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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (1): 83-87, 1999 *Research Article*

Influence of Cadmium, Lead and Zinc on the Growth and Metal Content in Ryegrass

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Abstract
A greenhouse experiment was conducted to evaluate the effects of cadmium (Cd), lead (Pb) and zinc (Zn) on the growth and heavy metal content in ryegrass plants. The metals were applied separately at three different levels i.e. Cd at 10, 30 and 60 µg g^{-1}; Pb at 50, 100 and 150 µg g^{-1} and Zn at 75, 150 and 225 µg g^{-1} soil. Results demonstrated a significant inhibitory effect of metals on the growth and dry matter yields of ryegrass plants. The concentrations of metals both in roots and shoots increased significantly with Cd, Pb and Zn addition to the soil. The adverse effects of metals were more pronounced on roots than on the above ground plant parts. Among the tested metals, Pb displayed the greatest suppressing effect followed by Cd and Zn, indicating their relative toxicity in the order: Pb>Cd>Zn. It was suggested that the solubility of lead compound, presence of acetate in the soil medium, low pH of the soil and the relative tolerance of ryegrass to Cd were the factors contributing to the toxicity trend observed in the study.

Introduction
Heavy metal contamination of soils is a world-wide problem of increasing importance and great environmental concern (Alloway, 1990; Kabata-Pendias and Pendias, 1992). Three metals often cited for their deleterious effects on both human and plant metabolism are cadmium (Cd), lead (Pb) and zinc (Zn). The excessive uptake of these elements by crop plants from the soil creates dual problem. Crop yields are reduced due to the inhibition of metabolic processes in plants (Foy *et al*. 1978; Costa and Morel, 1993; Aery and Jagetiya, 1997). In addition, the crops so contaminated serve as a source of heavy metals in our food supply (Somers, 1974; Singh *et al*. 1995; Sajwani *et al*. 1996). Therefore, the proplems posed by increased amounts of these metals in the environment demand a thorough understanding of their phytotoxic effects in a soil-plant system.

Most of the studies conducted so far to investigate the effects of Cd, Pb and Zn on plant growth and their possible entry into the food chain have been confined to application of these metals via fly ash or sewage sludge (Adriano, 1986; Alloway, 1990). Thus, information regarding the direct application of these metals to soil in various forms and their uptake by plants is clearly lacking. Moreover, although differences in uptake of trace elements among plant species and their cultivars have long been established (Adriano, 1986), there is not enough data on Cd, Pb and Zn toxicity in ryegrass, a forage crop of considerable importance. In view of the above, the main objectives of this study were; to evaluate the effects of Cd, Pb and Zn on the growth of ryegrass plants and to determine the accumulation and distribution of these metals in the ryegrass tissues.

Materials and Methods
A greenhouse experiment was conducted in the department of Soil Science, Zhejiang Agricultural University, Hangzhou, P. R. China. A red soil (Ultisol) collected at 0-15 cm of the fields surface from the Long you County, Zhejiang Province of P. R. China was brought to the laboratory, hand-picked to remove discrete plant residues and large soil animals (earthworms etc.), passed through 2 mm screen and homogenized. A sub-sample of the soil was taken, air-dried, ground and analyzed for various physic-chemical properties listed in Table 1. The total metals (Cd, Pb and Zn) in the soil were measured by an atomic absorption spectrophotometer (AAS) after aqua regia digestion (Soon and Abboud, 1993). The available metals were extracted with DTPA solution adjusted to pH 7.3 and analyzed by ASS.

Table 1: Some properties of the red soil used in the experiment

Water holding capacity (g kg^{-1})	421.80
Total Pb (mg kg^{-1})	52.25
Total organic carbon (g kg^{-1})	10.08
Total Zn (mg kg^{-1})	60.00
Organic matter (g kg^{-1})	17.40
Available Cd (mg kg^{-1})	B.D.L.*
pH	4.51
Available Pb (mg kg^{-1})	5.80
Total Cd (mg kg^{-1})	B.D.L.*
Available Zn (mg kg^{-1})	5.25

*Below Detection Limit

The plastic pots used in the experiment were washed thoroughly with distilled water prior to soil addition. 0.5 kg of soil was added to each plastic pot. The aqueous stock solutions of cadmium chloride, lead acetate and zinc acetate were applied to soil in appropriate volumes to achieve the desired cadmium (10, 30 and 60 µg g^{-1} soil), lead (50, 100 and 150 µg g^{-1} soil) and zinc (75, 150 and 225 µg g^{-1} soil) concentrations, respectively. The soil in each pot was mixed thoroughly and incubated for two weeks under natural conditions to maintain the uniform metal concentration throughout the soil sample. A set of three pots without metal application but incubated for the same period of time served as the control. The basal doze of nitrogen, phophorus and potassium (NPK) at the rate of 200-100-100 mg kg^{-1} was applied to each pot including the control. Liquir ammonia and potassium dihydrogen phosphate were used as sources of N, P and K.

Seeds of the ryegrass were sown and thinned three days after the germination to maintain ten seedlings pot^{-1}. The pots were watered at regular intervals to maintain the soil moisture around 50 percent of the soil water-holding capacity (WHC). Distilled water was used for irrigation throughout the crop growth period. Fifty days after the germination of seeds, data on plant height were recorded and the plants were taken out carefully from each pot along their roots. After washing carefully to remove the adhering soil particles, plants were cut off to separate into roots and shoots. The plant samples were then oven-dried at 60°C until a constant weight and the data on dry root and shoot yields were recorded. The dried plant material was digested in nitric-perchloric acid mixture and the Cd, Pb and Zn content of the digest were determined by atomic absorption spectroscopy. The statistical analysis of the data regarding the analysis of variance (ANOVA) and linear regression were performed by Statistix 4.1 software.

Results

Effects on Growth and Dry matter Yield: The data pertaining to the effect of cadmium, lead and zinc on the growth and dry matter yield of ryegrass plants is presented in Table 2. The addition of cadmium at the tested levels did not cause any significant decline in plant height compared with the control. The addition of lead at 100 and 150 µg g^{-1} soil resulted in a significant ($p<0.05$) reduction in plant height by 41.4 and 67.3 percent, respectively, relative to the control. The zinc application at 75 µg g^{-1} soil caused a slight (non-significant) improvement in plant height with reference to the control. While, the addition of 225 µg Zn g^{-1} soil showed a significant ($p<0.05$) decline in plant height by 9.7 percent, against the control.

The application of cadmium at 10 µg g^{-1} soil caused a significant ($p<0.05$) decline in the root yield (6.6 percent), but a non-significant reduction in the shoot yield, compared with the control. The higher cadmium levels (30 and 60 µg g^{-1} soil) showed marked declines in the root yield (18.1 and 34.3%, respectively) and shoot yield (7.9 and 16.1%, respectively), against the control. The addition of lead at all the levels (50, 100 and 150 µg g^{-1} soil)

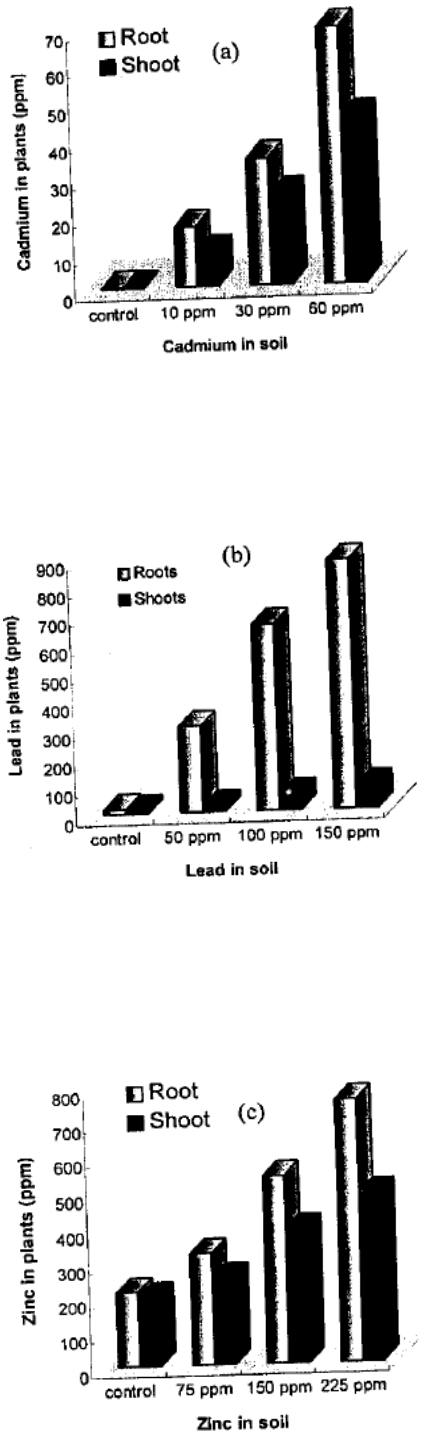

Fig 1: Canmium (a), lead (b) and zinc (c) concentration in ryegrass plants as affected by soil applied metals

Table 2: Growth and dry matter yield of ryegrass exposed to various concentrations of cadmium, lead and zinc

Metal ($\mu g\ g^{-1}$ soil)	Plant height (%)	Reduction (%)	Dry matter production (mg $plant^{-1}$)			
			Root	Reduction (%)	Shoot	Reduction (%)
Control	27.80	-	100.00	-	89.90	-
Cadmium						
10	27.60	0.7	93.40	6.6	86.20	4.1
30	27.10	2.5	81.90	18.1	82.80	7.9
60	25.90	6.8	65.70	34.3	75.40	16.1
$LSD_{0.05}$	3.45		8.15		5.80	
Lead						
50	24.80	10.8	39.00	61.0	61.30	31.8
100	16.30	41.4	7.40	92.6	18.10	79.9
150	9.10	67.3	2.90	97.1	8.50	90.5
$LSD_{0.05}$	4.68		6.34		6.66	
Zinc						
75	30.10	8.3*	102.20	2.2*	107.60	19.7*
150	27.30	1.8	84.10	15.9	83.70	6.9
225	25.10	9.1	43.80	56.2	64.80	27.9
$LSD_{0.05}$	4.56		8.62		7.65	

*Increase

resulted in drastic declines in the root (61, 92.6 and 97.1 percent, respectively) and shoot (31.8, 79.9 and 90.5 percent, respectively) yields, against the control. A slight (non-significant) increase in roots yield (2.2%) and a marked increase in the shoots yield (19.7%) was observed due to zinc addition at 75 $\mu g\ g^{-1}$ soil. The higher levels of zinc (150 and 225 $\mu g\ g^{-1}$ soil) however resulted in significant ($p<0.05$) declines in the roots (15.9 and 56.2%, respectively) and shoots (6.9 and 27.9 percent, respectively) yields, compared with the control.

Table 3: Relationship between soil applied metals and their concentration in plant tissues

Metal	Regression equation	r
	Root	
Cd	Y = 1.78+1.110 x	0.991
Pb	Y = 22.27+5.823 x	0.992
Zn	Y = 179.80+2.437 x	0.977
	Shoot	
Cd	Y = 1.09+0.729 x	0.989
Pb	Y = 6.34+0.475 x	0.950
Zn	Y = 181.10+1.301 x	0.973

y-Concentration of metal in plant tissue (µg/g)
x-Concentration of applied metal (µg/g soil)

Effect on Metal Concentration in Plant Tissues: The effect of cadmium, lead and zinc application on metal concentration in ryegrass plants is shown in Fig. 1. It is evident that the metal content both in roots and shoots increased significantly ($p<0.05$) with the Cd, Pb and Zn addition to the soil. The regression analysis of the data revealed highly significant correlation between the soil applied metals and their concentrations in plant tissues (Table 3). The figure also indicated that Cd and Zn were proportionately more translocated to shoots, while the lead remained mainly concentrated in the roots.

Table 4: Relationship between metal concentration in plant tissue and tissue yield

Metal	Regression equation	r
	Root	
Cd	Y = 100.20 - 0.506 x	0.920
Pb	Y = 88.75 - 0.112 x	0.887
Zn	Y = 131.17 - 0.107 x	0.873
	Shoot	
Cd	Y = 89.49 - 0.305 x	0.757
Pb	Y = 92.72 - 1.148 x	0.899
Zn	Y = 123.42 - 0.112 x	0.633

y-Tissue yield (mg/plant)
x-Concentration of metal in plant tissue (µg/g)

Table 5: Relationship between soil applied metals and relative reduction in dry matter yield

Metal	Regression equation	r
	Root	
Cd	Y = 1.24+0.553 x	0.999
Pb	Y = 47.47+0.361 x	0.841
Zn	Y = -35.10+0.389 x	0.959
	Shoot	
Cd	Y = 1.28+0.242 x	0.991
Pb	Y = 8.70+0.587 x	0.880
Zn	Y = -42.56+0.317 x	0.995

y-Relative reduction in plant tissue (%)
x-Concentration of applied metal (µg/g soil)

An inverse relationship was observed between the metal concentrations in plant tissues and their respective dry matter yields (Table 4). In case of Cd and Pb, a significant ($p<0.05$) correlation was observed between the metal concentration in roots and shoots and the roots and shoots yield. For Zn, the relationship between the tissue yield and the Zn concentration in tissue was not significant for roots, while it was significant ($p<0.05$) for shoots only.

Based on the relationship between the concentrations of soil applied metals and the relative reduction in dry matter yield (Table 5), the concentrations of Cd, Pb and Zn causing a 10 percent decline in roots yield were 15.8, -103.8, 115.9 µg g^{-1} soil, respectively. While, a 10 percent reduction in shoots yield was found to be at Cd, Pb and Zn concentrations of 36.0, 2.2 and 165.8 µg g^{-1} soil, respectively. This suggested that the relative toxicity of metals for the ryegrass was in the order: Pb>Cd>Zn. Moreover, the inhibitory effects of metals were more pronounced on roots than the shoots.

Discussion

Heavy metals are toxic to living organisms if present at higher concentrations (Adriano, 1986; Alloway, 1990). An excess of metal ions or of soluble metal chelates may induce a series of biochemical and physiological alterations in plants, among which membrane damage, alteration of enzyme activities and the inhibition of root growth are the characteristic features of heavy metal stress (Foy *et al.,* 1978; Lepp, 1981). These early events lead to a large range of secondary effects, such as disturbance of hormone balance, deficiency of essential nutrients, inhibition of photosynthesis, changes in photoassimilate translocation, alteration of water relations etc., which further enhance the metal-induced growth reduction (Barcelo and Poschenrieder, 1990). The present study demonstrated a significant inhibitory effect of cadmium, lead and zinc on the growth of ryegrass plants. This is in line with a number of reports published so far (Root *et al.,* 1975; Miller *et al.,* 1977; Allinson and Dzialo, 1981; Leita *et al.,* 1993; Jalil *et al.,* 1994; Yang *et al.,* 1996; Aery and Jagetiya, 1997).

The toxic effects of metals (Cd, Pb and Zn) in the present study were more pronounced on the dry matter yield of roots than on the growth of above ground plant parts (plant height and dry matter yield of shoots). In higher plants, roots are the first organ to contact the toxic metal concentrations, and roots usually accumulate significantly higher metal amounts than upper plant parts (Malone *et al.,* 1974; Guo and Marschner, 1995; Sajwani *et al.,* 1996). Thus, under long-term exposure, metals generally reduce root growth more intensively than shoot growth, decreasing the root/shoot ratio. Furthermore, different metals may inhibit root growth by different mechanisms. The inhibition of root cell elongation by Zn, Cu and Pb (Wainwright and Woolhouse, 1977; Lane *et al.,* 1978) and the extension of cell cycle by Zn (Powell *at al.,* 1986) have been responsible for the reduced root growth. The poorly mobile metals such as Pb, Cr, Al, Cu or Ba reduce root growth more intensively than the other relatively mobile metals in plants, which is also evident from the preseht results. A highly significant correlation between the root yield and plant height ($r = 0.92$) and root yield and shoot yield ($r = 0.98$) suggested that the reduction in above ground plant parts was mainly due to the inhibitory effect of metals on plant roots.

In the present study, the application of lead exerted the greatest biocidel effect than either of the cadmium or zinc applied separately at the tested metal levels. The relative decrease in the growth of ryegrass plants in relation to per unit increase in metals concentration was in the order: Pb>Cd>Zn. This observation is apparently contrary to the previous reports (Pahlsson, 1989; Kalyanaraman and Sivagurunathan, 1993; Aery and Jagetiya, 1997), where the toxicity of these metals (Cd, Pb and Zn) has been found to be as: Cd>Pb>Zn. Several factors might contribute to this apparent controversy between the previous findings and the results of this experiment. Cook and Hendershot (1996) reported that the toxicity of lead depends mainly on the solubility of lead compound used in the experiments. Khan and Frankland (1983) observed adverse effects (chlorotic radish seedlings) on vegetable plants at lead concentration of 50 µg g^{-1} applied in the form of lead chloride, but at 500 µg g^{-1} soil when applied as lead oxide. The solubility of lead compound (lead acetate) used in this study is considerably higher than those [$PbCl_2$ or $Pb(NO_3)_2$] applied in most of the studies reported above (Weast, 1988) and therefore, might be the major cause of the greater biocidal effect of lead observed in the present case. The presence of acetate in the soil medium (Khan and Huang, 1999), low pH (4.51) of the soil (Miller *et al.,* 1977; Reddy and Patrick, 1977; Singh *et al.,* 1995) and the relative tolerance of ryegrass to cadmium (Yang *et al.,* 1996) could be the other factors contributing to the toxicity trend observed in this study.

Acknowledgment

The first author gratefully acknowledges the award of Ph.D scholarship and financial support by the State Education Commission of People's Republic of China and the Ministry of Education, Pakistan to carry out this research work.

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