Pak. J. Bot., 39(2): 451-462, 2007.

TOXIC EFFECTS OF HEAVY METALS ON EARLY GROWTH AND TOLERANCE OF CEREAL CROPS

TARIQ MAHMOOD[1*], K.R. ISLAM[2] AND S. MUHAMMAD[3]

[1]Department of Agronomy, University of Arid Agriculture, Rawalpindi, Pakistan,
[2]Soil and Water Resources, The Ohio State University South Centers, Piketon, OH 45661, USA
[3]Department of Soil Science and Soil Water conservation, University of Arid Agriculture, Rawalpindi, Pakistan

Abstract

Metals have strong influence on development and growth of crops. To simulate how cereal crops are affected and/or tolerated from heavy metal contamination by disposal of unregulated wastes as soil amendments, the nutrient culture experiment was conducted with barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) at control (0), 1, 5, and 10 µM of copper (Cu), zinc (Zn), lead (Pb), magnesium (Mg) and sodium (Na). A 4 x 3 factorial experiment for each metal was set-up in a completely randomized design with four replications. Seed germination, total root numbers, root length, shoot height, and root: shoot ratio of seedlings were measured and integrated to calculate a metal tolerance index for each crop. Among the metals, Cu exerted the most adverse effects on seed germination, early growth and tolerance of crop seedlings followed by Zn and Pb. Wheat and rice seedlings were more susceptible to metal toxicity than barley. The effect of 10 µM Cu, Pb and Zn was more pronounced on crop seedlings especially on wheat and rice.

Introduction

Metals in terrestrial ecosystems are important for their influence on development and growth of plants (Lepp, 1981, Alloway, 1995, Hall & Williams, 2003). However, soil ecosystems are contaminated with heavy metals by human-induced activities (Naidu *et al*., 1996, Younas & Shahzad, 1998). A toxic concentration of heavy metals is not known in agricultural soils; however, land disposal of wastes as soil amendments for crop production is responsible for temporal accumulation of heavy metals in soil (Nriagu & Pacyna, 1988, Younas and Shahzad, 1998). Once present in the soil, the heavy metals are persistent (Alloway, 1995).

Pakistan is an agrarian county with high population growth. Soils are intensively cropped to meet the increasing demand for food production. However, soils of Pakistan are inherently low in fertility to support economic crop production (Rashid, 1993, Jamal *et al*., 2002). Due to high cost and scarcity of chemical fertilizers, the land disposal of agricultural, municipal and industrial wastes is widely practiced as a major and economic source of nutrients and organic matter for growing cereal crops by poor farmers in Pakistan (Rashid, 1993, Younas & Shahzad, 1998, Jamal *et al*., 2002). The waste generation in Pakistan has increased by 120% between 1980 and 1996 with a total of 16.2 TG generated in 1995 (Younas & Shahzad, 1998). The most reported heavy metals in waste amended agricultural soils are Cu, Pb and Zn (Nriagu & Pacyna, 1988, Younas & Shahzad, 1998, Jamal *et al*., 2002).

Copper is one of the most important micronutrient, essential for plant growth (Alloway, 1995, Hall & Williams, 2003). It is an integral component of numerous enzymes, and is actively involved in lignification (Hall & Williams, 2003). Zinc, on the other hand, is a non-redox micronutrient element, which has key structural and catalytic

---

[*]Corresponding address:E-mail:qiratm@yahoo.com

roles in many proteins and enzymes involved in energy metabolism (Sresty & Madhava Rao, 1999; Hall & Williams, 2003). Lead is neither an essential nor a beneficial element for plant growth (Alloway, 1995). However, temporal accumulation of the heavy metals in waste amended agricultural soils at higher concentration can be toxic for plant growth due to their adverse effects on plant development and growth (Pahlsson, 1989, Ayaz & Kadioglu 1997).

Growing cereal crops on widespread unregulated waste amended agricultural soils may become a food security problem because toxic concentration of heavy metals may accumulate in the food chain (Mitchell *et al*., 1978, Algeria *et al*., 1991, Younas & Shahzad 1998, Munzuroglu & Geckil, 2002) or cause failure of crops. In addition, crops which have the ability to tolerate may accumulate greater concentration of heavy metals and become environmental and public health issues (Stefanov *et al*., 1995, Munzuroglu & Geckil, 2002). However, the adverse effects of heavy metals on cereal crops in response to widespread land disposal of unregulated wastes as agricultural soil amendments have received a very limited attention in Pakistan (Mahmood, 1995, Mahmood & Islam, 2005). The objective of the nutrient culture study was to evaluate how graded concentration of Cu, Pb, Zn, Na and Mg affect seed germination, growth and tolerance of barley, rice and wheat when grown in waste amended soils.

Materials and Methods

Reference metals: Analar grade sulfate and chloride formulations of metals viz., $CuSO_4.5H_2O$, $PbCl_2$, and $ZnSO_4.7H_2O$ were used in solution concentrations of control (0), 1, 5, and 10 µM for Cu, Pb and Zn, respectively. In order to identify whether or not the possible adverse effects of individual metal on seed germination, growth characteristics and tolerance of crop seedlings were due to selected metals or associated anions (SO_4^{-2} or Cl^-), analar grade $MgSO_{4.}7H_2O$ and NaCl were used under similar concentration. The pH of all the individual metal treatment solutions was maintained at 7.0 ± 0.1.

Seed germination: Twenty-five seeds of barley, rice and wheat were randomly selected to use for germination test in sterilized disposable polystyrene Petri dishes. The seeds placed in each dish were equally spaced from each other among three successive layers of filter paper. The crease side of the seed was kept next to the paper and the seed was placed with embryo end towards the bottom of the paper, and coleoptile's pointing upwards. The filter paper method was used to reduce the masking effects of counter anions associated with the metals (Mahmood & Islam, 2005). A 10 ml sample of the individual metal treatment solution was added to the respective Petri dishes as per experimental treatments and the Petri dishes were covered with parafilm followed by 4 d incubation at 20 ± 2°C. After incubation, germinated seeds with 5 mm radical length were separated and counted to calculate percent germination.

Seedlings growth: The germinated seeds were loosely rolled in filter paper to form tube, and placed upright in 250 ml sterilized polystyrene beaker. To each beaker, a 100 ml sample of metal solution was added as per experimental treatments. The beakers containing germinated seeds were then incubated at 25 ± 2°C with 90 ± 2% relative humidity for a period of 3 d. The solution level in the beaker was maintained by adding freshly prepared solution. After incubation, the plumule (shoot height) and radical length (root length) and the number of roots were measured for all the seedlings.

Metal tolerance index: The data on seed germination, total number of roots, root length, shoot height and root: shoot ratio of barley, rice and wheat seedlings were integrated into a simple index (T_{index}) to evaluate their tolerance against or susceptibility to the composite effects of metals. Data of individual growth parameter (G_x) was normalized (G_i) relative to the maximum value (G_{max}) of that parameter in the data set, $G_i = (G_x\, G_{max}^{-1})$. Suing all the Gi's and then divided by the total number of Gi's calculated the $T_{index} = \Sigma(G_x\, G_{max}^{-1})\, n^{-1}$. The T_{index} of crop seedlings ranged from ≥ 0 to ≤ 1, with ≤ 1 being highly tolerant and ≥ 0 having extremely susceptible to the composite effects of heavy metal.

Statistical analysis: Data were organized in a 4 x 3 factorial combination and analyzed by using ANOVA procedure of the SAS (Anon., 2001). Simple and interactive effects in response to type and concentration of heavy metals on seed germination, growth and tolerance characteristics of barley, rice and wheat seedlings were separated by an F protected LSD test at $p \leq 0.05$.

Results and Discussion

Seed germination and seedlings growth: Among the metals used, the Mg and Na did not affect seed germination or growth of barley, rice and wheat seedlings (Table 1-5). On the other hand, [Cu] had the consistent adverse effects followed by Zn and Pb on seed germination and growth of crop seedlings (Table 1-3). Increasing [Cu] significantly inhibited the germination of seeds (Table 1). The inhibitory effect of [Cu] on seed germination was more pronounced on rice than on wheat and barley, respectively. In the 10 µM Cu treatment, the wheat and rice seed germination was reduced more than 35 and 60%, respectively over the control treatment. Seed germination did not affect significantly in response to Pb and Zn concentration (Table 2-3).

The number of roots of all crop seedlings did not increase consistently in response to metal concentration (Table 1-5). In wheat, the number of roots per seedlings increased significantly by 50% at 10 µM Cu over the control seedlings but root numbers of barley and rice seedlings did not increase significantly in response to Cu. The Pb, on the other hand, increased the root number (100%) in rice seedlings. Progressive increases in Zn significantly increased (50%) the root number of wheat seedlings as compared to seedlings grown in control treatment. Root length of all crop seedlings was significantly affected by increasing Cu, Pb and Zn. On average, the effect of Cu on seedlings root length was more pronounced than Pb and Zn, respectively. The 10 µM Cu significantly reduced the root length of both barley and wheat by 50% and rice by 40% than the control seedlings. The root length of all crop seedlings at both 5 and 10 µM Pb was significantly less (30-50%) than that of the control seedlings. At 10 µM Zn, the root length of barely, rice and wheat seedlings reduced more than 50, 40 and 60%, respectively than the root length of the control seedlings. At higher Cu, Pb and Zn, the root tips of all the crop seedlings had changed their color from brown to dark brown, and the roots were found hairless, stunted, thick, curled and brittle with numerous small lateral branches (data not shown). On the other hand, the roots of the seedlings growing in control or lower concentration of metal treatments were white with profuse root hairs and longer lateral branches.

Table 1. Seed germination, root growth, shoot height and root: shoot ratio of barley, rice and wheat in response to copper concentration.

Cu conc. (µM)	Germination of seeds (%)	Number of roots/plant	Root length (mm)	Shoot height (mm)	Root/shoot ratio
Barley					
Control	73a	6a	135.5a	87.1b	1.55a
1	70a	6a	110.1b	93.3ab	1.18b
5	70a	7a	85.6c	98.7a	0.87c
10	68b	7a	60.6d	100.0a	0.60d
Mean	71A	6A	104.3A	92.9A	1.15B
Rice					
Control	93a	2a	80.3ab	34.2ab	2.35a
1	78b	2a	76.0b	33.1ab	2.30a
5	55c	2a	60.7c	32.1b	1.89b
10	34d	3a	50.1d	32.3b	1.56c
Mean	71A	2B	70.5B	33.8B	2.08A
Wheat					
Control	82a	4b	102.1a	87.1a	1.17a
1	86a	5ab	70.5b	87.0a	0.80b
5	60b	5ab	45.9c	60.8b	0.75bc
10	50c	6b	30.3d	50.2b	0.60c
Mean	72A	5A	69.9B	74.1A	0.90C

Means followed by the same upper case letter in the column were not significantly different at the $p<0.05$. Means separated by same lower case letter within metal concentration treatments in the column for each crop were not significantly different at the $p<0.05$.

Table 2. Seed germination, root growth, shoot height and root: shoot ratio of barley, rice and wheat in response to copper concentration.

Pb conc. (µM)	Germination of seeds (%)	Number of roots/plant	Root length (mm)	Shoot height (mm)	Root/shoot ratio
Barley					
Control	82a	6a	125.1a	85.3b	1.47a
1	76a	6a	115.4a	86.7b	1.34a
5	76a	7a	80.1b	100.2a	0.80b
10	76a	7a	80.1b	95.2ab	0.84b
Mean	77B	6A	105.6B	91.2A	1.17B
Rice					
Control	95a	2b	75.1ab	50.2a	1.50a
1	93a	2b	82.2ab	52.1a	1.58a
5	95a	3ab	70.1b	52.0a	1.35a
10	95a	4a	45.4c	48.5a	0.94b
Mean	94A	3B	71.4C	50.4B	1.41A
Wheat					
Control	94b	4a	135.0a	110.0a	1.23a
1	92b	4a	130.0a	100.1ab	1.30a
5	95b	5a	115.1b	97.2bc	1.19a
10	95b	5a	90.2c	90.1c	1.00b
Mean	92A	5A	121.0A	100.6A	1.20B

Means followed by the same upper case letter in the column were not significantly different at the $p<0.05$. Means separated by same lower case letter within metal concentration treatments in the column for each crop were not significantly different at the $p<0.05$.

Table 3. Seed germination, root growth, shoot height and root: shoot ratio of barley, rice and wheat in response to copper concentration.

Zn conc. (µM)	Germination of seeds (%)	Number of roots/plant	Root length (mm)	Shoot height (mm)	Root/shoot ratio
			Barley		
Control	85a	6a	120.0a	85.1b	1.41a
1	89a	6a	115.1a	83.0b	1.39a
5	80a	6a	70.1b	108.2a	0.65b
10	85a	7a	60.3c	100.2a	0.60b
Mean	83A	6A	98.6A	93.2A	1.09B
			Rice		
Control	93a	2a	75.1ab	47.3a	1.60a
1	94a	2a	78.2ab	46.2a	1.70a
5	92a	3a	68.1bc	37.1b	1.84a
10	93a	3a	52.2c	36.1b	1.44a
Mean	93A	2B	71.6B	43.2B	1.65A
			Wheat		
Control	96a	4b	132.4a	100.0ab	1.32a
1	90a	4b	131.1a	95.2b	1.38a
5	95a	5ab	80.2b	92.1b	0.87b
10	95a	6a	55.2c	90.1b	0.61c
Mean	92A	5A	106.6A	96.6A	1.09B

Means followed by the same upper case letter in the column were not significantly different at the $p<0.05$. Means separated by same lower case letter within metal concentration treatments in the column for each crop were not significantly different at the $p<0.05$.

Table 4. Seed germination, root growth, shoot height and root: shoot ratio of barley, rice and wheat in response to copper concentration.

Mg conc. (µM)	Germination of seeds (%)	Number of roots/plant	Root length (mm)	Shoot height (mm)	Root/shoot ratio
			Barley		
Control	69a	6a	132.6a	90.1a	1.47a
1	69a	6a	137.2a	91.0a	1.51a
5	70a	6a	136.1a	91.2a	1.49a
10	70a	6a	137.0a	92.2a	1.49a
Mean	71B	6A	134.8A	90.0A	1.50B
			Rice		
Control	93a	2a	78.9a	34.0a	2.29a
1	90a	2a	82.5a	37.0a	2.22a
5	91a	2a	84.9a	36.2a	2.34a
10	90a	2a	85.1a	36.0a	2.36a
Mean	91A	2C	83.3C	36.0B	2.30A
			Wheat		
Control	93a	4a	105.0a	90.2a	1.17a
1	94a	4a	108.4a	90.0a	1.20a
5	93a	5a	110.2a	94.2a	1.17a
10	92a	5a	110.1a	97.3a	1.13a
Mean	91A	4B	106.8B	91.4A	1.17B

Means followed by the same upper case letter in the column were not significantly different at the $p<0.05$. Means separated by same lower case letter within metal concentration treatments in the column for each crop were not significantly different at the $p<0.05$.

Table 5. Seed germination, root growth, shoot height and root: shoot ratio of barley, rice and wheat in response to copper concentration.

Na conc. (µM)	Germination of seeds (%)	Number of roots/plant	Root length (mm)	Shoot height (mm)	Root/shoot ratio
			Barley		
Control	82a	6a	126.8a	85.7	1.48a
1	79a	6a	130.2a	83.0a	1.57a
5	78a	7a	134.0a	97.0a	1.38a
10	79a	7a	140.0a	99.0a	1.41a
Mean	78B	6A	131.8A	90.8A	1.45A
			Rice		
Control	90a	2a	80.9a	54.0a	1.48a
1	93a	2a	81.0a	53.2	1.53a
5	96a	3a	87.2a	56.0a	1.55a
10	94a	3a	92.0a	45.7	2.04a
Mean	93A	2C	85.0B	51.6B	1.66A
			Wheat		
Control	90a	4a	135.0a	106.0a	1.27a
1	94a	4a	134.0a	95.6	1.41a
5	93a	5a	126.6	95.8	1.33a
10	95a	5a	135.0a	92.7	1.47a
Mean	91A	4B	133.0A	98.8A	1.35A

Means followed by the same upper case letter in the column were not significantly different at the $p<0.05$. Means separated by same lower case letter within metal concentration treatments in the column for each crop were not significantly different at the $p<0.05$.

Shoot height of wheat seedlings decreased significantly at 10 µM Cu, however, barley shoot height increased by 15% than that of the control seedlings (Table 1). The quadratic shoot height of barley was observed with increasing Pb, while the wheat shoot height decreased by 15% with increasing Pb (Table 2). The effect of [Zn] on shoot height of barley seedlings followed a pattern similar to that observed for [Pb]. In contrast, the shoot height of rice seedlings at both 5 and 10 µM Zn was about 18% less than those of the control seedlings (Table 3). A significant inhibition of shoot height of wheat was also observed at 10 µM Zn.

The root: shoot ratio of barley, wheat and rice seedlings at 10 µM Cu were 60, 50, and 30% less, respectively than that of the control seedlings. In barley, the root: shoot ratio at 5 µM Pb was 30% less, while in rice and wheat seedlings there were non-significant differences in root: shoot ratio than that of the control seedlings (Table 2). A significant decrease (20 - 45%) in root: shoot ratio of seedlings at 10 µM Pb compared to control seedlings was observed for all three-crops (Table 2). Significant inhibition in root: shoot ratio of barley (55 - 65%) and wheat (28 - 50%) was found at both 5 and 10 µM Zn concentration than control seedlings (Table 3).

Since SO_4 and Cl ions from Mg and Na salts had no significant effect on seed germination, a consistent inhibition of seed germination especially rice and wheat under increasing $CuSO_4$ is possibly due to consequences of greater absorption of Cu by the seeds during imbibitions and subsequent toxicity (Fernandez & Henriques, 1991; McBride, 2001). The Cu-induced oxidative stress has been identified in severe reduction of enzyme activities involved in the seed metabolic processes related to germination (Mahmood, 1995, Ayaz & Kadioglu, 1997). A lack of consistent adverse effects exerted by Pb and Zn on seed germination is most probably related to interspecies differences in seed coat structures for regulating metal absorption. It is reported that plant seeds have inherent capability for selective absorption of metals in nature (Stefanov *et al*., 1995). This suggests that seed coat structures of barley, rice and wheat may have selectively reduced Pb and Zn absorption from the solution to minimize their adverse effects on germination. Various degrees of permeability of seed coats to metals lead to a range of seed germination inhibitions (Wierzbicka & Obidziniska, 1988).

The adverse effects of heavy metals especially Cu have reportedly caused structural and morphological changes of roots as well as inhibition of root hair growth of seedlings (Pahlsson, 1989; Fernandez & Henriques, 1991). Adverse effects of Cu on roots are related to severe reduction in the elongation growth of the longest root as well as root plasma membrane permeability of the seedlings was reported (Wainwright & Woolhouse, 1977, Nriagu & Pacyna, 1988; McBride, 2001). Significant inhibition of root length of all crop seedlings at higher Pb is also reported (Hasnian *et al*., 1993). Although Pb cannot penetrate the seed testa but Pb may have affected the root growth after radical emergence (Obroucheva *et al*., 1998). The root growth inhibition by Pb toxicity is most probably resulted from non-selective suppressive of both cell division and cell elongation of the seedlings (Ivanov *et al*., 1988).

The stunted and poorly developed root system (fibrous) of all the crop seedlings at higher Cu, Pb and Zn are likely related to partial disorder of metabolic processes of the seedlings (Pahlsson, 1989; Obroucheva *et al*., 1998; Dinev, 1988; Breckle, 1991). The toxic effects of Cu often affected mitotic activity and cell division of roots with a subsequent increase in the root number of seedlings (Hall & Williams, 2003). Increasing Pb induced a more compact distribution of lateral roots along a shorter branching zone due to a reduced length of mature cells in the primary root (Obroucheva *et al*., 1998). As a result of the inhibition of primary root growth by Pb, a shorter branching zone with more compact location of lateral roots have occupied the space much closer to the root tips (Obroucheva, 1998).

A number of studies have reported that plant seedlings responded quickly to a higher concentration of metals in terrestrial ecosystems by changing in their growth rates and root branching patterns compared to shoot growth (Stiborava *et al*., 1986; Dinev, 1988; Breckle, 1991; Hasnian *et al*., 1993). The change in root growth characteristics is probably due to the consequences of the direct exposure of the radical to metal toxicity and preferential accumulation of metals in the emerging roots followed by slow mobility to the plant shoots (Godzik, 1993; Fargasova, 1994). Such an effect can be explained as the affected roots may cause a slower movement of metals to the shoots (Fargasova, 1994). A consistent change in the barley and wheat root: shoot ratio in response to metals especially Cu and Zn is most probably related to greater inhibition of roots by metal toxicity than shoots. Metal induced changes in the structure and morphology of the roots such as absence of root hairs, stunted and fibrous root growth, and thickening or browning of roots, may be responsible to cause a decreased root: shoot ratio of the seedlings.

Metal tolerance of barley, rice and wheat seedlings: Increasing Cu, Pb and Zn had significant adverse effects on metal toxicity tolerance all crop seedlings (Figs. 1-3). The tolerance index of barley, rice and wheat seedlings was inversely related to Cu, Pb and Zn. The effect of Cu was more adverse on all crop seedlings than Pb and Zn, respectively. Wheat seedlings tolerance significantly decreased by both Cu and Pb than rice and barley, respectively. On the other hand, Mg improved the tolerance of barley. A significant decrease in tolerance ability of the crop seedlings is possibly due to greater inhibition of seed germination followed by root length and shoot height in response to the composite adverse effects of metal toxicity especially Cu by oxidative stress.

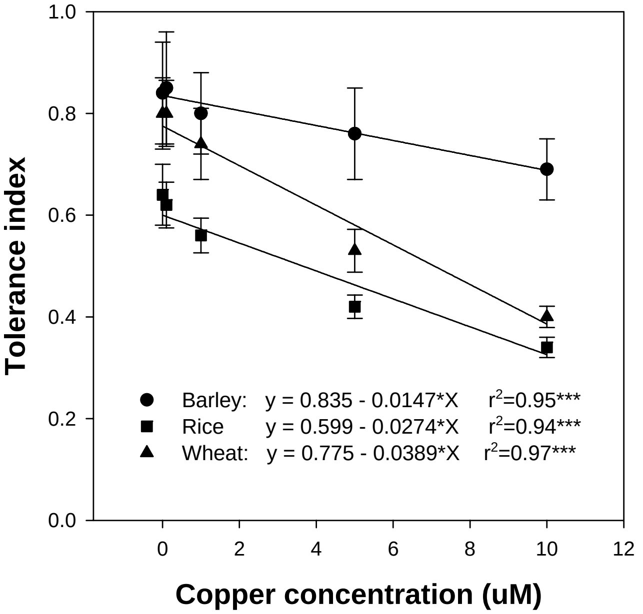

Fig. 1. Tolerance index of barley, rice and wheat seedlings in response to Cu concentration.

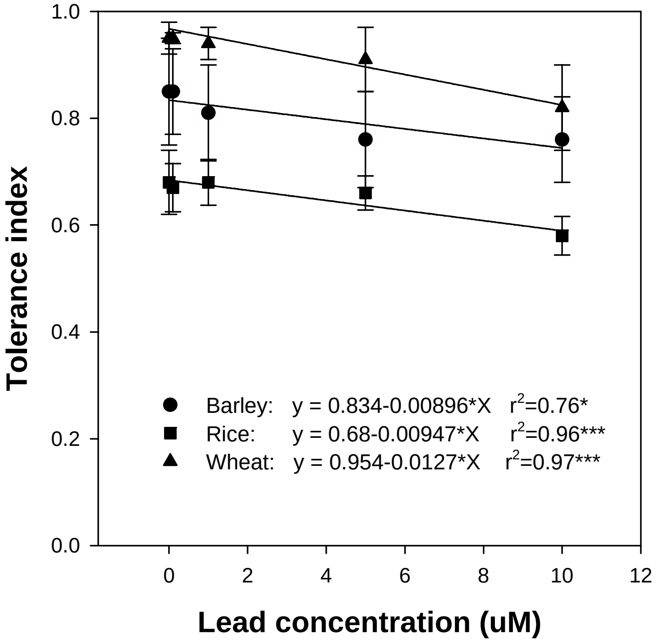

Fig. 2. Tolerance index of barley, rice and wheat seedlings in response to Pb concentration

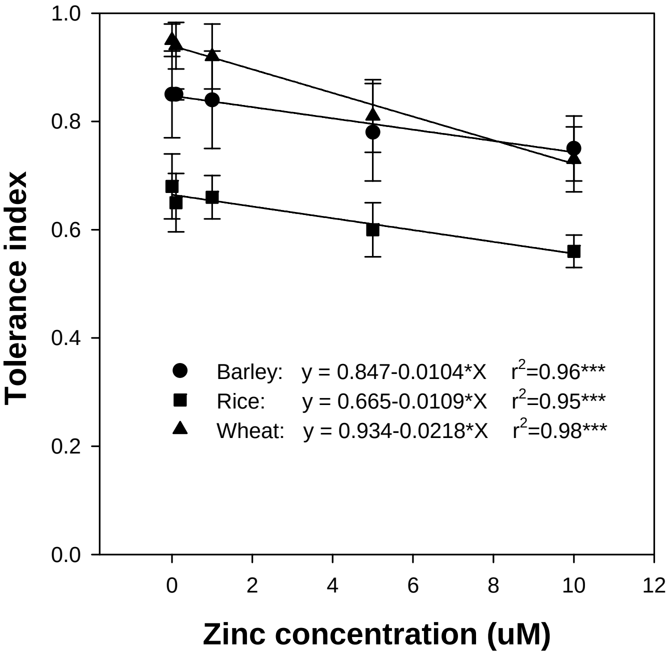

Fig. 3. Tolerance index of barley, rice and wheat seedlings in response to Zn concentration.

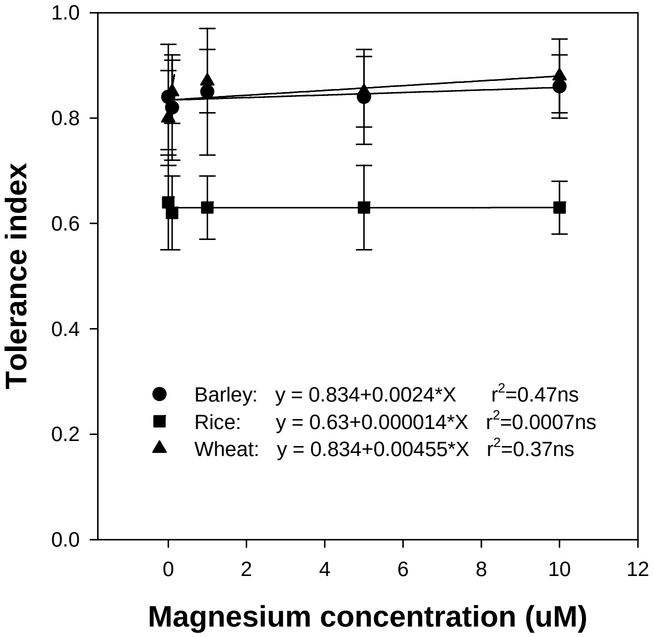

Fig. 4. Tolerance index of barley, rice and wheat seedlings in response to Mg concentration.

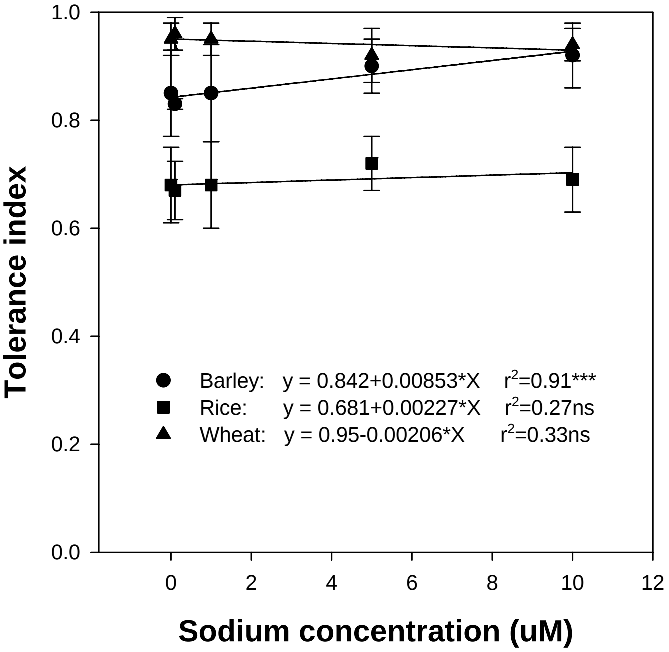

Fig. 5. Tolerance index of barley, rice and wheat seedlings in response to Na concentration.

Metal induced changes in the seed metabolic processes followed by structural and morphological properties of the roots are possibly related to a substantial decrease in metal tolerance ability of the crop seedlings.

Conclusions

Increasing concentration of heavy metals significantly inhibited seed germination and early growth of barley, rice and wheat seedlings. Seed germination was affected more followed by root length, root: shoot ratio, and shoot height of the seedlings. The overall inhibitory effects of metals calculated as tolerance index were more pronounced on wheat and rice than on barley seedlings. The inhibitory effect on cereal crop seedlings was more pronounced by Cu than Pb and Zn and was not due to SO_4 or Cl. The metals can be ranked in the order of greatest inhibitory effects on cereal crops as follows: Cu>Zn>Pb.

References

Algeria, A., R.F. Barbara, R. Errecalde, Farre and M. Lagarda. 1991. Environmental Cd, Pb and Ni Contamination: Possible Relationship between Soil and Vegetable Content. *Fresenius J. Anal. Chem*., 399, 654.

Alloway, B.J. 1995. *Heavy Metals in Soils*. Blackie Academic and Professional, Glasgow.

Anonymous. 2001. *SAS Users' Guide*, R 8.2, SAS Institute, Inc., Cary, NC.

Ayaz, F.A. and A. Kadioglu.1997. Effects of Metals Zn, Cd, Cu and Hg on the soluble protein bands of germinating *Lens esculenta* L. seeds, *Tr. J. Bot*., 21: 85-88.

Breckle, S.W. 1991. *Growth under Stress*: Heavy Metal. In: *Plant Roots: The Hidden Half.* (Eds.): Y. Waisel, A. Eshel and U. Kafkafi. Marcel Dekker, New York, pp. 351-373.

Dinev, N. 1988. Effects of heavy metals (Cu, Zn, Cd) on the growth of oat plants. *Soil Sci. Agrochem. Ecol*., 33: 5-9.

Fargasova, A. 1994. Effect of Pb, Cd, Hg, As and Cr on germination and root growth of *Sinapsis alba* seeds. *Bull. Environ. Contam. Toxicol.,* 52: 452-456.

Fernandez, J.C. and F.S. Henriques. 1991. Biochemical, physiological, and structural effects of excess copper in plants. *Botan. Rev.,* 57: 246-273.

Godzik, B. 1993. Heavy metals content in plants from zinc dumps and reference area. *Pol. Bot. Stud*., 5: 113-132.

Hall, J.L. and L.E. Williams. 2003. Transition metal transporters in plants. *J. Expt. Bot.,* 54: 2601-2613.

Hasnian, S., S. Yasmin and A. Yasmin. 1993. The effect of lead resistant *Pseudomonads* on the growth of *Triticum aestivum* seedlings under lead stress. *Environ. Pollut.,* 81: 179-184.

Ivanov, V.B., E.I. Bystrova, N.V. Obroucheva, O.V. Antipova, M. Sobotik and H. Bergmann. 1988. Growth response of barley roots as an indicator of Pb toxic effects. *J. Appl. Bot*., 72: 140-143.

Jamal, A., N. Ayub, M. Usman and A.G. Khan. 2002. Arbuscular mycorrhizal fungi enhance Zn and Ni uptake from contaminated soil by soybean and lentil. *Int. J. Phytorem*., 4: 205-221.

Lepp, N.W. 1981. *Effects of Heavy Metals on Plants.* Vol. I and II, Applied Science, London.

Mahmood, T. 1995. *Physiology of Metal Ion-induced Effects on Germination and Seedling Growth in Cereals.* Ph.D. Thesis, University of Edinburgh, UK.

Mahmood, T. and K.R. Islam. 2005. Rice seedlings response to copper toxicity and acidity. *J. Plant Nutr*. (In press).

McBride, M.B. 2001. Cupric ion activity in peat soil as a toxicity indicator for maize. *J. Environ. Qual*., 30:78-84.

Mitchell, G.A., F.T. Bingham and A.L. Page. 1978. Yield and metal composition of lettuce and wheat grown on soil amended with sewage sludge enriched with Cd, Cu, Ni, and Zn. *J. Environ. Qual*., 165-171.

Munzuroglu, O. and H. Geckil. 2002. Effects of metals on seed germination, root elongation, and coleoptiles and hypocotyls growth in *Triticum aestivum* and *Cucumis sativus*. *Arch. Environ. Contam. Toxicol.,* 43: 203-213.

Naidu, R., R.S. Kookuna, D.P. Oliver, S. Rogers and M.J. McLaughlin. 1996. Contaminants and the soil environment in the Australasia-Pacific Region, Dordrecht *Kluwer Acad. Publ*.

Nriagu, J.O. and J.M. Pacyna. 1988. Quantitative assessment of worldwide contamination of air, water and soil by trace-metals. *Nature,* 333: 134-139.

Obroucheva, N.V., V.B. Bystrova, O.V. Ivanov, M.S. Antipova and I.V. Seregin. 1998. Root growth responses to lead in young maize seedlings. *Plant Soil*, 200: 55-61.

Pahlsson, A.B. 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to Vascular Plants. *Water Air Soil Pollut*., 47: 287-319.

Rashid, A.1993. Nutritional disorder of rapeseed mustard and wheat grown in pothowar Area. Land Resources Institute, NARC, Islamabad, Pakistan. 105 p.

Sresty, T.V.S. and R.K.V. Madhava 1999. Ultrastructural alterations in response to zinc and nickel stress in the root cells of pigeon pea. *Environ. Exp. Bot.,* 41: 3-13.

Stefanov, K., K. Seizova, N. Yanishlieva, E. Marinova and S. Popov. 1995. Accumulation of Pb, Zn and Cd in plant seeds growing in metalliferous habitats in Bulgaria. *Food Chem*., 54: 311-313.

Stiborava, M., M. Doubravova, A. Brezinova and A. Friedrich. 1986. Effect of heavy metal ions on growth and biochemical characteristics of photosynthesis of barley (*Hordeum vulgare* L.) *Photosynthetica*, 20: 418-425.

Wainwright, S.J. and H.W. Woolhouse. 1977. Some physiological aspects of copper and zinc tolerance in *Agrostis tenuis* Sibth: Cell elongation and membrane damage. *J. Expt. Bot*., 28: 1029-1036.

Wierzbicka, M. and J. Obidziniska. 1988. The effect of lead on seed imbibitions and germination in different plant species. *Plant Sci*., 137: 155-171.

Younas, M. and F. Shahzad. 1998. Assessment of Cd, Ni, Cu, and Pb pollution in Lahore, Pakistan. *Environ. Intern*., 24: 761-766.

(Received for publication 20 November 2006)