BRIEF COMMUNICATION

Dry matter and leaf structure in young wheat plants as affected by cadmium, lead, and nickel

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Abstract

The effects of 1 mM cadmium, lead and nickel on dry mass, Cd, Pb, and Ni contents, and changes in leaf structure in young wheat plants were studied. In leaves, Cd content was highest, followed by Pb and Ni, in roots Cd content was also highest, but followed by Ni and Pb. Roots accumulated considerably larger amounts of the three heavy metals than leaves. Largest reductions of leaf and root mass were obtained with Cd. Pb and Ni effects were almost equal. Ni excess had a strong negative effect on mesophyll thickness, while Cd mostly reduced the number and size of vascular bundles and vessel diameter. High Pb reduced the diameter of vessels causing their different deformations.

Additional key words: anatomy, epidermis, heavy metals, mesophyll, *Triticum aestivum*, vascular bundles.

Almost all plants are sensitive to increased concentrations of heavy metals. Leaves of heavy metal treated plants are smaller and chlorotic (Khan and Khan 1983, Kastori *et al.* 1998), while roots show abnormal growth and deformation (Wilkins 1978, Kastori *et al.* 1997). The photosynthetic apparatus is particularly susceptible to heavy metal toxicity (*e.g.* Rebechini and Hanzely 1974, Clijsters and Van Assche 1985, Ouzounidou *et al.* 1997).

The effect of heavy metals on cell division and elongation, meristem cell cycle, and hormonal regulation of plant growth and development was also reported

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Received 12 July 1998, *accepted* 17 January 1999.
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(Burzynski and Jacob 1983, Wierzbicka 1988). The effect of different heavy metal ions on the morphological and anatomical structure of plants may be as different as their chemical properties, especially valence, ion radius, capacity to form organic complexes, accumulation and distribution in plants (Kabata-Pendias and Pendias 1984). Taking the above in consideration, this paper compares the effect of excess Cd, Pb and Ni on the anatomical structure of the lamina of young wheat plants.

Experiments were done with young wheat plants (*Triticum aestivum* L.) cv. Renesansa. Before sowing, seeds were rinsed with de-ionized water and kept 5 h in 1 mM $CaSO_4 . 2 H_2O$. Seeds were germinated in the dark, at 25 °C, on a sterilized quartz sand, and watered daily with demineralized water. Seven days later the seedlings were transferred to half-strength Hoagland's solution, and grown to the stage of three leaves. Thereafter, the plants were exposed to 1 mM Cd, Pb or Ni (applied as $CdCl_2$, $PbCl_2$ and $NiSO_4$) until they developed the fourth leaf. Control plants were grown in half-strength Hoagland's solution.

Cross sections of fourth leaf lamina, developed at heavy metal excess, were made by freezing microtome. Microscopical measurements in main vein zone included: main vein thickness, main vascular bundle size, vessel diameter of main vascular bundle, while at 1/4 lamina width mesophyll thickness (between the veins), lateral vein thickness, large lateral bundle size, vessel diameter of lateral bundle, small lateral bundle size, epidermal and bulliform cell size were measured. Measurements also included total number of vascular bundles. Data are means of measurements on 10 leaves and 50 cells per treatment.

To determine dry mass of shoots and roots, plant material was dried at 105 °C to constant mass. The contents of the investigated metals were then assessed in dried samples which were digested in a mixture of concentrated nitric and perchloric acid (1:3). Cd, Pb and Ni contents in plant digests were determined by standard atomic absorption spectrometry (*Spectra AA-600, Varian*, Mulgrave, Australia). The data were analyzed by Duncan's multiple test alpha level.

Table 1. Effect of 1 mM Cd, Pb and Ni on corresponding heavy metal content [mg g^{-1}(d.m.)] and dry mass [mg $plant^{-1}$)] of wheat roots and leaves. Heavy metal contents are means of three replications, dry mass means of 32 plants ± SE.

Organs	Heavy metal contents			Dry mass			
	Cd	Pb	Ni	Control	Cd	Pb	Ni
Roots	2816 ± 220	1034 ± 97	2366 ± 201	21.2 ± 1.7	10.9 ± 1.0	18.4 ± 1.4	17.8 ± 1.9
Shoots	846 ± 91	280 ± 21	218 ± 19	50.4 ± 4.2	28.5 ± 3.1	34.2 ± 2.7	31.5 ± 2.9

Significant differences were found in heavy metal contents between leaves and roots (Table 1). Their descending order was Cd, Ni, Pb in leaves and Cd, Pb, Ni in roots. The accumulation of all three heavy metals was considerably higher in roots than in leaves of young wheat plants. It is supposed that the roots of the actively growing plant provide a barrier which restricts the movement of heavy metals to the aboveground plant parts (Ernst 1982, Wierzbicka 1987).

The heavy metals significantly decreased dry mass of leaves and roots of young wheat plants (Table 1). Jalil *et al.* (1994) and Titov *et al.* (1995) already reported unfavourable effects of Cd and Pb on wheat plant mass. Cadmium reduced the root and leaf mass to the largest extent, while the effects of Pb and Ni were similar. The analyzed heavy metals reduced leaf mass to a greater extent than root mass despite the considerably smaller concentrations in leaves.

Heavy metals affected cell division and differentiation. The heavy metals used caused significant reductions of lamina thickness, especially at main and lateral veins (Table 2). As veins are mainly composed of vascular and mechanical tissues, their partitioning in total lamina structure was lowered. Pb treatment resulted in the smallest changes of lamina thickness. Data on mesophyll thickness show that the photosynthetic tissue exhibited stability to Pb. The above results are in agreement with those of Gašić *et al.* (1992) and Janjatović *et al.* (1993). The thinnest mesophyll was found in the plants treated with Ni and Cd. Toxic concentrations of Ni significantly disintegrated the mesophyll cells occurring between vascular bundles. Thinner chlorenchyma after Cd treatment is possibly due to its negative effect on cell division and elongation.

Table 2. The effect of 1 mM Cd, Pb and Ni on leaf anatomy. Means that are not followed by the same letter are significantly different at the 0.05 level.

Parameter		Control	Cd	Pb	Ni
Lamina thickness on main vein [μm]		315 a	215 c	261 b	212 c
Lamina thickness on lateral vein [μm]		213 a	161 c	194 b	168 c
Mesophyll thickness [μm]		167 a	121 c	138 b	115 c
Vascular bundles number		16.5 a	12.2 b	16.2 a	15.8 a
Main vascular bundle size [μm]	height	104 a	80 c	91 b	83 c
	width	99 a	74 d	87 b	81 c
Lateral v. bundle size [μm]	height	91 a	61 d	74 b	67 c
	width	81 a	57 c	69 b	61 c
Small lateral v. bundle size [μm]	height	41.7 a	33.2 c	36.8 b	36.4 c
	width	42.4 a	33.5 c	27.9 d	36.8 b
Vessel diameter of main v. bundle [μm]	height	24.8 a	16.7 c	18.2 d	18.5 b
	width	24.1 a	16.9 c	18.0 bc	19.1 b
Vessel diameter of lateral v. bundle [μm]	height	20.1 a	13.8 b	13.9 b	14.4 b
	width	19.8 a	15.7 b	15.2 b	15.7 b
Adaxial epidermal cell size [μm]	height	23.7 a	24.3 a	22.3 b	25.5 a
	width	27.0 a	21.6 c	24.7 b	23.6 bc
Abaxial epidermal cell size [μm]	height	26.3 a	24.0 c	24.0 c	29.4 a
	width	32.1 a	27.2 b	26.1 b	25.4 b
Bulliform cell size [μm]	height	40.8 a	38.1 ab	33.9 c	35.5 bc
	width	31.7 a	28.7 b	24.3 c	24.7 c

Heavy metals reduced the number and size of vascular bundles in the lamina. The smallest size and number of bundles were found in the Cd-treated plants. Vessel diameter underwent the largest reduction when Cd was used (Table 2). Similar changes were reported for different plant species (Lamoreaux and Chaney 1977, Barceló *et al.* 1988). In our investigation a similar effect was also obtained with Pb. The results of Janjatović *et al.* (1991) also indicated a decreasing vessel diameter under the influence of toxic concentrations of Pb. The most evident effect of Ni was in mesophyll cells.

The analyzed heavy metals reduced the size of epidermal cells, in particular the abaxial ones. The reduction was more evident in cell width than in cell height. Unlike Pb and Cd, Ni increased the height of cells in both adaxial and abaxial epidermes. The treated plants had smaller bulliform cells. More significant reductions of their height and width were obtained with Pb and Ni than with Cd.

Our results point out a specific effect of heavy metals on the individual lamina tissues.

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