



Influence of Zinc and Cadmium on Physiological and Biochemical Characteristics of Maize (*Zea mays* L.)

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ABSTRACT

This research was carried out as pod cultivation to evaluate effect of different level of zinc and cadmium on agro physiological traits of Maize via factorial experiment based on completely randomized design with three replications along 2012-2013. The factors included different level of Zinc ($Zn_1=0$ or Control, $Zn_2=15$ and $Zn_3=30$ mg.kg⁻¹) and Cadmium ($Cd_1=0$ or control, $Cd_2=10$, $Cd_3=20$ and $Cd_4=30$ mg.kg⁻¹). The results showed that zinc and cadmium yield, chlorophyll a and b, cell membrane stability, malon di aldehyde and tyrosine significantly affect the probability of a percent. In soil treated with increasing concentrations of the increased seed yield and highest performance 30 mg treatment was achieved. Fell sharply with increasing cadmium performance and best results of treatment was observed. Among different concentrations of zinc, the best treatment to improve seed yield and biochemical characteristics was the treatment with 30 mg.kg⁻¹ zinc. In cadmium treatment, contrary to zinc treatment, as the concentration increased the studied traits decreased significantly, so that the best results were obtained by the control treatment. The results of the interaction effect of zinc and cadmium on the studied traits indicated the reduction of adverse effects of cadmium on corn by zinc, but the best results of the interaction effect of zinc and cadmium on all traits were associated with the Zn_3Cd_1 treatment.

Keywords: Chlorophyll, Corn, Micro nutrient, Seed yield.

INTRODUCTION

The indiscriminate increase of the population, increasing demand for food and inadequate food resources have caused a huge portion of the people of the world face hunger and malnutrition. The way to deal with this phenomenon is the optimum use of the resources, facilities and the development of the cultivation of the high yield products. Among the food supplying products corn with regard to specific nutritional characteristics is one of the promising products to deal with the phenomenon which is cultivated in a wide area (Kang and saltveit, 2002). Corn is one of the important crops of cereal family with short growing period and high yield. Corn is high expectation plants and also is considered strategic crop for Iran country (Ghazvineh and Yousefi, 2012). On the other hand, soil fertility is an important factor, which determines the growth of plant. Soil fertility is determined by the presence or absence of nutrients macro and micronutrients, which are required in minute quantities for plant growth (Zayed *et al.*, 2011). Maize is the third most important cereal grain worldwide after wheat and rice. It is referred to as the cereal of the future for its valuable nutritional facts in human diet (Enyisi *et al.*, 2014). Annual production of cereals in 2014 was more than 2.4 billion tons. The 872 million tons of cereal production was maize production. According to the reports, Iran's share of world maize production is only 1.250 million tons (FAO, 2014). Zinc with the atomic number 30, the is the twenty-third most abundant element on Earth. Zinc is an essential micro element for plants and its participation in plant metabolism as a structural component or activator of the several enzymes has proved it to be necessary for normal growth of plant. In addition, zinc is effective in revival of plant water

regulators, synthesis of proteins, and metabolism of the nitrogen and carbohydrates. Zinc element belongs to the ribosome components, hence the reduction of protein due to lack of zinc element is attributed to the reduction of ribosomal RNA. Zinc plays a role in activating the enzymes of the higher plants such as anhydride and alkaline phosphatase (Bukvice *et al.*, 2003). Also, Zn is required for regulation and maintenance of the gene expression to induce tolerance of environmental stresses in plants (Cakmak, 2000). Amongst crops, maize shows the high sensitivity to Zn deficiency for its physiological requirements (Marschner, 1995). By application of the nitrogen fertilizers, zinc deficiency can be increased in maize. Also, nitrogen integrated with zinc improved plant height and yield in maize (Xia *et al.*, 2004). Zinc have main role in synthesis of the proteins, enzyme activating, oxidation and revival reactions and metabolism of the carbohydrates. By utilizing of fertilizers contain zinc and other micronutrients, performance on quality of crops is increasing and with shortage of this elements due to decline in plant photosynthesis and destroy RNA, amount of solution carbohydrates and synthesis of protein decreased and then performance and quality of crop will be decreased (Mousavi *et al.*, 2007). Shojaei and Makarian (2015) examined the effect of foliar application of zinc in three levels (control, 5, 10 grams per liter of zinc oxide) and water stress at three levels (full irrigation, irrigation off once at 50% flowering stage, irrigation off once at 50% podding stage) on the yield and yield components of mung bean. They reported that foliar application of zinc significantly increased traits such as the number of pods per plant, 1000 seed weight, and biological yield. Foliar ap-

plication with ten grams of zinc oxide at flowering and podding stages increased the yield 3.6% and 5.4%, respectively in comparison to lack of its application. Zinc element activates the plant enzymes by carbohydrate metabolism, maintaining the integrity of the cellular membranes, protein synthesis and regulation of the auxin synthesis (Marschner, 1995). Among the heavy metals cadmium is more important due to its high mobility and bioavailability in soil and its toxicity at the low concentrations. Environmental pollution of cadmium began since 100 years ago with the start of human activities such as mining, industrial and agricultural activities and the importing waste (Alloway, 1995). Cadmium is a heavy metal which is known today as a major cause of environmental pollution with very high level of toxicity for animals and plants. The high half life of the element in the human body (10 to 30 years) has caused cadmium to be the most susceptible metal to accumulation in the body. That is why this element is highly toxic to humans (Wieczorek *et al.*, 2005). About 75% of the cadmium in the human food chain comes through seed and the vegetables (Hani and Pazira *et al.*, 2011). Due to the use of industrial plants and the municipal waste water, such as the indiscriminate use of the pesticides and high levels of the chemical fertilizers particularly phosphate in arable lands led to more availability of cadmium. In other hand as a result of its consumption in the various industries such as the industrial paint production, plastics, pesticides, fungicides, herbicide, another poison, batteries, photography, water, metal coating and the melting industry, cadmium element distributes in the environment. The maximum amount of cadmium tolerable to humans that is introduced by FAO is 70 micro grams

per day (Zhang *et al.*, 2002). Cadmium is not essential for plants growth, but it will be toxic element in the plants leaves at concentrations of the 5 to 30 mg.kg⁻¹ (Clarke *et al.*, 2002). Another researcher reported Cd concentrations more than 5 until 10 mg.kg⁻¹ have toxic effects on growth and yield of plants. (Sauerbeck, 2012). Previous studies show that the among seeds, wheat (especially between durum varieties) is able to absorb the cadmium more than other cereal (rye, barley, oats). Cadmium uptake in durum wheat varieties is more than the bread varieties which is probably due to apoplastic links at the roots of these plants. Increased cadmium accumulation in durum wheat may be due to its more transition by the phloem vessels, in addition the amount of cadmium in durum wheat seed is higher than the bread wheat this trend may be due to genetic differences between bread and durum wheat (Li *et al.*, 2011). Thalooth *et al.* (2005) stated that cadmium consumption has reduced chlorophyll content in sunflower. Dhopte and Manuel (2002) reported that adding zinc to the medium increased cadmium toxicity and increased chlorophyll and photosynthesis rate. They concluded that zinc reduces the harmful effects of cadmium by improving photosynthesis and thus improves the photosynthesis and internal interactions. Staggenborg *et al.* (2008) reported that the consumption of zinc in water stress conditions had a positive and significant impact on growth, yield and yield components of plants. The use of cadmium in the barley causes leaves chlorosis, roots browning and reduction of the amount of chlorophyll in leaves and as the iron concentration was more than critical level, reduction of chlorophyll was attributed to reduction of photosynthetic compounds. The essential micro nutri-

ents required by the plant should not be overlooked (Staggenborg *et al.*, 2008). The role of micronutrients such as zinc involved from very simple to very complex reactions. Zn plays a very important role in plant metabolism by influencing the activities of the hydrogenase and carbonic anhydrase and stabilization of ribosomal proteins (Tisdale, 1984). Cadmium and Zn are elements having similar electronic configuration and valence and hence similar environmental properties. Zinc is often associated with Cd, and the processing and subsequent release of Zn to the environment is normally accompanied by Cd (Goyer, 1997; Verougstraete *et al.*, 2003). Thus there is a real need for analysis of the adaptive eco-physiology, biochemistry and reliable technologies of Cd–Zn interaction in the environment (Duruibe *et al.*, 2007). The interaction between metals like Cd and Zn is varied and can be difficult to predict. For example, in *Phaseolus vulgaris* L., the effect of Cd on the accumulation of Zn depended on the tissue concentrations of Zn. It was higher in roots and lower in shoots when Cd was added to solution culture (Chaoui *et al.*, 1997). The interaction may also depend on the nutritional status of the plant. Addition of Cd to Zn-deficient soil resulted in reduced concentrations of Zn in *Triticum aestivum* L. and *Triticum turgidum* L. var durum, whereas the addition of Cd to soils with adequate Zn either stimulated or had no effect on the uptake of Zn (Koleli *et al.*, 2004). Podar *et al.* (2004) found a similar effect for *Brassica juncea* (L.) grown in Cd contaminated soils, but Zn induced reduction in accumulation of Cd occurred only in soils supplemented with up to 340 mg Zn per kg soil; a higher dose of Zn (705 mg.kg⁻¹) stimulated Cd accumulation. It is reported that, some other crops from the Brassicaceae family (cauliflower,

kale, canola and cabbage) can also take up more metal than other plant species without visible toxicity symptoms (Zayed and Terry, 2003). Another study found no interaction between the two metals for *Glycine max* (L.) grown in Cd and Zn-amended soil (White and Chaney, 1980). Hart *et al.* (2002) determined that Zn and Cd are transported by a common carrier at the root plasma membrane, which has a higher affinity for Cd than for Zn. Therefore, Cd and Zn ions should experience competitive inhibition. Considering the importance of corn in Iran and other area under its cultivation in country and the implementation of the program to increase maize production. This research was carried out to study the effect of soil application of zinc and cadmium on the agro physiological characteristics of corn.

MATERIALS AND METHODS

Field and Treatment Information

This research was carried out as pot cultivation to evaluate effect of different level of zinc and cadmium on agro physiological and biochemical traits of Maize via factorial experiment based on completely randomized design with three replications along 2012-2013. The factors consisted different level of Zinc (Zn₁=0 or Control, Zn₂=15 and Zn₃=30 mg.kg⁻¹) and Cadmium (Cd₁=0 or control, Cd₂=10, Cd₃=20 and Cd₄=30 mg.kg⁻¹). The place of research was located in IAU of Ahvaz at latitude 31°20'N, longitude 48°40'E and altitude of 22m above the sea level. The soil of pot had clay-silty texture with pH=8.

Lab Management

First 20 cm diameter pots were prepared and 4 kg soil was weighed for each pot and after applying the treatments in soil and using other nutrients with regard to the soil test, pots were

prepared for cultivation. For each treatment two 5 kg pots were considered. After preparing the potting medium, cultivation was done in July 16, 2011. In each pot five seeds were planted and thinning was done in 3-to-4-leaf stage and two plants remained in each pot. Plants were irrigated every five days and the plants were allowed to grow under greenhouse conditions. Then, in order to apply water deficit treatment, irrigation-off was done at early flowering stage uniformly in all treatments and pots containing the plant.

Measured Traits

Lipid peroxidation in terms of malondialdehyde (MDA) content was determined for evaluation of membrane damage generated by drought stress treatments. MDA content was determined according to the method of (Cakmak and Horst, 1991). Fresh root and leaf tissues were weighed to 0.2 g and homogenized with liquid nitrogen by the addition of 1 mL of 5% trichloro acetic acid (TCA). The homogenates were transferred to tubes and centrifuged at the 12,000 rpm for 15 min at room temperature. Freshly prepared 0.5% thiobarbituric acid (TBA) in 20% TCA and supernatant, in equal volumes, were put into Eppendorf tubes and incubated for 25 min at 96°C. The tubes were placed in an ice bath and then centrifuged at 10,000 rpm for 5 min. Absorbance of the supernatant was determined at 532 nm, and the correction for nonspecific turbidity was performed by subtracting the absorbance at 600 nm. MDA contents were calculated using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$. In order to calculate the leaf chlorophyll content, 1 g punched fresh leaf sample was grinded along with 40 mL acetone 80% (v/v) until it was well smoothed. The resulted green liquid was transferred through Whatman paper

No. 2. Eventually, the final liquid volume using acetone 80% reached to 100 ml. Light densities of Chlorophyll extract were read using Spectrophotometer at 645, 663 and 652 nm wavelengths. Chlorophyll a, b and total as $\text{mg} \cdot \text{g}^{-1}$ leaf fresh weight were calculated according to (Dhopte and Manuel, 2002):

Equ. 1. Mg chlorophyll a = $[12.7 (D663) - 2.69 (D645)] \times V/1000$ w Mg chlorophyll b = $[22.9 (D645) - 4.68 (D663)] \times V/1000$ w.

Where, D of Light densities of Chlorophyll extract, V is final volume of chlorophyll extract in acetone 80% and w is leaf fresh weight as gram (Arnon, 1949). Measuring the stability of cell membranes was done according to the protocol of (Gnanasiri *et al.*, 1990). 1 gram of leaf pieces was weighed with sensitive scale and washed with distilled water and then placed in falcons inside the content of 10 ml of distilled water. One replication of each genotype was placed in a hot water bath at a temperature of 52 °C for 1 hour. (T₁) and control falcons were stored at 10 °C (C₁). 10 ml of distilled water was added to each falcon and then were kept at 10 °C for 24 hours. Then, the samples were transferred to room temperature and electrical conductivity of the solution was measured by EC meter. After that, the falcons were autoclaved at 100 °C for 15 minutes (T₂ and C₂) and their electrical conductivity was measured again. Finally, cell membrane stability was calculated using the following formula: **Equ. 2.** CMS (%) = $[1 - (T_1/T_2) / 1 - (C_1/C_2)] (100)$.

To measure D-tyrosine two young lower leaves were separated from the plant and were placed in a cool chamber whose floor was covered by ice and were transported to the laboratory. Immediately after the transfer, they were washed with distilled water and then entered 0.16 M Tris phosphate buffer with pH= 7.5 and then were crushed and homogenized. Then, the similar volume

of the same buffer containing digitoxin and wall digesting enzyme were permitted to do the digestion process of cell wall membrane. Finally, 0.5 ml of the homogenized solution was taken to be measured by method and the rate of protein was determined in mg per liter. After that, amount of D tyrosine was evaluated in remaining of solution according to above method. In this method, rate of activity was measured based on reaction to chromatography liquid (Gaines *et al.*, 1982).

Statistical Analysis

In order to analyze the data, SAS (Ver. 9.2) software was used and to compare treatment means Duncan test at 5% probability level was used.

RESULTS

Seed yield

The ANOVA results in this study showed that the use of Zinc and Cadmium and their interaction had a significant effect on seed yield at 1% probability level (Table 1). The highest seed yield was obtained Zn_3Cd_1 (688 $gr.plant^{-1}$) and lowest one belonged to interaction effect of the control (non use zinc) treatment and 10, 20, 30 mg cadmium (by the average of 246, 246, and 214 $gr.plant^{-1}$, respectively) (Table 2).

Chlorophyll a

The ANOVA results showed that chlorophyll a was significantly affected by different concentrations of zinc, cadmium and interaction effect of treatment at 1% probability level (Table 1). The mean comparison result showed that the highest rate of chlorophyll a was belonged to Zn_3Cd_1 (1.7 $mg.gr^{-1}$ of leaf tissue) and lowest one belonged to Zn_1Cd_4 (1.35 $mg.gr^{-1}$) (Table 2).

Chlorophyll b

The ANOVA results showed that chlorophyll b was significantly affected by different concentrations of Zinc and cadmium and their interaction at 1% probability level (Table 1). The highest rate of chlorophyll b was related to Zn_3Cd_1 (1.3 $mg.gr^{-1}$ of leaf tissue) and the lowest one was associated to Zn_1Cd_4 (with average of 1.13 mg per gram of leaf tissue) (Table 2).

Cell membrane stability

The ANOVA results indicated that cell membrane stability significantly affected different levels of zinc and cadmium at 1% probability level, but their interaction had a significant effect at 5% probability level (Table 1). Result of mean comparison of interaction effect of treatment revealed the Zn_3Cd_1 treatment had highest cell membrane stability (82.72%) and lowest one was belonged to Zn_1Cd_4 (52.80%) (Table 2).

Malondialdehyde (MDA)

The ANOVA results showed that the malondialdehyde was significantly affected by different concentrations of zinc, cadmium and their interaction at 1% probability level (Table 1). Mean comparison result of interaction effect of zinc and cadmium showed that the Zn_3Cd_4 treatment had the highest malondialdehyde (18 $\mu mol.mg^{-1}$ FW) and lowest one was obtained to the Zn_1Cd_1 (11 $\mu mol.mg^{-1}$ FW) (Table 2).

D-tyrosine

The ANOVA results showed effect of treatment on D-tyrosine was significant at 1% probability level, but their interaction had a significant effect at 5% probability level (Table 1).

Table 1. The ANOVA results of the effect of zinc and cadmium on the studied traits

S.O.V	df	Seed yield	Chlorophyll a	Chlorophyll b	Cell membrane stability	Malondi aldehyde	D-tyrosine
Year (Y)	1	ns	ns	ns	ns	ns	ns
Y*Rep	4	552.6	0.09	0.06	1.5	1.03	0.065
Zinc (Zn)	2	**	**	**	**	**	**
Cadmium (Cd)	3	**	**	**	**	**	**
Zn*Cd	6	**	**	**	*	**	*
Y*Zn	2	ns	ns	ns	ns	ns	ns
Y*Cd	3	ns	ns	ns	ns	ns	ns
Y*Zn*Cd	6	ns	ns	ns	ns	ns	ns
Error	44	446.8	0.02	0.013	2.20	6.70	0.030

ns, * and ** mean non-significant, significant at 5% and 1% probability levels respectively.

Table 2. Mean comparison of the studied traits at different concentrations of zinc and cadmium

Treatment	Seed yield (gr.plant ⁻¹)	Chlorophyll a (mg.gr ⁻¹)	Chlorophyll b (mg.gr ⁻¹)	Cell membrane stability (%)	Malondi aldehyde (μmol.mg ⁻¹ FW)	D-tyrosine (μmol.mg ⁻¹ FW)	
Zn ₁	Cd ₁	278.25 ^{ah}	1.66 ^b	1.20 ^d	60.84 ^e	11.00 ^h	3.70 ^{cd}
	Cd ₂	246.25 ⁱ	1.56 ^e	1.18 ^{de}	57.18 ^f	13.00 ^f	4.20 ^{bc}
	Cd ₃	246.00 ⁱ	1.40 ⁱ	1.18 ^{de}	54.16 ^g	13.30 ^{ef}	4.70 ^b
	Cd ₄	214.25 ⁱ	1.35 ^j	1.13 ^f	52.80 ^h	14.70 ^d	5.30 ^a
Zn ₂	Cd ₁	399.25 ^e	1.52 ^g	1.25 ^c	59.57 ^e	12.60 ^{fg}	3.50 ^{de}
	Cd ₂	365.00 ^f	1.59 ^d	1.25 ^c	59.46 ^e	14.70 ^d	4.00 ^c
	Cd ₃	373.25 ^f	1.55 ^{ef}	1.24 ^c	57.17 ^f	15.90 ^c	4.50 ^b
	Cd ₄	317.75 ^g	1.48 ^h	1.19 ^d	52.99 ^{gh}	17.40 ^b	5.20 ^a
Zn ₃	Cd ₁	688.75 ^a	1.70 ^a	1.30 ^a	82.72 ^a	13.60 ^e	2.60 ^f
	Cd ₂	615.25 ^b	1.61 ^c	1.28 ^b	79.27 ^b	16.00 ^c	3.30 ^e
	Cd ₃	603.25 ^c	1.63 ^c	1.28 ^b	75.74 ^c	16.50 ^c	3.70 ^{cd}
	Cd ₄	535.00 ^d	1.54 ^f	1.22 ^{cd}	71.93 ^d	18.20 ^a	4.20 ^{bc}

*Numbers with similar letters in each column are not significantly different at 5% probability level via Duncan test.

Different level of Zinc (Zn₁=0 or Control, Zn₂=15 and Zn₃=30 mg.kg⁻¹).

Different level of Cadmium (Cd₁=0 or control, Cd₂=10, Cd₃=20 and Cd₄=30 mg.kg⁻¹).

The means comparison of interaction effect of zinc and cadmium on D-tyrosine characteristic indicated that the Zn₁Cd₄ treatment had the highest amount of D-tyrosine (5.3 μmol.mg⁻¹ FW) and the lowest amount of D-tyrosine was belonged to the 30 mg.kg⁻¹ zinc and non use of cadmium element treatment (2.6 μmol.mg⁻¹ FW) (Table 2).

DISCUSSION

Seed yield

The results showed that the consumption of cadmium significantly reduced the seed yield, straw yield, and total yield but the consumption of zinc significantly increased them. Cadmium consumption alone reduced the seed yield as much as 54.26%, while the use of zinc reduced the yield by 14.42%.

The interaction effects of cadmium and zinc were significant negatively. Bukvice *et al.* (2003) have reported that the use of zinc sulfate increases the seed yield.

Chlorophyll a, b

Stated that cadmium consumption reduced chlorophyll content in sunflower (Zangin and Munzuroglu, 2006). Found that the use of cadmium in the barley caused leaves chlorosis, roots browning and reduction of the amount of chlorophyll in leaves and since the iron concentration in leaves was more than critical level, reduction of chlorophyll was attributed to reduction of photosynthetic compounds (Vassilev *et al.*, 2002). Reported that adding zinc to the medium reduced cadmium toxicity and increased the rate of chlorophyll and photosynthesis. They concluded that by improving photosynthesis, zinc element reduces the harmful effects of cadmium and consequently improves photosynthesis and internal interactions (Hassan *et al.*, 2005). With the arrival of heavy metals in to the chloroplasts and their high accumulation in these organelles, oxidative stress might occur which cause some damage such as chloroplast peroxiation. They also can disrupt the structure and function chlorophyll biosynthesis (Srivastava *et al.*, 2006). Chaab *et al.* (2016) by alleviate the cadmium toxicity on maize crop by the application of humic acid and compost reported indicated that enhancement of cadmium concentration in soil decreased root and shoot dry weight, chlorophyll content and relative growth rate of the plant. Heavy metals produce oxy radicals in plants. These radicals cause widespread damage to membranes and associated molecules, including the chlorophyll pigments (Mireles *et al.*, 2004).

Cell membrane stability

One of the most obvious effects of such heavy metals in plants is the induction of oxidative stress and development of active oxygen radicals. Reactive oxygen species (ROS) as the main factors that cause oxidative stress have high oxidizing power, threaten biological molecules, affect different cell processes and lead to cells death by disrupting the normal metabolism of plant cells (Kang and Saltiveit, 2002).

Malondialdehyde (MDA)

The amount of Malondialdehyde (MDA) which is created in the tissues under tension is known as the marker of lipids peroxidation reported that as the concentration of zinc increased the rate of Malondialdehyde increased in plant which was consistent with the results of this research (Wang *et al.*, 2009). Heavy metals in soil solution and disruption of root system after absorption can damage cell membrane wall under the influence of Malondialdehyde increase (Ishikawa *et al.*, 2010).

D-tyrosine

The increase of cadmium absorption by plant can influence the rate of chemical markers such as MDA and D-tyrosine in water scarcity conditions and if this trend continues it will lead to the loss of plant cells (Apel and Hirt, 2010). Zinc is also able to neutralize the destructive effects of cadmium, so that it has been reported that by the interaction of zinc and cadmium, cadmium concentrations in corn decreased about %5 as compared to the control treatment. Therefore, studying these effects can be effective in decreasing the amount of markers in cells. After oxygen free radicals attack to proteins, some changes will occur in special place of amino acids and causes analysis of peptide chain.

Sensitivity of peptide amino acids to oxidative attack is different and different forms of activated oxygen are different from each other in terms of reactivity potential.

CONCLUSION

The results showed that zinc and cadmium treatment significantly affected the seed yield and biochemical traits (such as chlorophyll a and b, cell membrane stability, malondialdehyde and D-tyrosine) at 1% probability level. In this experiment, zinc significantly reduced the effects of cadmium on corn. Among different concentrations of zinc, the best treatment to improve seed yield and biochemical characteristics was the treatment with 30 mg.kg⁻¹ zinc. In cadmium treatment, contrary to zinc treatment, as the concentration increased the studied traits decreased significantly, so that the best results were obtained by the control treatment. The results of the interaction effect of zinc and cadmium on the studied traits indicated the reduction of adverse effects of cadmium on corn by zinc, but the best results of the interaction effect of zinc and cadmium on all traits were associated with the Zn₃Cd₁ treatment.

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