Int. J. Environ. Sci. Tech.
© Autumn 2005, Vol. 2, No. 3, pp. 269-274

Germination and seedling growth of corn (*Zea mays l.*) under varying levels of copper and zinc

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Received 22 July 2005; revised 5 August 2005; accepted 29 August 2005; available onlined 30 September

Abstract

The heavy metal tolerance in corn *(Zea mays* L.*)* var. 'Neelum' was assessed at germination and seedling growth after having subjected it to different concentrations of $CuSO_4$ and $ZnSO_4$. Germination was not affected by any of the metal tested, whereas initial growth was strongly inhibited by increasing concentrations of $ZnSO_4$. Seedlings developed toxicity symptoms in the presence of both metals but more chlorotic and necrotic regions were observed at varying levels of $ZnSO_4$ than $CuSO_4$. The metal accumulation was concentration dependent. *Z. mays* seedlings accumulated more copper in roots but greater contents of zinc in their shoots. On the basis of results presented here, it can be concluded that the cultivar of the species tested has shown a marked sensitivity to the presence of small amounts of metals present in the growth medium. The data support the assumption that metal sensitivity is probably due to strong tendency of the species to accumulate them. This justifies that the corn variety 'Neelum' is not suitable for the cultivation under situations where water and soil suffer from occasional and/ or transitory metal pollution.

Key words: *Corn, heavy metals, copper, zinc, germination, seedling growth*

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Introduction

Unrestricted mining, municipal waste disposal practices and extensive use of agrochemicals have resulted in the addition of large amounts of heavy metals at many places of the world (Ernst 1996, Mullar *et al.* 2000). These metals persist indefinitely in soil thereby posing an ever-increasing threat to human health and agriculture (Leyval *et al.* 1995). Metal toxicity primarily depends on plant species as they exhibit considerable genetic variation in their ability in tolerating amounts and the concentration of specific heavy metals (Vojtechova and Leblova 1991). Some heavy metals are essential micronutrients for plants but their excess may result in metabolic disorders and growth inhibition in most of the plant species (Claire *et al*, 1991). The apparent damage of plant tissues due to excessive amount of heavy metals in the growth medium can be used as an indicative of toxic effects of metals (Mullar *et al.* 2000). Corn ranks third in the global cereal production and is utilized as food, feed and fodder. Large quantities of corn are used in extracting oil, manufacturing cellulose products, and mild abrasives. There are studies about the response of crop species to heavy metals reporting mechanisms that are responsible for their tolerance or sensitivity (Chatterjee and Chatterjee 2000, Ernst 1996, Lindon and Henriques 1992, Mullar *et al.* 2000). Avoidance of metal uptake or its accumulation in plant tissues without developing any toxicity symptoms is considered as metal tolerance in plants. Sensitive species may lack this mechanism and show toxicity symptoms and poor development. The present study was aimed at determining heavy metal tolerance in corn and to draw parallels between tolerance and metal accumulations. Heavy metal tolerance was tested at germination and seedling growth of corn as these are the key events for the establishment of plants under any prevailing environment (Welbaum *et al.* 1998).

Materials and Methods

Seed of corn (*Zea mays* L. var. Neelum) was obtained from ICI Pakistan Ltd, Multan. The germination of the seeds was more than 98%. Seeds were surface sterilized in 5% sodium hypochlorite solution for 10 minutes before use, to avoid fungal contamination. Seeds were then washed thoroughly with deionized water. $CuSO_4$ and $ZnSO_4$ solutions were prepared containing one thousand ppm of Cu^{+2} and Zn^{+2}. Different concentrations (3, 6, 9, 12 ppm) of both metals were prepared by further dilution of the 1000 ppm solutions.

Thirty petri dishes (8 cm diam) were washed with deionized water and lined with filter paper (Whatman No. 1) for germination study. Each petri dish received 20 seeds of corn variety Neelum and 20 mL of treatment solution. Treatments comprised of control (deionized water), 3, 6, 9, and 12 ppm of Cu^{+2} and Zn^{+2}. The petri dishes were arranged in a completely randomized block design with three replicates of each metal treatment. The experiment was conducted in a growth chamber at 25^{oC}, 12 hours light /12 hours dark period, (illumination of 2500 lux, Philips T2 40W/33 lamp). The seeds were observed for germination each day and recorded. Seeds were considered germinated when both radicle and plumule had emerged to about 0.2 cm.

For seedling experiment, 20 pre-germinated seeds were transferred to 30 plastic beakers filled with 100 mL of Cu^{+2} and Zn^{+2} solutions of different concentrations as used for germination experiment. An equal quantity of polystyrine beads was added to each beaker. This experiment was arranged in a complete randomized block design and each block contained three replicates for each metal concentration. The beakers were aerated during the course of seedling development. Seedlings were allowed to grow for 12 days then they were taken out from the solutions and washed carefully. The measurements were made for root and shoot length. Necrotic and chlorotic regions were observed using Swift Stereomicroscope with an eyepiece graticule of 10x magnitude.

Metal contents were determined after rinsing of seedlings for five minutes under a permanent flow of deionized water and then dried at 110^{oC} for 24 hours. Samples were ashed by heating at 600^{oC} for six hours and digested in HCl. The concentration of metal in the seedling was measured by atomic absorption spectroscopy (Varian AAS, 1475). It was expressed in ppm on the basis of fresh weight using the following formula,

$$\text{ppm (f)} = \frac{\text{ppm (d) x d.w.}}{\text{f.w.}}$$

where, d.w. = Dry weight and f.w. = Fresh weight

The biological absorption coefficient (BAC) for the fresh weight was determined with the formula,

$$\text{BAC} = \frac{\text{ppm (fresh)}}{\text{ppm (sol)}}$$

ppm (sol) = Concentration of the metal in the original solution.

Data were subjected to a two-way analysis of variance in order to elucidate differences between two metals and treatment levels.

Results

Germination of *Z. mays* seed was not affected by any of the solutions tested. Similarly, the rate of germination was constant both in the absence and presence of these metals (Table 1). Germination started on the fourth day and was completed by seventh day after placing seeds in the petri dishes. Root and shoot lengths of 12 days old seedlings at varying concentrations of Cu^{+2} and Zn^{+2} are presented in Table 1. The longest roots were observed at control for both the metals. The shortest roots were observed at 12 ppm of $CuSO_4$. There was a marked difference ($P< 0.00$) in root length at varying concentrations of $CuSO_4$. The extent of decline was significantly greater for Cu^{+2} than for Zn^{+2}. The root length was not much influenced by varying concentrations of $ZnSO_4$ (Table 1). Analysis of variance indicated a significant ($P< 0.001$) differential effect of both salts on the development of root. Similarly, varying concentrations of metals had a significant ($P< 0.01$) adverse effect on root length (Table 3).

The two metals ($P< 0.05$) and their varying s ($P< 0.001$) had a significant effect on shoot development (Table 3). The shortest shoot length was observed at the highest salt concentration (12 ppm) of $ZnSO_4$. However, longer shoots of *Z. mays* were recorded under $CuSO_4$ (Table 1). A considerable ($P<0.001$) effect of both metals on shoot/root ratio is evident (Table 3). This ratio was greater for varying concentrations of $CuSO_4$ while smaller ratios were observed at different concentrations of $ZnSO_4$ (Table 1). The effects of copper and zinc on seedling health were observed after the 12$^{th.}$ day of growth by stereoscopic microscope (Table 1). The increasing concentrations of both metals had caused a significant ($P<0.001$) damage to seedling health (Table 3). Although more necrotic region on the seedlings of Z. mays seedlings was observed in the presence of $ZnSO_4$ than $CuSO_4$ but the difference was non-significant (Table 3). The development of chlorotic region was observed in the presence of both the metals, which significant ($P<0.001$) influence on the growth of the seedlings (Table 3). Under varying concentrations of $ZnSO_4$, considerably greater chlorotic region was developed as compared to $CuSO_4$ (Table 1). The higher concentrations of both the metals caused significant (P<0.001) seedling damage (Table 3).

The metal content of the shoot of *Z. mays* grown in the presence of 3, 6, 9, and 12 ppm of Cu^{+2} was 0.27, 0.30, 0.27, and 0.37 ppm of fresh weight, respectively. The BAC was 0.48, 0.27, 0.08 and 0.066 (Table 2) and % absorption was 10, 20, 56.7 and 75 (Fig. 1) for each of the respective concentrations. In the presence of different concentrations of Cu^{+2}, roots accumulated 0.41, 0.43, 0.49 and 0.60 metal content at 3, 6, 9,and 12 ppm respectively; and the BAC observed was 0.13, 0.081, 0.047 and 0.05 for above concentrations respectively (Table 2). Maximum Cu^{+2} absorption was observed in shoot at 12 ppm (Fig. 1).

In the presence of different concentrations of Zn^{+2}, roots accumulated 0.24 and 0.22 ppm of Zn^{+2}, at 3 and 12 ppm that was highest at 6 ppm (0.42). The BAC observed was 0.09 and 0.018 for these concentrations respectively. The percent absorption in shoot was the highest at 12 ppm (Fig. 1). For the seedlings of *Z. mays* grown in the presence of 3, 6, 9 and 12 ppm of Zn^{+2}, the metal contents of the shoot were 0.29, 0.51, 0.44 and 0.40 respectively.

Table 1: Overall mean values (± S.E.) for various traits of *Zea mays* seedlings grown under different concentrations of copper and zinc

	Control	3	6	9	12
Cu^{+2} (Metals concentration (ppm))					
Germination (%)	99.5±0	97.9±0.056	98.2±0.052	97.5±0.067	97.6±0.093
Root length (cm)	3.49±0.11	3.39±0.17	3.15±0.06	2.22±0.13	1.25±0.12
Shoot length (cm)	6.28±0.01	6.07±0.24	5.35±0.15	5.23±0.17	4.25±0.16
Shoot/root ratio	1.79±0.04	1.79±0.12	1.69±0.18	2.35±0.25	3.4±0.14
Necrotic region (mm)	0.1±0.001	1.5±0.006	2.5±0.007	6.9±0.05	8.3±0.06
Chlorotic region (mm)	0.1±0.001	1.0±0.016	2.3±0.011	2.5±0.017	2.8±0.016
Zn^{+2}					
Germination (%)	99.5±0	98.3±0.08	98.7±0.018	98.1±0.085	97.5±0.026
Root length (cm)	3.55±0.16	3.51±0.11	3.24±0.03	3.15±0.05	2.45±0.11
Shoot length (cm)	6.25±0.02	4.26±0.11	3.34±0.08	3.02±0.02	2.19±0.06
Shoot/root ratio	1.76±0.11	1.21±0.16	1.03±0.15	0.95±0.01	0.72±0.12
Necrotic region (mm)	0.2±0.0025	2. 0±0.001	12.3±0.005	14.5±0.009	15.3±0.008
Chlorotic region (mm)	0.1±0.001	6.7±0.04	8.5±0.019	11.5±0.014	12.3±0.012

Table 2: Metals content of *Zea mays* seedlings grown under different concentrations of copper and zinc

	Cu^{+2}				Zn^{+2}			
	Metals concentration (ppm)							
Root	3	6	9	12	3	6	9	12
Metal content (fresh weight) ppm	0.41	0.43	0.49	0.60	0.24	0.42	0.18	0.22
Biological absorption coefficient	0.13	0.081	0.047	0.05	0.09	0.085	0.02	0.018
Shoot								
Metal content (fresh weight) ppm	0.27	0.30	0.27	0.37	0.29	0.51	0.44	0.40
Biological absorption coefficient	0.48	0.27	0.08	0.066	0.08	0.046	0.048	0.04

Table 3: Analysis of variance (mean squares) for various growth attributes of *Zea mays* seedlings grown under different concentrations of copper and zinc

Characters	MS_{Metal}	Significance	$MS_{Conc.}$	Significance	MS_{Inter}	Significance
Germination (%)	248.90	N.S.	39.10	N.S.	142.07	N.S.
Root length (cm)	21.98	***	1.07	**	4.10	***
Shoot length (cm)	4.74	*	9.67	***	1.19	N.S.
Shoot/root ratio	12.36	***	0.78	N.S.	0.65	N.S.
Necrotic region (mm)	0.78	N.S.	2.05	***	0.53	N.S.
Chlorotic region (mm)	4.25	***	1.09	***	4.25	***

*, **, *** denote significance at P<0.05, P<0.01 and P<0.001 respectively, N.S. = non significant

MS_{Metal} = Mean square metal, $MS_{Conc.}$ = Mean square metal concentration, MS_{Inter} = Mean square interaction

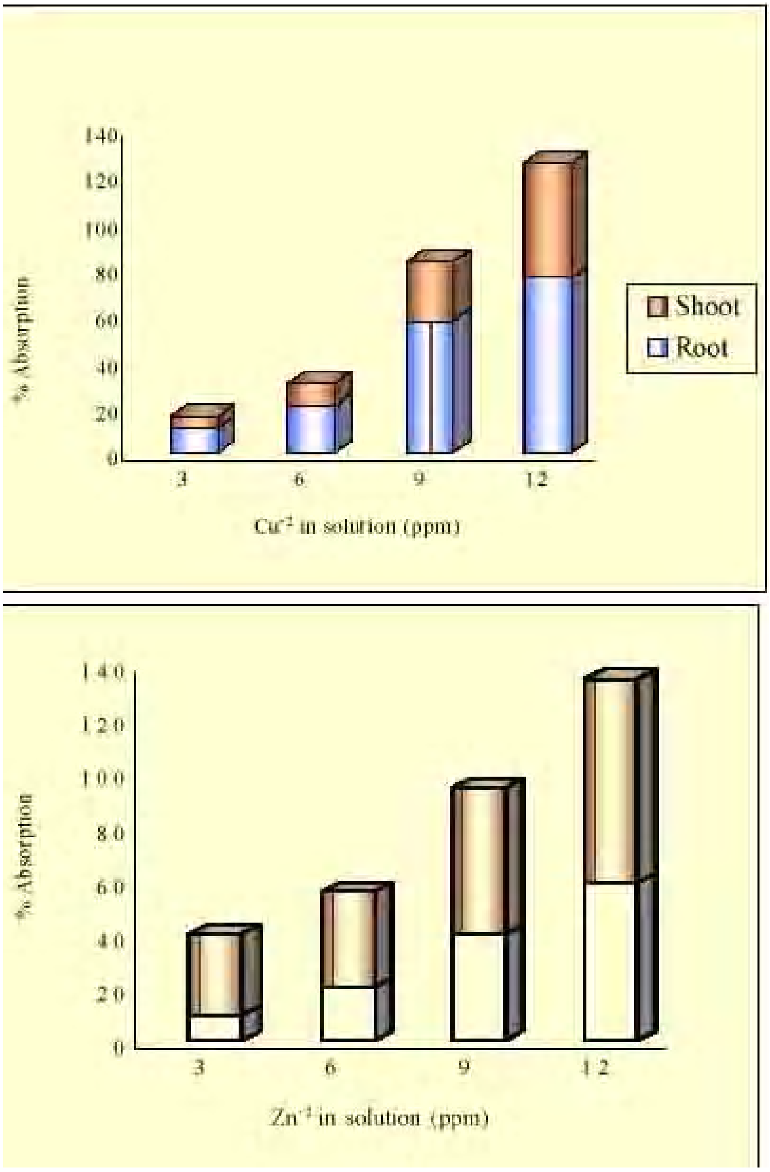

Fig. 1: Percent absorption of *Zea mays* seedlings grown under different concentrations of copper and zinc

The BAC was 0.08, 0.046, 0.048 and 0.04 for each of these respective concentrations (Table 2) and the % absorption being 30, 36, 55 and 76 (Fig. 1).

Discussion and Conclusions

The present results showed that the germination of *Z. mays* seeds was not influenced by the presence of Cu^{+2} and Zn^{+2} in the culture medium. The toxicity difference of the two metals was not evident at seed germination. The results of seed germination clearly indicated that the increasing concentrations of these two metals did not influence the germination. The insignificant effect of metal toxicity on germination suggests that seed uses its own reserves during germination process and this process is not influenced by the presence of metal ions in the culture medium (Stefani *et al.* 1991). Therefore, the presence of small concentration of heavy metals in the culture medium cannot account for inhibition of germination in *Z. mays*. These results are consistent with other herbaceous species like *Triticum vulgare* and *Avena sativa* (Fiussello, 1973) and in arboreal gymnosperms *Piceae rubens,* and *Abies balsamica* (Scherbatskoy *et al*. 1987). The presence of heavy metals in the culture medium had a significant adverse effect on seedling growth and health. The study showed reduction in root and shoot length, and development of chlorotic and necrotic area. The highest concentration of Zn^{+2} prevented the shoot growth. The growth of root was arrested in the presence of 12 ppm of $CuSO_4$. The presence of Cu^{+2} and Zn^{+2} affected the growth of *Z. mays* seedlings; the reduction in shoot growth was directly related to the heavy metal concentration (Table 1). Walley *et al.* (1974) reported similar effects in a number of other plant species.

The presence of Cu^{+2} in the culture medium showed a stronger effect on root growth than shoot while the reverse was true for Zn^{+2}. This suggests some preventive mechanism for the reduced translocation of Cu^{+2} from root to shoot (Nishizono *et al.* 1989). The distribution of the two metals was different between root and shoot as shown in Table 2. Cu^{+2} seem to concentrate in the roots while Zn^{+2} appeared to be more diffusible than Cu^{+2}. Stefani *et al.* (1991) similar pattern of distribution of heavy metals in *Juncus acutus* seedlings.

All the concentrations of Zn^{+2} and Cu^{+2} caused the necrosis of shoot. However, actual toxicity of any of these metals was not ascertained. Nevertheless, the development of necrotic region depended on concentration. These findings agree with the observations of Chatterjee and Chatterjee (2000), who reported similar effects of cobalt, chromium and copper on cauliflower. The chlorosis caused by the presence Cu^{+2} was in a minor extent as compared to Zn^{+2}. This may be explained by variable action of these metals on chloroplast. These assumptions are supported by independent studies by Becerril *et al.* (1988) and Barcelo *et al.* (1988).

The accumulation of metals in roots and shoots varied greatly. The amount of Cu^{+2} accumulated increased with its availability in the medium. However, the extent of Zn^{+2} deposition was the maximum at 6 ppm. Therefore, Zn^{+2} uptake seems to be promoted by lower ionic concentration in the culture solution as reported by Sela *et al.*, (1989). Early seedling development of Z. *mays* appeared to be prone to the presence of small amounts of metals in the culture medium. The data support the assumption that metal sensitivity is probably due to strong tendency of this variety to accumulate heavy metals. This justifies that the corn variety 'Neelum' is not suitable for the cultivation under situations where water and soil suffer from occasional and/ or transitory metal pollution.

Acknowledgement

Thanks are due to ICI Pakistan Ltd, Multan, Pakistan for supplying corn seed variety Neelum for testing for heavy metals.

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