Phytotoxic effects of Heavy metals (Cr, Cd, Mn and Zn) on Wheat (Triticum aestivum L.) Seed Germination and Seedlings growth in Black Cotton Soil of Nanded, India

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Available online at: www.isca.in

Received 25th April 2013, revised 2nd May 2013, accepted 20th May 2013

Abstract

To assess the phytotoxicity of chromium (Cr), cadmium (Cd), manganese (Mn) and zinc (Zn) to wheat, experiments were carried out in Black Cotton soil (of Nanded city, India) fed with different aqueous concentrations (2, 4, 6, 8 and 10 mg/L) of aforementioned metal ions over the period of eight consecutive days. The phytotoxic effects on seed germination, root, shoot, seedling growth, seedling vigor index, tolerance indices of wheat (Triticum aestivum L.) were studied. All results, when compared to control, show heavy metals adversely affecting the normal growth of plants by reducing seed germination and decreasing root and shoot length. The toxic effects of selected heavy metals to seed germination can be arranged in the rank order of inhibition as: Zn>Cd>Mn>Cr. The toxicity of all heavy metals to young seedlings was found similar to seeds and their effects on seedlings increase with their increased concentrations in the aqueous medium or soil ecosystem. The present results thus exemplify a model system to screen for various concentrations of heavy metals for their phytotoxic effects and also screen for the seeds able to counteract the deleterious effects of such heavy metals in various types of irrigation waters and agricultural soils.

Keywords: Phytotoxicity, heavy metals, triticum *aestivum* L., germination, tolerance indices, seedling vigor index.

Introduction

Heavy metals exist in nature, but their elevated level due to anthropogenic activities represents serious ecological, environmental, and financial issues¹. Heavy metal contaminated sites require immediate remediation and thus pose a major technological and financial problem worldwide². The problem is seriously ecological, because these metals due to their bioaccumulation can enter food chains and the biological cycle. They can eventually affect plants and animals including humans^{3,4}.

Soil ecosystems and irrigation water are contaminated with heavy metals by human-activities, e.g. unrestricted mining, municipal waste, industrial and automotive emissions, extensive use of agrochemicals, etc⁵. The heavy metals can be in dissolved form or immobilized. The immobilized heavy metals are known to be more hazardous to plants than those dissolved⁵. The former group includes Mn, Fe, Cd, As, Pb and Hg. The metals most damaging to crops are Cd, Cu, Mo, Ni, Pb and Zn⁶. Most of these metals precipitate readily in agricultural soil. Soluble metals such as Al or Mn in high concentrations are present in acid soils while elevated levels of metals such as Cr, Cd, Zn, Cu or Pb may be present in places contaminated by human-induced activities. Particularly interesting amongst the heavy metals are

Cr, Cd, Mn and Zn which at low doses are considered as essential micronutrients for the growth of plants and animals, but at higher concentrations cause toxic effects such as metabolic disorders and growth inhibition^{7,8}.

Upon literature survey, we found scientific reviews that examine the effect of heavy metals on plant growth and function 9,10 . The trace metals exerting toxic effects on plants have been studied for over a century by now but there remains confusion within the literature with regards to their concentrations as micronutrients and as components inducing phytotoxic effects 11 . For example, Taylor and Foy found 30 μM Cu enough for reducing growth of wheat (Triticum aestivum L.) by 50%, whereas Wheeler et al. reported only 0.5 μM Cu required for a 50% growth reduction in the same species 12,13

Wheat is the most vantage and pivotal cereal for the world population including India. Like many plants, wheat at seed germination and seedling stages are sensitive to environmental factors. The current investigation use a range of different aqueous concentrations of four selected heavy metals (Cr, Cd, Mn and Zn) for investigating their phytotoxic effects on the seed germination and seedling growth of wheat (Triticum *aestivum* L.) in Black Cotton soil.

Material and Methods

Wheat (Triticum aestivum L.) – the test plant: Wheat (Triticum aestivum L.) was selected as a model plant system. Wheat is recommended for such experiments by the Guideline for testing of chemicals, Terrestrial plant test: 208: Seedling emergence and seedling growth test. Paris: Organisation for Economic Co-operation and Development (OECD)¹⁴. Certified and healthy seeds of wheat (Triticum aestivum L.), being widely planted all over the Maharashtra State of India, were used in this study. Seeds were kindly provided by Nanded Agricultural Production Market Committee's supplier. To prevent fungal contamination, seeds were soaked in 1 vol% H₂O₂ for 15 min and washed five times with double distilled water immediately before use. Prior to use, the glassware was acid washed. Singleuse plastic containers were used in the phytotoxicity trials.

Black cotton soil: Black soil is internationally known as 'tropical black earths' or 'tropical chernozems.' In India, black soil is spread across Maharashtra State, Malwa Plateau i.e. western parts of Madhya Pradesh and south-eastern parts of Rajasthan, interior parts of Gujarat, parts of Andhra Pradesh, parts of Tamil Nadu and Deccan Lava Plateau.

The soil and vegetation are, as usual, related to climate and the geology. The soil is formed from the igneous and black basalt rock of the Deccan plateau. The volcanic activity, which had taken place in the region centuries ago, and the breaking of igneous rocks are considered to have given the soil black colour, composition, fertility and texture. Black soil, locally termed as Regur, is commonly called as the Black Cotton soil (BC soil) because it is best suited for the cultivation of cotton. The physical properties of BC soil might vary from place to place. The soil is rich in humus and organic matter. The black color in BC soil is considered to be due to titanium oxide in small concentration. The BC soil has a high percentage of clay, which is predominantly montmorillonite in structure and black or blackish grey in color. This mineral is responsible for swellshrink behaviour of the soil. This soil has a reputation for being highly moisture-retentive and thus responds well to irrigation. In addition to iron, the BC soil contains fairly high quantities of lime, magnesia and alumina.

For this research work, the natural BC soil has been collected from Nanded city area. Nanded is a District headquarters located in the south-eastern part of Maharashtra State. It is situated in Deccan Trap, a part of Deccan Plateau of India. The Geographical coordinates of Nanded are: Latitude: 19⁰ 9' 0" North and Longitude: 77⁰ 20' 0" East.

Heavy metals and their different concentrations: The stock solutions of chromium (Cr), cadmium (Cd), manganese (Mn) and zinc (Zn) were prepared at concentrations of 2, 4, 6, 8 and 10 mg/L by using standard APHA methods¹⁵. K₂Cr₂O₇, Cd metal, KMnO₄, Zn metal powder were used in these preparations as sources of Cr, Cd, Mn and Zn respectively. The seeds were then allowed to germinate in sterilized Petri dishes,

on Whatmann filter-paper moistened with 10 ml of selected heavy metal test solution. Seed germination rate was noted down every 24 hours upto eight days. The Petri dishes, covered with a net, were kept in laboratory under ambient conditions. The length of shoot and root were recorded by using a centimetre scale, % phytotoxicity for shoot and root of 8 day old seedlings were calculated by the formula given by Chou and Lin¹⁶. All the Petri dishes with 10 seeds were kept under optimum agricultural farm conditions by their exposure to ambient temperature, air and light conditions in the laboratory. A control experiment uses double-distilled water to irrigate BC soil with wheat seed and the water is devoid of any metal concentration.

Germination parameters and their determination: Experiments were carried out to determine whether or not the supply of aqueous concentrations Cr, Cd, Mn and Zn influence seed germination, seedling growth including shoot and root length, seedling indices, etc. The tests were performed on 10 wheat seeds exposed to increasing concentrations of 2, 4, 6, 8 and 10 mg/L. For each metal concentration and control groups 10 seedlings were used. Radicle and plumule lengths were noted down. All the analyses were made in triplicates.

Statistical analysis: Mean standard deviations, variance, minimum, maximum, standard error, and correlation coefficient were calculated from at least three replicates. Statistical significance was determined. After 8 days, 10 seedlings of each Petri dish were sampled to measure the root length and shoot height using a centimetre-scale (against a black background). Germination was not considered until the lengths of radicle measured more than 2 mm and other relevant calculations were made based on Soltani $et\ al^{17}$.

Vigor Indices were determined by the following formula given by Iqbal and Rahmati¹⁸.

The Vigor Index (VI) was calculated using the formula,

VI = (mean root length + mean hypocotyls/shoot length) X % germination.

Seedling vigor index (SVI) was determined as per formula given by Bewly and Black $as^{19}\,$

Seedling Vigor Index = % of Germination X Mean seedling length (cm)

The meaning of the above formulae for VI and SVI are one and the same.

Tolerance indices (T.I.) were determined through use of the following formula given by Iqbal and Rahmati, ¹⁸

T.I. = (Mean root length in metal solution / Mean root length in distilled water) x 100

The length of shoot and root were recorded by using a centimeter scale, % Phytotoxicity for shoot and root of seedlings were calculated by the following formula given by Chou and Lin¹⁶:

Results and Discussion

All seeds germinated the next day. The result of the study is compiled in table-1. Chromium, cadmium, manganese and zinc treatments exerted toxicity on seed germination and seedling growth in Triticum aestivum L. as compared to control. Figures 1-8 summarize the results of the effects of metals on seed germination, root, shoot, seedling growth, seedling vigor index, tolerance indices, and phytotoxicity of root and shoot of Triticum aestivum L. Seed germination, seedling length, root length, shoot length, and seedling Vigor index in Triticum aestivum L. significantly decreased at 10 mg/L of Cr, Cd, Mn and Zn treatment as compared to control. Results obtained from the germination studies indicated that the Triticum aestivum L. showed higher germination percentage, seedling growth at 2 mg /L metal level in the medium. Statistically results showed that all the heavy metals significantly affected root, shoot and seedling length of wheat.

Seed germination: Seed germination was found reduced at higher metal concentration (figure-1). Control showed 100% germination whereas other reduced average germination ranged from 32-80%. Reduction in germination percentage of wheat at higher concentrations may be attributed to the interference of metal ions. The percent mean values of seed germination are 80±24.49, 64±32.86, 64±26.07 and 32±22.8 (Mean ± Standard Deviation) for chromium, cadmium, manganese and zinc respectively.

Shoot length: Shoot length decreased with increasing the concentrations of all the heavy metals tested. The average shoot length of Triticum *aestivum L.* decreased from 4.2-1.0, 4.25-2.0, 4.6-1.8 and 3.7-00 cm due to increased concentration of Cr, Cd, Mn and Zn respectively (figure-2).

Root length: The average root length of Triticum *aestivum L.* decreased from 1.37-0.6, 1.02-0.5, 3.2-0.56 and 4.9-00 cm due to increased concentration of Cr, Cd, Mn and Zn respectively (figure-3). Root length of wheat was significantly inhibited at all the treatments of heavy metals as compared to control.

Seedling length: Metals at higher concentrations suppressed the seedling growth of the Triticum *aestivum* L. (figure-4). The average seedling length of Triticum *aestivum* L. decreased from 5.57-1.6, 5.27-2.5, 7.8-2.36 and 8.6-00 cm due to increased concentration of Cr, Cd, Mn and Zn respectively. The toxicity of all heavy metals to young seedlings was found similar to seeds and their effects on seedlings increase with their increased concentrations in the aqueous medium or soil ecosystem.

Phytotoxicity of shoot: Phytotoxicity of shoot was decreased at lower concentration (2 mg/L) and increased at higher concentration (10 mg /L). The Phytotoxicity of shoot of Triticum *aestivum* L. increased from 19.23-80.76, 18.26-61.53, 11.53-65.38 and 28.84-100 % due to increased concentration of Cr, Cd, Mn and Zn respectively. Figure-5 depicts the effect of metals on our phytotoxicity studies of shoot (%) in Triticum *aestivum* L.

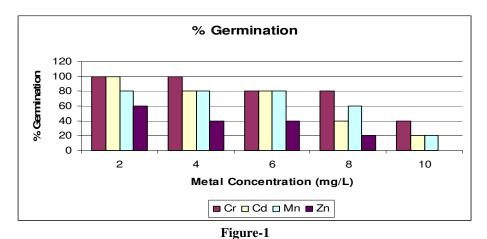
Phytotoxicity of root: Phytotoxicity of root was decreased at lower concentration (2 mg/L) and increased at higher concentration (10 mg/L). The Phytotoxicity of root of Triticum *aestivum* L. increased from 72.6-88, 79.6-90, 36-88.8 and 2.0-100 % due to increased concentration of Cr, Cd, Mn and Zn respectively. The effect of metals on phytotoxicity of root (%) in Triticum *aestivum L*. is plotted in figure-6.

Seedling vigor index: Seedling Vigor index was increased at lower metal concentration (2 mg/L) and decreased at higher concentration (10 mg/L). The average seedling Vigor index of Triticum *aestivum L*. decreased from 557-64, 527-50, 624-47.2 and 516-00 due to increased concentration of Cr, Cd, Mn and Zn respectively. The effect of different metals on seedling Vigor index is shown in figure-7.

Tolerance index: The seedlings of Triticum *aestivum* L. were tested for tolerance to heavy metals, using different aqueous concentrations of chromium, cadmium, manganese and zinc. The figure-8 shows indices of tolerance for Triticum *aestivum* L. at different treatments of metal. Tolerance index decreased from 27.4 to 12, 20.4 to 10, 64 to 11.2 and 98 to 0 due to treatments with increased concentrations of Cr, Cd, Mn and Zn respectively.

Statistical average value of different studied parameters of Triticum aestivum L. are shown in table-2. Correlation coefficient matrix among the parameters was calculated and correlations between various parameters were worked out. The correlation coefficient for analysed parameters is presented in table-3. The significance of the observed correlation coefficients has been tested. Significant positive and negative correlations among the parameters were determined. Out of a total of 36 correlations between parameters, 12 were found to have negative (inverse) correlations. The tolerance index and mean root length shows highest correlation coefficient 0.9999 among all other parameters.

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Percentage decrease in seed germination (%) at different concentrations of metals in Triticum aestivum L

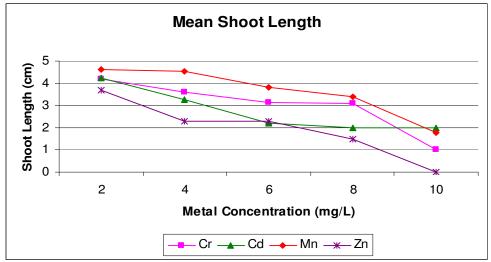


Figure-2
Effect different concentrations of metals on shoot length of Triticum aestivum L

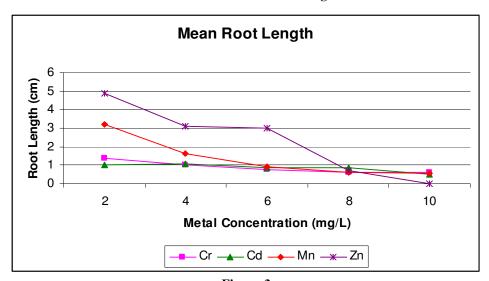


Figure-3
Effect different concentrations of metals on on root length of Triticum aestivum L.

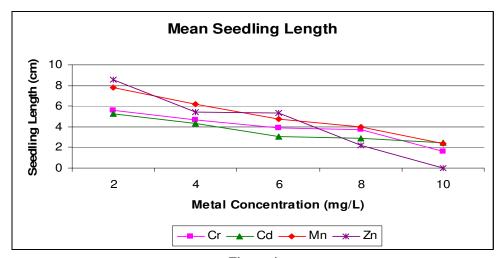


Figure-4
Effect of different concentrations of metals on seedling length of Triticum aestivum L

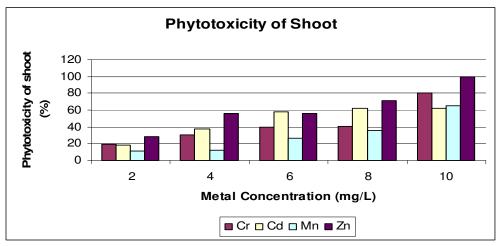


Figure-5
Effect of metals on phytotoxicity of shoot (%) in Triticum aestivum L

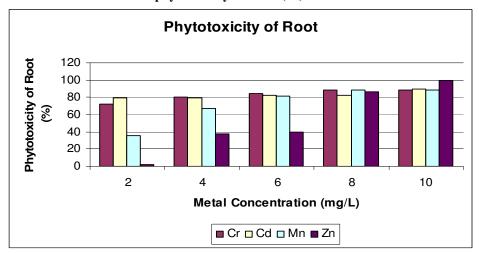


Figure-6
Effect of metals on phytotoxicity of root (%) in Triticum aestivum L

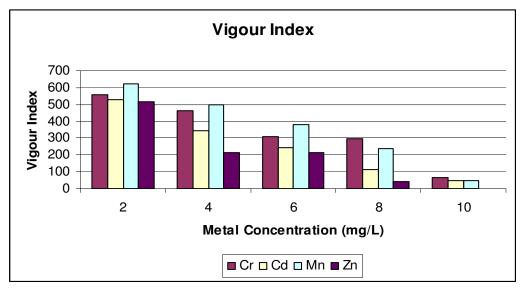


Figure-7
Effect of different metals on seedling Vigor of Triticum *aestivum L*

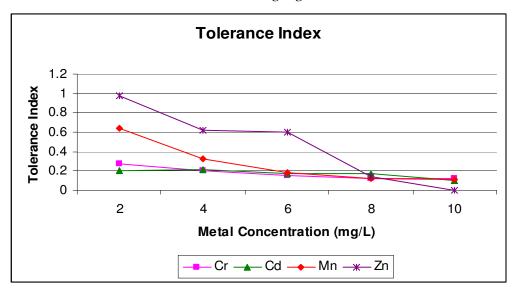


Figure-8
Indices of tolerance for Triticum *aestivum* L. at different treatments of metals

Discussion in Details: There are previous reports presented by a number of authors that clearly indicate a negative impact of heavy metals on seed germination²⁰⁻²⁶. It could be helpful to test the seed security and plant growth in heavy metal polluted water and soil ecosystems. In our experiments, wheat (Triticum aestivum L.) is easily grown in the laboratory mimicking farm conditions for its use in toxicity tests. Seeds of wheat germinated the next day under all the trace metal treatments.

The main objective of the research was to determine the effect of aqueous concentrations of chromium (Cr), cadmium (Cd), manganese (Mn) and zinc (Zn) on Triticum aestivum L. wheat variety in BC soil (of Nanded city India). The change of plant growth at the germination and seedling stage under heavy metal

stress is once again regarded as an important index to evaluate plant tolerance to heavy metals. This limitation impact observed depends mainly on the type of heavy metal; the concentration applied to the wheat species in BC soil. We found that increasing concentrations of heavy metals gradually decreasing germination percentage, germination index and vigor index as compared to control seeds. In case of zinc, with concentration of 10 mg/L, it caused deadly poisoning to the plant.

Characteristics and phytotoxicity of heavy metals Cr, Cd, Mn and Zn: Chromium: Several industries involving electroplating, metallurgy, tanning, paints, pigments, chemicals and paper and pulp production have contaminated soil and water with chromium (Cr) and thus has threatened the environmental

health²⁷. We know that Cr is a strong toxic metal and its ions are tightly bound to humus and clay particles and are more or less insoluble in soils, such as in the BC soil. Among the most stable and common forms of chromium, Cr (VI) is considered the most toxic form of Cr, which usually occurs associated with oxygen as chromate (CrO4²⁻) or dichromate (Cr2O⁻⁷) oxy anions that have a long residence time and high solubility in water.

Cadmium: Cd is one of the most highly dispersed metals by anthropogenic activities⁵. The agricultural soils are contaminated by fertilizer impurities (Cd²⁺), use of refuge-derived compost and sewage sludge (Cd²⁺). Cadmium is easily taken up by plants because, geochemically, it is quite mobile element in water and soil ecosystems. Cadmium has a bad reputation for being highly toxic and threatening to plant growth²⁸.

Manganese: Mn is an essential micronutrient for plants. Lack of Mn is damaging for chloroplasts because it affects the watersplitting system of Photo System, which provides the necessary electrons for photosynthesis. However, Mn in excess is damaging to the PS. Thus, Mn is i. as an essential micronutrient; and ii. a toxic element when it is in excess²⁹. Its toxicity is

favoured in acid soils. With decreasing pH, the amount of exchangeable manganese i.e. mainly Mn²⁺ form increases in the soil solution. Mn concentrations in excess in plant tissues can alter various processes including utilization of other mineral elements (Ca, Mg, Fe and P) and cause oxidative stress. The threshold of Mn injury as well as the tolerance to an excess of this metal is highly dependent on the plant species.

As manganese is present in several oxidation states (0, II, III, IV, VI and VII), its biogeochemistry is complex in soils. The most preferred forms of Mn in biological systems are with II, III and IV oxidation states. Among these, divalent manganese (Mn II) is the most soluble species of Mn in soil and Mn II and Mn IV are almost insoluble³¹. Oxides of Mn can co-precipitate with iron oxides and exhibit amphoteric behaviour.

Zinc: Zn is essential for living organisms, because it is present in the enzymes composition catalyzing important life processes. The primary source of zinc in soil is the weathering of rocks. Though Zn was once not considered to be highly toxic, phytotoxicity of zinc is usually reported in acid and heavily sludged soils⁵.

Table-1
Effect of different concentrations of heavy metals on seed germination, seedling growth, seedling indices and their phytotoxicity on Triticum aestivum L

Metal concentration	Germination (%)	Mean shoot length (cm)	Mean root	Mean seedling	Seedling Vigor	Phyto- toxicity	Phyto- toxicity	Tolerance index		
(mg/L)			length	length (cm)	index	of root	of shoot			
			(cm)			(%)	(%)			
Control	100	5.2	5.0	10.2	1092	00	00	00		
CHROMIUM METAL										
2	100	4.2	1.37	5.57	557	72.6	19.23	27.4		
4	100	3.62	1.0	4.62	462	80.0	30.38	20.00		
6	80	3.12	0.77	3.89	311.2	84.6	40.0	15.4		
8	80	3.1	0.6	3.7	296	88.0	40.38	12.0		
10	40	1.0	0.6	1.6	64	88.0	80.76	12.0		
CADMIUM METAL										
2	100	4.25	1.02	5.27	527	79.6	18.26	20.4		
4	80	3.25	1.05	4.30	344	79.0	37.5	21.0		
6	80	2.2	0.87	3.07	245.6	82.5	57.69	17.5		
8	40	2.0	0.87	2.87	114.8	82.5	61.53	17.5		
10	20	2.0	0.5	2.5	50	90.0	61.53	10.0		
			MANGANI	ESE METAL						
2	80	4.60	3.2	7.8	624	36.0	11.53	64.0		
4	80	4.55	1.65	6.2	496	67.0	12.5	33.0		
6	80	3.8	0.93	4.73	378.4	81.4	26.92	18.6		
8	60	3.37	0.60	3.97	238.2	88.0	35.19	12.0		
10	20	1.8	0.56	2.36	47.2	88.8	65.38	11.2		
ZINC METAL										
2	60	3.7	4.9	8.6	516	2.0	28.84	98.0		
4	40	2.3	3.1	5.4	216	38	55.76	62.0		
6	40	2.3	3.0	5.3	212	40	55.76	60.0		
8	20	1.5	0.7	2.2	44	86	71.15	14.0		
10	00	00	00	00	00	100	100	00		

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Table-2 Statistical average values of different studied parameters

Statistical average values of different studied parameters										
	Metal	Germination	Shoot length	Root length	Seedling length	Seedling Vigor index	Phyto- toxicity of root	Phyto- toxicity of shoot	Tolerance index	
Mean	Cr	80	3.008	0.868	3.876	338.04	82.64	42.15	17.36	
	Cd	64	2.74	0.862	3.602	256.28	82.72	47.302	17.28	
	Mn	64	3.624	1.388	5.012	356.76	72.24	30.304	27.76	
	Zn	32	1.96	2.34	4.3	197.6	53.2	62.30	46.8	
	Cr	24.49	1.209	0.325	1.4692	187.68	6.5013	23.25	6.5	
Standard	Cd	32.86	0.9908	0.2187	1.1513	189.51	4.378	19.054	4.37	
Deviation	Mn	26.07	1.1437	1.1032	2.0846	224.25	22.064	21.994	22.06	
	Zn	22.8	1.352	1.9831	3.3015	202.83	39.66	26.00	39.66	
Variance	Cr	600	1.4621	0.1056	2.1586	35223.8	42.268	540.58	42	
	Cd	1080	0.9817	0.0478	1.3256	35916.5	19.167	363.07	19	
	Mn	680	1.3081	1.217	4.3456	50289.2	486.82	483.77	486	
	Zn	520	1.828	3.933	10.9	41140.8	1573.2	676.18	1573	
Minimum	Cr	40	1	0.6	1.6	64	72.6	19.23	12	
	Cd	20	2	0.5	2.5	50	79	18.26	10	
	Mn	20	1.8	0.56	2.36	47.2	36	11.53	11.2	
	Zn	0	0	0	0	0	2	28.84	00	
Maximum	Cr	100	4.2	1.37	5.57	557	88	80.76	27.4	
	Cd	100	4.25	1.02	5.27	527	90	61.53	20.4	
	Mn	80	4.6	3.2	7.8	624	88.8	65.38	64	
	Zn	60	3.7	4.9	8.6	516	100	100	98	
Standard Error	Cr	10.98	0.53	0.14	0.65	84.16	2.91	10.42	2.91	
	Cd	14.73	0.44	0.09	0.51	84.93	1.95	8.54	1.95	
	Mn	11.69	0.51	0.49	0.93	100.5	9.89	9.86	9.89	
	Zn	10.22	0.6	0.88	1.47	90.95	17.78	11.65	17.78	

Table-3 Correlation Matrix for different variables

Variables	Mean shoot length (cm)	Mean root length (cm)	Mean seedling length (cm)	Seedling Vigor index	Phytotoxicit y of root (%)	Phytotoxicity of shoot (%)	Toleran ce index	Germin ation (%)
Mean shoot length (cm)	1	-	-	-	-	-	-	-
Mean root length (cm)	0.391138	1	-	-	-	-	-	-
Mean seedling length (cm)	0.837099	0.83089	1	-	-	-	-	-
Seedling Vigor index	0.933034	0.499856	0.861234	1	-	-	-	-
Phytotoxicity of root (%)	-0.39095	-1	-0.83078	-0.49971	1	-	-	-
Phytotoxicity of shoot (%)	-1	-0.3912	-0.93714	-0.93303	0.391014	1	-	-
Tolerance index	0.390952	0.999999	0.830778	0.499708	-1	-0.39101	1	-
Germination (%)	0.848876	0.134488	0.593174	0.862864	-0.13451	-0.84886	0.13451 2	1

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Phytotoxic effects of Cr. Cd. Mn and Zn on seed germination and seedling growth in wheat: Migration of heavy metals from polluted air and (surface) water to agricultural soil ecosystem is a ubiquitous problem. In this research paper, authors strived to understand the toxic effect of aqueous concentrations of heavy metal (chromium, cadmium, manganese and zinc) on the germination and seedling growth of Wheat in BC soil. The stock solutions of heavy metals at concentrations of 2, 4, 6, 8 and 10 mg/L were prepared using potassium dichromate (K₂Cr₂O₇), cadmium metal powder, potassium permanganate (KMnO₄), zinc metal powder as sources of Cr, Cd, Mn and Zn respectively. These affect the biogeochemistry of Cr, Cd, Mn and Zn in soil and subsequently the plausible reasons for phytotoxic effects of their treatments on seed.

Results showed that all the heavy metals individually affected root, shoot and seedling length of wheat as compared to control. Causes of growth inhibition could be many and varied. However, factors affecting cell division and cell expansion might have played a key role from the morphogenetic view point. We believe the heavy metal treatments decrease germination capability, seedling growth through inhibition of cell enlargement due to heavy metals.

Chromium affects seed germination. In the present research, the effect of aqueous chromium (Cr VI) solution on seed germination and seedling growth of wheat is studied and found that the Cr (VI) might have interfered with metabolic process and caused toxicity to the plant by reducing its root growth. As the chromium concentration increases from 2 mg/L to 10 mg/L, the seed germination decreases from 100% to 40% and seedling vigor indices decrease from 557 to 64. The increasing chromium concentrations increase the phytotoxicity of shoot and root and decrease the tolerance indices.

Cadmium treatment exerted more toxic effects on Triticum *aestivum* L. seedlings as compared to chromium. As the cadmium concentration increases from 2 mg/L to 10 mg/L, the seed germination decreases from 100% to 20% and seedling vigor indices decrease from 527 to 50. The increasing cadmium concentrations increase the phytotoxicity of shoot and root and decrease the tolerance indices.

Increasing manganese concentrations from 2 mg/L to 10 mg/L decreases the seed germination from 80% to 20% and seedling vigor indices decrease from 624 to 47.2. The increasing manganese concentrations increase the phytotoxicity of shoot and root and decrease the tolerance indices.

In some previous studies, zinc salts such as zinc chloride is proved to be less harmful to the germination of seeds. These results were confirmed by Somova and Pechurkin who showed a high tolerance of plants to zinc salts³⁰. In the present research work, Triticum *aestivum* L. showed decreasing percentage of germination from 60% to 00% over the increasing

concentrations made of zinc metal powder. The seedling vigor indices decrease from 516 to 00. The increasing manganese concentrations increase the phytotoxicity of shoot and root and decrease the tolerance indices.

Looking at the results obtained, the rank order of inhibition to wheat seed germination exerted by the toxicity of metals can be arranged as: Zn>Cd>Mn>Cr. The experimental results were interpreted using standard statistical methods. Correlation coefficient matrix among the parameters was calculated and correlations between various parameters were worked out. The significance of the observed correlation coefficients has been tested to determine significant positive and negative correlations among the parameters. The tolerance index and mean root length shows highest correlation coefficient 0.9999 among all other parameters. Also the mean shoot lengths and seedling vigor indices show good correlation i.e. 0.933034.

Conclusion

The results obtained from the present research work have shown that the treatments of heavy metals (such as Cr, Cd, Mn and Zn) to wheat (Triticum aestivum L.) seed induce dose-dependent inhibition of germination capability and seedling growth. In this case study, the germination and root length in emergence were adversely affected by aqueous concentrations of heavy metals fed to wheat seeds in Black Cotton soil of Nanded city (India). All results, when compared to control, show gradual reduction in seed germination and the decrease in growth of root and shoot with increasing concentrations of these heavy metals. The inhibition was stronger in root than in shoot. The toxic effects of selected metals to seed germination can be arranged in the rank order of inhibition as: Zn>Cd>Mn>Cr. The increasing heavy metal concentrations increase the phytotoxicity of shoot and root and decrease the tolerance indices and the seedling vigor indices. These methods and results thus provide a model system to screen for various concentrations of heavy metals for their phytotoxic effects and also screen for the seeds able to counteract the deleterious effects of such heavy metals in various types of irrigation waters and agricultural soils.

Acknowledgement

The author *Isak Rajjak Shaikh* is thankful to the National Chemical Laboratory (NCL), Pune, India for providing library facilities.

References

- 1. Sumner M.E. and Noble A.D., Soil acidification: the world story. In: Rengel Z, ed. *Handbook of soil acidity*, New York, USA: Marcel Dekker, 1–28 (2003)
- CEI: Soil remediation technologies: assessment, clean-up, decommissioning, rehabilitation. *Canadian Environmental Industries* (Energy and Environmental Industries Branch) (2005), available at: http://www.ic.gc.ca/eic/site/ea-ae.nsf/eng/ea02201.html

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- 3. Kabata-Pendias A. and Pendias H., Trace elements in soils and plants, CRC Press, London, UK (2001)
- Mertz W., Trace elements in human and animal nutrition. San Diego, California: Academic Press, fifth ed., Vol. 1-2 (1987)
- Alloway B.J., Heavy Metals in Soils, Blackie Academic and Professional, Glasgow, UK (1995)
- Lasat M.M., Phytoextraction of toxic metals. A review of biological mechanism, Journal of Environmental Quality, **31**, 109-120 (**2002**)
- Jarup L., Hazard of heavy metal contamination, Br. Med. Bull., 68, 167-182 (2003)
- Azevedo R.A. and Lea P.J., Toxic metals in plants, Braz. J. Plant Physiol., 17, 1 (2005)
- Clemens S., Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants, Biochimie **88,** 1707–1719 (**2006**)
- 10. Babula P., Adam V., Opatrilova R., Zehnalek J., Havel L. and Kizek R., Uncommon heavy metals, metalloids and their plant toxicity: a review, Environmental Chemistry Letters, **6**, 189–213 (**2008**)
- 11. Jensen G.H., Toxic limits and stimulation effects of some salts and poisons on wheat, Botanical Gazette, 43, 11–44 (1907)
- **12.** Taylor G.J. and Foy C.D., Differential uptake and toxicity of ionic and chelated copper in Triticum aestivum, Canadian Journal of Botany-Revue Canadienne De Botanique, **63**, 1271–1275 (**1985**)
- 13. Wheeler D.M., Power I.L. and Edmeades D.C., Effect of various metal ions on growth of two wheat lines known to differ in aluminium tolerance, Plant and Soil 155/156, 489-492 (1993)
- **14.** OECD Guideline for testing of chemicals, Terrestrial plant test: 208: Seedling emergence and seedling growth test. Paris: Organisation for Economic Co-operation and Development (2003)
- 15. APHA, Standard Methods for the Examination of Water and Wastewater. American Public Health Association, 20th edition, Washington, D.C. (1998)
- 16. Chou C.H. and Lin H.J., Autointoxication mechanism of Oriza sativa L Phytotoxic effects of decomposing rice residues in soil, *J. Chem. Ecol.*, **2**, 353-367 (**1976**)
- 17. Soltani A.M. and Gholipoor E., Zeinali, Seed reserve utilization and seedling growth of wheat as affected by drought and salinity, Environmental and Experimental Botany, 45, 195-200 (2006)
- 18. Iqbal M.Z. and Rahmati K., Tolerance of Albizia lebbeck to Cu and Fe application, Ekologia (CSFR), 11, 427-430 (1992)

- 19. Bewly J.D. and Black B.M., Germination of seeds. In: Physiology and biochemistry of seed germination, (Ed.): AA Khan, Springer Verlag, New York, pages 40-80 (1982)
- 20. Athar R., Ahmad M., Heavy Metal Toxicity: Effect on Plant Growth and Metal Uptake By Wheat, and on Free Living Azotobacter. Water, Air, & Soil Pollution, 138, 165-180 **(2002)**
- 21. Sharma D.C. and Mehrota S.C., Chromium toxicity effects on wheat (Triticum aestivum L. cv. HD 2204, Indian J. *Environ. Health*, **35**, 330-332 (**1993**)
- 22. Jamal S.N., Iqbal M.Z., Athar M., Phytotoxic effect of aluminum and chromium on the germination and early growth of wheat (Triticum aestivum) varieties Anmol and Kiran, Int. J. Environ. Sci. Tech., 3(3), 411-416 (2006)
- 23. Wang M. and Zhou Q., Single and joint toxicity of chlorimuron ethyl, cadmium and copper on wheat Triticum aestivum, Ecotoxicol. Environ. Saf., 60(2), 169-175 (2005)
- 24. Haiou Wang, Guangrong Zhong, Guoqing Shi, and Fangting Pan, Toxicity of Cu, Pb, and Zn on Seed Germination and Young Seedlings of Wheat (Triticum aestivum L.), Li D, Liu Y, and Chen Y (Eds.): CCTA (2010), Part III, IFIP AICT 346: pp. 231–240 © IFIP International Federation for Information Processing (2011)
- 25. Asgharipour M.R., Khatamipour M., and Razavi-Omrani M., Phytotoxicity of Cadmium on Seed Germination, Early Growth, Proline and Carbohydrate Content in Two Wheat Varieties, Advances in Environmental Biology, 5(4), 559-565 (2011)
- 26. Datta J.K., Bandhyopadhyay A., Banerjee A., and Mondal N.K., Phytotoxic effect of chromium on the germination, seedling growth of some wheat (Triticum aestivum L.) cultivars under laboratory condition, Journal Agricultural Technology, **7(2)**, 395-402 (**2011**)
- 27. Zayed A.M., Terry N., Chromium in the environment: factors affecting biological remediation, Plant soil, 249, 139-156 (2003)
- 28. Iqbal M.Z. and Mehmood T., Influence of cadmium toxicity on germination and growth of some common trees, Pak. J. Sci. Ind. Res., 34, 140-142 (1991)
- 29. Millaleo R., Reyes-Díaz M., Ivanov A.G., Mora M.L., and Alberdi M., Manganese as Essential and Toxic Element For Plants: Transport, Accumulation and Resistance Mechanisms, J. Soil Sci. Plant Nutr., 10(4), 476-494 (2010)
- 30. Somova L.A. and Pechurkin N.S., The influence of microbial associations on germination of wheat seeds and growth of seedlings under impact of zinc salts, Advances in *Space Research*, **43**, 1224 – 1228 (**2009**)