Toxic Effects of Heavy Metals on Plant Growth and Metal Accumulation in Maize (Zea maysL.)

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ABSTRACT

Background: A pot experiment was conducted to determine the toxic effects of some heavy metals on the plant growth and seed yield of maize (Zea mays L.).

Materials and Metheds: Heavy metals Mn, Pb, Cd, Cr and Co individually and in combinations were added as chloride salts in solutions to the pots before sowing. The test plants for were harvested after 80 days of germination and evaluated for nitrogen, protein and heavy matal content was determined.

Resalts: Heavy metals caused significant decreases in growth and protein content. Cd was the most toxic metal followed by Co, Hg, Mn, Pb, and Cr. Protein content decreased from 16.0–68.4% in metal exposed plants at metal concentrations equivalent to those found in

Conclusion: Metal accumulation by seeds was directly related to the applied heavy metal with greater concentrations of metals found in cases where metals were added individually rather than in combinations. The toxic effects on the plant growth, nitrogen content in different plant parts, and protein content in seeds, exerted by two metals in combination were only as harsh as for the most toxic metal individually probably due to their antagonistic effects.

Keywords: Heavy metals, Metal accumulation, Maize, Phytotoxicity

INTRODUCTION

Industrial wastes are a major source of soil pollution that originate from mining industries, chemical industries, metal processing industries, and the like. These wastes include a variety of chemicals like heavy metals, phenolics etc. (1, 2). Use of industrial effluent and sewage sludge on agricultural land has become a common practice in the world as a result of which, these toxic metals can be transferred and concentrated into plant tissues from the soil. These metals have damaging effects on the plants themselves and may become a health hazard to man and animals. Above certain concentrations and over a narrow range, the heavy metals turn into toxins (3, 4). Moreover, these metals adversely affect natural microbial populations, leading to disruption of vital ecological processes (5,6,7). Currently, microorganisms are being used as potential bioindicators for the assessment of chemical risk to the ecosystem (8) and effects of heavy metals on the growth of plants and microorganisms have been investigated by several workers (9,10,11,12). Abiotic stresses like heavy metal stress, air pollutants stress etc., negatively affect processes associated with biomass production and seed yield in almost all major field grown crops (13). Every metal and plant interacts in a specific way, which depends on several factors such as type of soil, growth conditions, and the presence of other ions.

The objective of this study was to investigate the toxic effects of heavy metals on the growth of maize plants to determine the loss of agricultural productivity of a very important cereal crop.

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Table 1 The physico-chemical properties of soil and water used in the experiments

Physiochemical properties	**2006**	**2007**	**2008**
Soil characteristics			
Soil texture	Sandy loam	Sandy loam	Sandy loam
Sand (%)	20	23	19
Silt (%)	12	14	16
Clay (%)	65	63	62
Saturation percentage	27	29	26
Moisture percentage	17	18	15
Nitrogen (%)	0.53	0.47	0.55
Available P (mg kg^{-1})	6.6	7.0	7.3
K^+ (mg kg^{-1})	157	160	180
Ca^{2+} (mg L^{-1})	11.9	10.3	12.5
Soil pH	8.1	7.9	8.1
Electrical conductivity (dS m^{-1})	2.1	2.5	2.2
Cadmium (mg g^{-1})	Traces	Traces	Traces
Organic matter	1.15	1.21	1.23
Water characteristics			
Electrical conductivity (dS m^{-1})	0.82	0.81	0.84
PH	7.11	7.24	7.15
Sodium adsorption ratio	3.74	3.65	3.91
RSC	1.92	1.84	1.87

Table 2 Composition of Nutrient Solution applied to the plants (Hoagland and Arnon, 1950)

Macronutrients	**Concentration**
Calcium nitrate	3.54 Mm
Potassium nitrate	5.00 mM
Magnesium sulphate	2.00 Mm
Potassium dehydrogen phosphate	1.02 Mm
Micronutrients	
Boric acid	92.2 µM
Manganese sulfate	2.19 µM
Zinc chloride	1.62 µM
Copper sulfate	0.69 µM
Sodium molybdate	0.29 µM
Na-Fe-EDTA	0.15 µM

All the salts were dissolved and mixed after autoclaving separately. Final pH of the solution was adjusted at 6.7

MATERIALS AND METHODS

The soil in which the experiments have been conducted was a sandy clay loam and had received no exogenous input of metals. The physiochemical properties and the heavy metal concentrations of the soil are given in Table I. The soil was sieved (<2 mm) and homogenized and the test heavy metals were added as the solutions of their chloride salts. The amounts of heavy metals added were equivalent to normal, half and twice the concentrations found in the polluted soil in sargodha.

Table 3 Amounts of heavy metals added to the soil at various levels

Heavy Metal	Concentration of heavy metals (mg Kg^{-1}) at		
	3	6	9
Pb	98.00	196.19 (100.6)[a]	377.34
Mn	2559.8	5312.2 (1158.0)	10238.6
Co	368.8	738.4 (107.2)	1382.6
Hg	151.9	303.7 (112.6)	608.3
Cd	7.3	13.4 (5)	26.6
Cr	39.24	76.42 (20.1)	138.03

The values in parentheses indicate the concentrations of bio-available forms of heavy metals

Pot Experiment

A pot experiment was conducted with maize, Zea mays L. varNeelam as model crop. Five kilograms of soil were taken in each pot for the different treatments and three replicates were taken for each treatment. An extra set of pots which contained no added heavy metals were also taken which served as a control. The heavy metals Mn, Pb, Hg, Cd, Cr and Co individually and in combinations were added once as chloride salts in solution to the soil before sowing. Sufficient water was added to bring the soil to 50% of its water holding capacity. The soil was preincubated for 2 weeks before sowing, and was also fertilized with 120:60:50 kg ha^{-1} of nitrogen, phosphorus and potassium (NPK). Maize seeds obtained from the Ayub Agricultural Research Institute (AARI),Faisalabad, were washed with distilled water. Eight seeds of maize were sown in each pot. Seedlings were thinned to five plants per pot, and plants were watered as and when required. The pots were randomized on alternate weeks to minimize any positional effects.

Biomass Production

The test plants for biomass production were harvested after 80 days of germination. Roots and shoots were dried at 80 $^{\circ}$C for 18 hr and then weighed separately. The grade of growth inhibition (GGI) was evaluated by the comparison of dry matter production of metal treated and control plant tissues (14).

Seed Yield

Seed yield was also recorded at the harvest of the crop.

Determination of Available Soil Nitrogen

Available soil nitrogen of the treated and control soil was estimated by the Kjeldahl method using alkaline permanganate (15,16) after about four weeks of germination of seeds. The procedure involves distilling the soil with alkaline potassium permanganate solution and determination of the ammonia liberated which serves as an index of the available nitrogen status.

Determination of Nitrogen in Plant Parts

At harvest, the shoots and roots were dried at 80 ◦C for 18 hr, weighed and ground to pass through a 2 mm pore size stainless steel sieve and the nitrogen in roots and shoots was determined by the Kjeldahl method (17).

Determination of Protein Content in Seeds

At harvest, seeds were dried, weighed and ground. The protein content was then determined by the Kjeldahl method (17).

Heavy Metal Accumulation by Seeds

The heavy metal concentrations of the most toxic (Cd) and the least toxic (Cr) metals found in this study in the seeds of maize were determined in hot concentrated HNO3 digests of the ground seed samples. Heavy metals were determined by Atomic Absorption Spectrophotometer (AAS) model Unichem FP 1900 series.

Statistical Analysis

The results were analysed statistically by analysis of variance and critical difference (CD) at 5% level according to standard procedures (18).

RESULTS

Toxicity of heavy metals on the growth of maize is presented in Table 3. These data indicate that the heavy metals were toxic to the growth of maize plants. Shoots of plants had noticeable and gradual stunted growth. These symptoms were more obvious in treatments containing Cd alone and a combination of all the heavy metals (Table 4). The reduction in dry weight of maize plants as a result of treatment with heavy metals was minimum with Cr and Pb. Phytotoxic effect of heavy metals was in the following order:

Cd > Co > Hg > Mn > Pb > Cr

The higher the concentration of heavy metal in the soil, the greater was the toxic effect on the plant. The results in Table 3 show that the effects of combinations of two metals were not preservative, rather the effects were only as severe as the most toxic metal alone.

The lowest reduction in the seed yield was recorded with Cr at all the test doses and the highest was recorded in the plants having been treated with all the test metals (Table 3). Decrease in the seed yield was less than 40% with Cr as against 83.9% by Cd at 2× concentrations. Perusal of the data in Table 3 clearly indicates that though Cr appeared to be the least toxic metal, it also led to substantial losses (>40%) in the dry matter at 2× concentrations. Results in Table 3 show that the dry weights of shoot and root of maize plant, respectively, were reduced 63.4 and 70.5% by Cd; 58.5 and 55.8% by Co; 51.2 and 46.1% by Hg: 26.3 and 29.1% by Pb; 31.7 and 39.7% by Mn; 17.0 and 13.8% by Cr at 0.5× concentration. Mn appears to be less toxic than Co and Hg despite the relatively higher amount used in the study.

Table 4 Dry matter and seed yield of maize plant exposed to various levels of heavy metals added either individually or in combination

Heavy metal treatment	Dry weight of shoot (g/pot) at			Dry weight of root (g/pot) at			Seed weight (g/pot) at		
	3	**6**	**9**	**3**	**6**	**9**	**3**	**6**	**9**
Pb	4.03	3.49	1.90	3.51	2.81	1.40	5.04	3.98	3.09
Mn	3.85	3.15	1.75	3.05	2.78	0.94	4.44	3.56	2.81
Co	1.80	1.45	1.08	2.50	1.30	0.78	3.38	1.84	1.09
Cr	3.45	2.86	2.41	2.98	2.08	1.88	5.51	4.06	3.83
Hg	3.00	1.76	1.70	1.88	1.80	0.72	3.83	2.39	2.08
Cd	1.70	1.16	0.85	1.02	0.89	0.66	1.99	1.38	0.95
Hg + Cd	1.70	3.00	0.84	1.08	0.86	0.53	1.88	0.96	0.75
Hg + Cr	2.75	1.69	1.45	2.09	1.49	1.06	2.81	2.39	1.88
Cr + Cd	1.75	0.89	1.09	1.29	0.94	0.95	2.08	1.84	1.36
Hg + Cr + Cd	1.08	0.66	0.86	0.87	0.48	0.39	1.09	0.66	0.49
Hg + Cr+ Cd+ Co + Mn + Pb	0.85	0.56	0.44	0.42	0.35	0.17	0.84	0.43	0.33
Control	-	4.10	-	-	3.40	-	-	5.30	-
Statistical Analysis (F test)	Sig.[a]	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
CD[b] at 5%	0.78	0.71	0.69	0.65	0.65	0.60	1.40	1.40	0.72

Values are mean of three replicates
Sig. = Significantly different over control
CD = Critical difference

Table 5 Available soil Nitrogen mg/kg in rhizosphere soil of maize under different metal treatments

Heavy Metal	Available soil nitrogen at various levels of heavy metal treatment		
	3	6	9
Pb	98.0±6.0	94.2±2.3	92.3±5.3
Mn	97.7±6.1	92±1.5	94.0±2.6
Co	92.7±4.4	90±6.5	89.2±3.7
Cr	99±5.0	96±2.3	95.5±4.3
Hg	94±1.9	91±5.4	89.5±3.2
Cd	89.5±7.6	89±3.4	83.7±3.0
Cr + Cd	87.5±3.9	87±1.2	84.9±2.9
Hg + Cr	92.2±1.5	82±2.5	83.1±2.2
Cr + Cd	87.7±1.8	80.5±2.9	82.1±1.8
Hg + Cr + Cd	86.3±2.7	78±2.8	80.0±3.0
Hg + Cr + Cd + CO + Pb + Mn	84.5±2.8	77±2.6	75.8±1.9
Control	-	114.0±6.0	-
Statistical Analysis (F test)	N.S[b] .	N.S.	N.S.

Values are mean of three replicates
a ± Standard deviation
[b] N.S = Non significant

Results in Table 5 show that the reduction in nitrogen content in the soil with metal treatments at all the three concentrations was found to be statistically insignificant. The order of toxicity of various heavy metals was as under:

Cd > Co > Hg > Mn > Pb > Cr

The nitrogen content in shoots and roots decreased in metal treated plants. Data in Table 6 indicates that there was a significant reduction in nitrogen (%) in shoots and roots of maize plants compared with the control. In both the shoots and roots, the percent of nitrogen varied inversely with the amounts of
331 metals added, Cd and Co causing the greatest effect. The percent of nitrogen in shoot and root was reduced by 68.6 and 79.4% in the presence of Cd at 6mg concentration. Data in Table 7 indicates that heavy metal treatment under different concentrations resulted in decreased protein content in seeds with Cr recording the highest protein content in seeds. Protein content was significantly lower in metal treated seeds compared with the control.

The metal (Cd and Cr) accumulation pattern by maize seeds is given in Table 7. It was observed that as the concentration of Cd and Cr in the seeds increased, the yield was reduced and the metal accumulation in seeds was also found to be directly related to that applied to the soil. The concentrations of individual metals in seeds were usually found to be greater for metals added individually than in combinations of all metals in the pot.

Table 6 Percent nitrogen content in shoots and roots of maize plant as influenced by different heavy metal treatment

Heavy metal treatment	**% N in shoot at various concentrations of metal treatment**			**% N in root at various concentrations of metal treatment**		
	3	**6**	**9**	**3**	**6**	**9**
Pb	1.94	1.74	1.52	1.60	1.50	0.72
Mn	1.62	1.42	1.30	1.40	0.76	0.60
Hg	1.52	1.30	1.04	1.07	0.63	0.56
Co	1.44	1.11	1.00	1.0	0.72	0.56
Cd	1.32	1.0	0.82	0.96	0.63	0.45
Cr	2.10	1.96	1.80	1.90	1.59	0.40
Hg + Cr	2.10	1.80	1.61	1.20	1.01	0.92
Cr + Cd	1.43	2.02	1.02	1.06	0.83	1.00
Hg + Cr + Cd	1.10	1.29	0.80	0.52	0.45	0.26
Hg + Cr + Cd + Pb + Mn + Co	0.82	0.63	0.43	0.38	0.30	0.21
Control	_	2.40	_	_	1.80	_
Statistical Analysis (F Test)	Sig	Sig	sig	Sig	Sig	sig
CD at 5%	0.95	0.73	0.60	0.60	0.47	0.39

Values are mean of three replicates
Sig = Significantly different over control
CD = Critical difference

Table 7 Protein contents in maize seeds as affected by different heavy metals treatment

Heavy Metal treatments	Protein (%) in maize seeds at		
	3	6	9
Pb	20.0	17.6	15.1
Mn	18.2	14.5	12.0
Hg	16.5	13.5	10.0
Co	15.0	13.2	11.5
Cd	12.7	10.1	8.2
Cr	21.0	19.0	17.0
Hg + Cr	17.6	14.5	12.0
Cr + Cd	12.0	10.7	9.6
Hg + Cd	11.3	9.2	7.6
Hg + Cr + Cd	11.5	10.0	8.2
Hg +Cr +Cd+ Mn + Pb + Co	9.2	8.0	6.0
Control	-	23.0	-
Statistical analysis (F test)	Sig	Sig	Sig
CD at 5T	10.3	9.4	8.5

Values are mean of three replicates
Sig = Significantly different over control
CD = Critical difference

Table 8 Cd and Cr contents (mg/kg) in maize grains as influence by metals amendments added individually or in combination

Heavy metal treatment	Cd Concentration at			Cr concentration at		
	3	**6**	**9**	**3**	**6**	**9**
Cd	0.24±0.04[a]	0.44±0.09	0.90±0.20	-	-	-
Cr	-	-	-	0.95±0.14	3.74±0.22	12±0.2
Hg +Cr +Cd+ Mn + Pb + Co	0.13±0.03	0.33±0.10	0.53±0.20	0.74±0.43	0.67±0.17	9.9±4
Control	-	N.D. [b]	-	-	0.14±0.3	-

Values are mean of three replicates
a ± Standard deviation
[b] N.S = Non significant

DISCUSSION

Treatment of maize plant with heavy metals resulted in the decreased dry matter and seed yield, reduced nitrogen content in plant tissues, and lowered protein content in seeds. Table 3 indicates that heavy metals exerted an adverse effect on the growth and yield of maize plants substantiating the reported phytotoxicity of these metal ions (19). The effects on plants of environmental stresses are determined by the responses of the individual cells in which the integrity of structure and function is affected (20). In the present investigation, Cr was found to be the least but significantly phytotoxic metal as compared to other metals added individually in the soil. Low levels of phytotoxicity of Cr (III) have been attributed to its insolubility under most soil conditions (21), and it did not affect the plant growth unless the concentrations were very large (22).

Previous investigations indicated that Cr^{3+} added in sand culture of maize under glasshouse conditions brought about significant reduction in biomass, chlorophyll and activities of catalase and peroxidase while it enhanced the acid phosphatase and ribonuclease activities (23). Cd at all levels tested was found to be the most toxic metal for the maize crop and caused the most severe reduction in the dry weight of shoot, root and seed yield followed in order by Co, Hg and Mn. Previous studies have also demonstrated a relatively higher phytotoxicity of Cd and Co than that of Mn (24). In general, the reduction in the dry weight of roots was more severe than the dry weight of shoots following treatment with heavy metals added individually or in combination (Table 3). This is supported by the findings of Karataglis who reported that the influence of relatively higher amounts of Co, Mn, Pb, Hg, Cr and Cd in maize cv (25). Vergina resulted in depressed shoot growth but the most evident

Symptoms were on roots. Baccouch showed that the accumulation of carbohydrates in maize shoots treated with Hg might, at least in part be the cause of root growth inhibition (12).

Amendment of soil with the heavy metals at concentrations higher than the normal levels resulted in a conspicuous decrease of root and shoot biomass expressed in terms of dry weight (Table 3). It has earlier been reported that increasing Co supply resulted in decreased root biomass indicating the alterations of physiology and metabolism of test plants (26). Biomass loss (fresh weight) under metal treatment has also been reported by many workers (27,28).

When two heavy metals were added in combinations, instead of a preservative effect on the phytotoxicity, the effect was only as severe as for the most toxic metal alone. This might be due to the antagonistic effect of the two metals. Cd is reported to antagonize the inhibitory effect of Mn on the total amount of mineralized carbon (29). A significant decrease in the seed protein content was observed with heavy metal treatment in this study (Table 7). This is in accordance with the findings of Salgare and Acharekar who reported that growth performance, as well as pigment, carbohydrate and protein content showed a decreasing trend with increase in the level of industrial pollution (30). Decreased levels of protein content in heavy metal exposed tissues have been reported by many workers (31, 32). Relatively strong affinities of heavy metal ions for side chain ligands of protein indicate that enzyme and other functional proteins are one of the primary targets of metal toxicity (33). Our results on the metal accumulation by maize seeds are given in Table 8. The accumulation of metals in the seeds was greater at least in the case of 3mg concentration when the metals were added individually than in combination. This is in accordance with the findings of Smilde who demonstrated that the total amounts of metals in plant tissues were higher for metals added inividuallyly than for combined metals (34). It is an established fact that the soils and plants under waste water irrigation from various industries contained higher concentrations of heavy metals than those irrigated with tubewell water (35). Moreover, the heavy metals deposited in soil were bound preferentially to interaggregate soil material, and accumulation preferentially occurred in parts of the soil where plant roots were concentrated and in the forms easily accessible for plants (36). Cd is of particular concern to the human health as it is concentrated by many cereal and vegetables (leafy and roots) as well as fruits (37,38) which can lead to unexpected human intoxication when it is consumed (39). Albering showed that the legal standard for Cd as endorsed by commodities act was exceeded in maize crops grown in soil contaminated with heavy metals and the main exposure pathways for the general population was through the consumption of food crops grown in these soils (40). The different heavy metals used in this study were found to vary in their phytotoxic effects with Cd being the most toxic and Cr the least toxic. Therefore, the alkaline pH of the test soil in the present system presumably makes it easier to monitor the toxicity of heavy metals only. We can conclude that soils contaminated by heavy metals probably lead to substantial losses in dry matter and seed yield of maize plant.

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