{2+}, Cu^{2+} and Zn^{2+} caused significant reduction in number, fresh weight and dry weight of nodules in both the cultivars. Cu^{2+} was found to be more toxic than Cd^{2+} and Zn^{2+} treatments. In both the cultivars the reductions in these parameters were significantly enhanced with combined doses of Cu^{2+} and Cd^{2+} as compared to individual doses of Cd^{2+} however combined doses of Zn^{2+} and Cd^{2+} significantly recovered the Cd^{2+} induced reduction in these parameters (Table 1,2). Treatment of heavy metal suppresses the nitrogen metabolism inhibiting the activities of nitrogen assimilating enzymes [30]. The main toxic effects of heavy metal on nodule structure and function were the occlusion with glycoprotein of intracellular spaces of nodule cortex, alterations in symbiosomes, enrichment in Cd of cell walls and oxidative stress [31]. Higher heavy metal concentrations reduce the nitrogen fixing area and nitrogen fixing cells [32].

Pollen Fertility and Seed Fertility:

A pronounced reduction in pollen and seed fertility of both the cultivars was noticed under the influence of individual doses of Cd^{2+}, Cu^{2+} and Zn^{2+}. The treatments of Cu^{2+} were more effective than Cd^{2+} and Zn^{2+}. In both the cultivars combined treatment of Cu^{2+} with Cd^{2+} significantly increased the Cd^{2+} induced pollen and seed fertility reduction however, Zn^{2+} supplementation significantly suppressed the Cd^{2+} induced reduction in these parameters (Table 2). Similar results of pollen and seed fertility under the influence of these heavy metals were also observed by various workers [7,33].

Mitotic Index and Mitotic Anomalies

The individual doses of Cd^{2+}, Cu^{2+} and Zn^{2+} were significantly able to reduce the mitotic index but increased the mitotic anomalies in both the cultivars. Cu^{2+} was found to be more effective than Cd^{2+} and Zn^{2+} to cause such effects. Supplementation of Cu^{2+} to the Cd^{2+} significantly reduced the mitotic index but enhanced the mitotic anomalies as compared to the individual dose of Cd^{2+}. On the other hand, Zn^{2+} supplementation significantly alleviated the Cd^{2+} induced inhibition in mitotic index and decreased the Cd^{2+} generated mitotic anomalies (Table 2,3). Induction of mitotic anomalies under different heavy metal treatments may be due to chromatin agglutination [34] enhanced disturbances of spindle function [27], inhibition of DNA synthesis at S-phase of cell cycle, stickiness of chromosomes due to polymerization of chromosomal nucleic acid, bridge formation due to chromosomal stickiness or due to chromosomal breakage and reunion, and origin of micronuclei from lagging chromosome or form a chromosome fragment [35].

Thus, the heavy metals reaching to the soil through city waste water and industrial waste water contaminate the soil and are toxic to the plants growing in that area. This toxicity especially with heavy metal Cd^{2+} is altered when it is mixed up with other heavy metals i.e. Cu^{2+} and Zn^{2+}. It is interesting to note that the toxicity caused by Cd^{2+} is enhanced

by Cu^{2+} but recovered in the presence of Zn^{2+}. So the application of Zn^{2+} may be useful to recover the toxicity induced by Cd^{2+}. Further the lower doses of Zn^{2+} are more effective in the recovery of Cd^{2+} induced toxicity.

Table 1. Effects of individual and combined doses of Cd^{2+}, Cu^{2+}, Zn^{2+} in *Vigna radiata* (L.) Wilczek

Heavy Metal conc. (mg L^{-1})	Germination Percentage	Plant Height (cm.)	Fresh Weight (gm.)	Dry Weight (gm.)	Number of Nodules
PDM-139					
Control (Distilled Water)	92.4±1.08	61.44±0.95	49.23±0.72	16.22±0.36	23.2±0.52
Cd^{2+} 0.2	66.2*±0.95	42.20*±0.68	31.19*±0.58	12.00*±0.41	12.4*±0.36
Cu^{2+} 6.5	62.0*±0.89	40.26*±0.90	29.00*±0.41	11.20*±0.35	11.8*±0.52
Cu^{2+} 7.5	58.4*±0.83	36.38*±0.98	25.41*±0.53	9.70*±0.36	11.0*±0.40
Cu^{2+} 8.5	54.2*±0.77	31.26*±0.49	22.88*±0.61	8.82*±0.41	9.8*±0.33
Zn^{2+} 6.3	75.8*±1.21	52.20*±0.67	37.43*±0.72	14.13*±0.39	19.4*±0.67
Zn^{2+} 7.3	71.2*±0.72	46.94*±0.65	36.01*±0.64	13.43*±0.36	16.6*±0.36
Zn^{2+} 8.3	68.4*±1.19	44.40*±0.44	32.88*±0.69	12.90*±0.34	13.8*±0.44
Control (Cd^{2+} 0.2)	66.2±0.95	42.20±0.68	31.19±0.58	12.00±0.41	12.4±0.36
Cu^{2+} 6.5 + Cd^{2+} 0.2	56.8*±0.82	35.12*±0.56	25.36*±0.44	8.70*±0.30	10.6*±0.46
Cu^{2+} 7.5 + Cd^{2+} 0.2	53.2*±1.04	30.38*±0.44	22.36*±0.52	7.80*±0.29	9.6*±0.36
Cu^{2+} 8.5 + Cd^{2+} 0.2	50.4*±0.92	26.94*±0.61	20.05*±0.50	6.80*±0.17	8.0*±0.40
Zn^{2+} 6.3 + Cd^{2+} 0.2	80.2*±1.04	55.32*±0.79	40.40*±0.61	15.30*±0.37	21.0*±0.49
Zn^{2+} 7.3 + Cd^{2+} 0.2	74.4*±0.61	51.44*±0.75	38.91*±0.74	14.21*±0.29	17.8*±0.52
Zn^{2+} 8.3 + Cd^{2+} 0.2	71.0*±0.80	47.30*±0.73	37.01*±0.42	13.50*±0.35	14.8*±0.44
K-851					
Control (Distilled Water)	95.0±1.02	69.20±0.26	53.02±0.51	18.54±0.27	21.6±0.46
Cd^{2+} 0.2	67.2*±0.77	44.06*±0.83	33.43*±0.68	11.40*±0.31	11.8*±0.34
Cu^{2+} 6.5	64.0*±1.10	41.38*±0.78	30.21*±0.80	11.02*±0.39	11.0*±0.40
Cu^{2+} 7.5	59.8*±0.77	36.34*±0.91	26.92*±0.58	9.91*±0.37	9.4*±0.22
Cu^{2+} 8.5	55.2*±0.66	32.42*±0.48	24.34*±0.50	8.96*±0.31	8.6*±0.22
Zn^{2+} 6.3	79.2*±0.91	53.86*±0.72	41.42*±0.71	15.24*±0.37	16.6*±0.61
Zn^{2+} 7.3	71.4*±1.08	49.94*±0.84	38.06*±0.67	13.60*±0.33	14.0*±0.63
Zn^{2+} 8.3	68.4*±1.19	47.20*±0.43	36.37*±0.52	13.02*±0.34	12.4*±0.36
Control (Cd^{2+} 0.2)	67.2±0.77	44.06±0.83	33.43±0.68	11.40±0.31	11.8±0.34
Cu^{2+} 6.5 + Cd^{2+} 0.2	60.4*±0.96	37.16*±0.65	26.39*±0.59	9.40*±0.36	10.0*±0.28
Cu^{2+} 7.5 + Cd^{2+} 0.2	56.0*±0.75	31.38*±0.56	22.40*±0.61	8.50*±0.18	8.0*±0.28
Cu^{2+} 8.5 + Cd^{2+} 0.2	50.2*±0.52	28.24*±0.77	21.07*±0.49	7.70*±0.26	7.4*±0.22
Zn^{2+} 6.3 + Cd^{2+} 0.2	84.8*±1.15	59.32*±0.76	44.40*±0.67	16.42*±0.35	18.0*±0.57
Zn^{2+} 7.3 + Cd^{2+} 0.2	75.2*±1.04	54.12*±0.55	43.03*±0.56	15.01*±0.39	15.4*±0.54
Zn^{2+} 8.3 + Cd^{2+} 0.2	72.4*±0.78	49.80*±0.70	39.36*±0.53	13.81*±0.28	13.6*±0.46

* significant at 5% leve

Table 2. Effects of individual and combined doses of Cd^{2+}, Cu^{2+}, Zn^{2+} in *Vigna radiata* (L.) Wilczek

Heavy Metal conc. (mg L^{-1})	Fresh Weight of Nodules (gm.)	Dry Weight of Nodules (gm.)	Pollen Fertility (%)	Seed Fertility (%)	Mitotic Index (%)
PDM-139					
Control (Distilled Water)	0.173±0.018	0.067±0.014	96.28±1.24	4.62±0.23	10.22±0.31
Cd^{2+} 0.2	0.114*±0.010	0.034*±0.004	75.42*±0.85	3.10*±0.13	6.40*±0.21
Cu^{2+} 6.5	0.110*±0.010	0.033*±0.006	71.34*±0.93	2.92*±0.16	6.32*±0.22
Cu^{2+} 7.5	0.091*±0.013	0.027*±0.006	66.08*±0.77	2.70*±0.17	5.88*±0.19
Cu^{2+} 8.5	0.082*±0.011	0.022*±0.003	59.26*±0.89	2.44*±0.11	5.20*±0.14
Zn^{2+} 6.3	0.143*±0.017	0.050*±0.006	85.90*±0.90	3.72*±0.17	8.62*±029
Zn^{2+} 7.3	0.130*±0.014	0.045*±0.009	81.12*±0.97	3.52*±0.26	7.40*±0.23
Zn^{2+} 8.3	0.121*±0.012	0.039*±0.007	77.06*±0.62	3.20*±0.21	6.74*±0.25
Control (Cd^{2+} 0.2)	0.114±0.010	0.034±0.004	75.42±0.85	3.10±0.13	6.40±0.21
Cu^{2+} 6.5 + Cd^{2+} 0.2	0.084*±0.013	0.028*±0.006	66.40*±0.78	2.62*±0.15	5.60*±0.16
Cu^{2+} 7.5 + Cd^{2+} 0.2	0.070*±0.012	0.022*±0.004	59.14*±0.87	2.30*±0.10	5.02*±0.18
Cu^{2+} 8.5 + Cd^{2+} 0.2	0.062*±0.015	0.018*±0.006	55.06*±0.66	2.02*±0.11	4.10*±0.19
Zn^{2+} 6.3 + Cd^{2+} 0.2	0.160*±0.012	0.058*±0.011	90.16*±0.92	4.02*±0.26	9.12*±0.29
Zn^{2+} 7.3 + Cd^{2+} 0.2	0.151*±0.014	0.050*±0.009	86.38*±0.63	3.70*±0.21	7.88*±0.24
Zn^{2+} 8.3 + Cd^{2+} 0.2	0.130*±0.013	0.044*±0.009	81.06*±0.90	3.52*±0.13	7.14*±0.28
K-851					
Control (Distilled Water)	0.182±0.019	0.068±0.011	97.04±0.92	5.10±0.20	11.00±0.31
Cd^{2+} 0.2	0.110*±0.009	0.034*±0.006	70.18*±0.98	3.32*±0.15	7.20*±0.24
Cu^{2+} 6.5	0.110*±0.010	0.031*±0.006	67.20*±0.83	3.10*±0.15	7.02*±022
Cu^{2+} 7.5	0.100*±0.012	0.028*±0.005	63.28*±0.91	2.78*±0.16	6.54*±0.24
Cu^{2+} 8.5	0.090*±0.011	0.023*±0.003	59.86*±0.50	2.40*±0.12	5.30*±0.17

Zn^{2+} 6.3	0.140*±0.016	0.051*±0.010	86.30*±0.86	4.02*±0.19	8.84*±0.29
Zn^{2+} 7.3	0.134*±0.014	0.045*±0.009	78.32*±0.67	3.80*±0.21	8.10*±0.27
Zn^{2+} 8.3	0.123*±0.011	0.041*±0.007	73.06*±0.47	3.42*±0.17	7.54*±0.21
Control (Cd^{2+} 0.2)	0.110±0.009	0.034±0.006	70.18±0.98	3.32±0.15	7.20±0.24
Cu^{2+} 6.5 + Cd^{2+} 0.2	0.092*±0.014	0.028*±0.006	62.40*±0.94	2.72*±0.14	6.40*±0.20
Cu^{2+} 7.5 + Cd^{2+} 0.2	0.084*±0.015	0.023*±0.003	57.94*±0.72	2.54*±0.14	5.62*±0.18
Cu^{2+} 8.5 + Cd^{2+} 0.2	0.070*±0.012	0.019*±0.005	53.06*±0.60	2.00*±0.15	4.40*±0.20
Zn^{2+} 6.3 + Cd^{2+} 0.2	0.164*±0.013	0.057*±0.011	91.16*±0.57	4.32*±0.19	9.38*±0.30
Zn^{2+} 7.3 + Cd^{2+} 0.2	0.150*±0.014	0.051*±0.008	85.38*±0.81	4.10*±0.20	8.40*±0.24
Zn^{2+} 8.3 + Cd^{2+} 0.2	0.142*±0.014	0.046*±0.008	78.10*±0.82	3.82*±0.18	7.92*±0.26

* significant at 5% level

Table 3. Effects of individual and combined doses of Cd^{2+}, Cu^{2+}, Zn^{2+} in *Vigna radiata* (L.) Wilczek

Heavy Metal conc. (mg L^{-1})	Laggards	Bridges	Stickiness	Clumping	Total mitotic anomalies (%)
PDM-139					
Control (Distilled Water)	0.00	0.00	0.00	0.00	0.00
Cd^{2+} 0.2	0.21	0.31	0.67	0.73	1.92
Cu^{2+} 6.5	0.00	0.72	0.29	1.01	2.02
Cu^{2+} 7.5	0.32	0.00	0.77	1.13	2.22
Cu^{2+} 8.5	0.00	0.80	0.20	1.40	2.40
Zn^{2+} 6.3	0.43	0.00	0.25	0.64	1.32
Zn^{2+} 7.3	0.20	0.33	0.81	0.26	1.60
Zn^{2+} 8.3	0.50	0.55	0.00	0.75	1.80
Control (Cd^{2+} 0.2)	0.21	0.31	0.67	0.73	1.92
Cu^{2+} 6.5 + Cd^{2+} 0.2	0.00	0.35	0.72	1.13	2.20
Cu^{2+} 7.5 + Cd^{2+} 0.2	0.34	0.53	0.21	1.40	2.48
Cu^{2+} 8.5 + Cd^{2+} 0.2	0.00	0.40	0.83	1.49	2.72
Zn^{2+} 6.3 + Cd^{2+} 0.2	0.43	0.48	0.21	0.00	1.12
Zn^{2+} 7.3 + Cd^{2+} 0.2	0.19	0.40	0.49	0.30	1.38
Zn^{2+} 8.3 + Cd^{2+} 0.2	0.27	0.73	0.00	0.60	1.60
K-851					
Control (Distilled Water)	0.00	0.00	0.00	0.00	0.00
Cd^{2+} 0.2	0.29	0.17	0.51	0.85	1.82
Cu^{2+} 6.5	0.00	0.82	0.00	1.08	1.90
Cu^{2+} 7.5	0.42	0.46	0.40	0.96	2.24
Cu^{2+} 8.5	0.30	0.11	0.70	1.39	2.50
Zn^{2+} 6.3	0.32	0.52	0.36	0.00	1.20
Zn^{2+} 7.3	0.63	0.30	0.18	0.47	1.58
Zn^{2+} 8.3	0.12	0.30	0.51	0.79	1.72
Control (Cd^{2+} 0.2)	0.29	0.17	0.51	0.85	1.82
Cu^{2+} 6.5 + Cd^{2+} 0.2	0.00	0.34	0.50	1.28	2.12
Cu^{2+} 7.5 + Cd^{2+} 0.2	0.20	0.38	0.79	1.07	2.44
Cu^{2+} 8.5 + Cd^{2+} 0.2	0.12	0.19	1.10	1.39	2.80
Zn^{2+} 6.3 + Cd^{2+} 0.2	0.34	0.44	0.22	0.00	1.00
Zn^{2+} 7.3 + Cd^{2+} 0.2	0.38	0.30	0.00	0.64	1.32
Zn^{2+} 8.3 + Cd^{2+} 0.2	0.50	0.00	0.44	0.56	1.50

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