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BRIEF COMMUNICATION

Steady presence of cadmium and nickel affects root anatomy, accumulation and distribution of essential ions in maize seedlings

I. MAKSIMOVIĆ*[1], R. KASTORI*, L. KRSTIĆ** and J. LUKOVIĆ**

*Faculty of Agriculture, University of Novi Sad, and Institute of Field and Vegetable Crops, Novi Sad, Trg D. Obradovića 8, 21000 Novi Sad, Serbia**
*Faculty of Sciences, Department of Biology and Ecology, University of Novi Sad, Trg D. Obradovića 2, 21000 Novi Sad, Serbia***

Abstract

When growing in the field, plants are exposed to the effect of heavy metals as soon as the seed comes into contact with the soil solution. Therefore, we found important to study the effect of Cd and Ni on maize exposed to these heavy metals since sowing. The aim of this work was to examine which anatomical changes are induced by continuous intoxication of young maize root system with 0.1 mM Cd and Ni, thus modifying its growth and capacity for water and nutrient uptake. Concomitantly, the effect on concentration and distribution of Cd, Ni and some essential ions (Ca, Mg, Zn, Fe, Cu and Mn) was studied.

Additional key words: continuous heavy metal intoxication, mineral nutrition, *Zea mays*.

There are many experimental data on the effect of Cd or Ni on young maize plants, treated with heavy metals after germination (*e.g*. Rauser and Meuwly 1995, L'Huillier *et al.* 1996, Lagriffoul *et al*. 1998, Nyitrai *et al*. 2002, Seregin *et al*. 2003, 2004, Souza and Rauser 2003, Wójcik and Tukiendorf 2005, Wang *et al.* 2007). However, under field conditions, plants are exposed to the effect of heavy metals as soon as the seed comes into contact with the soil solution. Therefore, we found it important to examine the effect of Cd and Ni added already to the water used for imbibition of maize seeds. The same Cd and Ni concentrations were steadily present during the entire experiment. We studied the effects of these treatments on seedlings' morphology, anatomy of primary seminal roots and accumulation and distribution of Cd, Ni, and some essential ions in the seedlings. Chosen Cd and Ni concentrations are approximately ten times higher than those usually found in metal-contaminated soils. In our preliminary experiments 0.1 mM Cd and Ni had an effect on plants, but this effect was not lethal, which resembles environmental conditions.

Maize (*Zea mays* L.) seeds, hybrid NS-303, were germinated in demineralized water (control) or in the presence of 0.1 mM $CdCl_2$ or 0.1 mM $NiSO_4$ dissolved in demineralized water. Uniform 2-d-old seedlings were transferred on half-strength Hoagland solution (Hoagland and Arnon 1950) to which was added either 0.1 mM $CdCl_2$, 0.1 mM $NiSO_4$ or demineralized water (control) and grown in a greenhouse for 7 d, under 12-h photoperiod (irradiance of 190 μmol m^{-2} s^{-1}, day/night temperature of 25 ± 2/17 ± 2 °C, relative humidity of 85 %). Nutrient solution was changed every day and plants were aerated regularly. Each variant was set in seven replications with 8 plants in each replication.

Nine days after sowing, plants were analyzed. Fresh matter was measured and dry matter after drying the samples at 70 °C to constant mass. Total leaf area was measured by an automatic area meter (*LI-3000*, *Li-Cor*, Lincoln, USA). Concentrations of Cd, Ni, Ca, Mg, Zn, Fe, Cu and Mn were determined by atomic absorption spectrophotometer (*Varian SpectrAA 600*, *Varian Techtron*, Victoria, Australia).

For anatomical analyses, 2 cm long segments of the middle part of the primary seminal root were excised, fixed in 5 % glutaraldehyde and kept at 4 °C until needed (Jones *et al*. 2003). Cross sections were made using *Leica*

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[1] Author for correspondence; fax: (+381) 21 450 620; e-mail: ivanam@ib.ns.ac.yu

CM 1850 (*Leica Microsystems*, Nussloch, Germany) cryostat, at temperature -18 to -20 °C, at cutting intervals of 25 μm. Light microscopy observations and measurements were made using *Image Analyzing System Motic 2000* (Motic Microscopes, Germany) on ten root cross sections and fifty cells per treatment. The data were statistically processed by analysis of variance and means with standard errors (SE) were calculated using program *STATISTICA version 7.0*. Differences between the treatments were determined using Duncan's test.

Cd and Ni modified young maize morphology and anatomy. Total root length and primary seminal root length were significantly smaller than in the control (Table 1). These results are in agreement with Wójcik and Tukiendorf (2005) and Široká *et al*. (2004) who also found that maize root growth was reduced in the presence of Cd. According to Wang *et al*. (2007) Cd had stimulatory effect on maize root elongation at 0.01 mM and 0.1 mM Cd concentrations during the first five days, while root length decreased with an increasing Cd concentration (1 mM) and duration of treatment.

Both Cd and Ni reduced fresh root biomass and fresh and dry shoot biomass, while dry root biomass was not significantly affected. Percentages of root and shoot dry matter significantly increased in treated plants. Cd and Ni less affected shoots than roots of seedlings. The height and total leaf area of plants grown in the presence of Cd and Ni were reduced, most probably as a consequence of the toxic Cd effects in roots. Our results are consistent with those of several studies on plant growth reduction in the presence of Cd (Ouzounidou *et al*. 1997, Seregin *et al*. 2004, Sterckeman *et al*. 2004, Wójcik and Tukiendorf 2005, Österås and Greger 2006, Dražić *et al*. 2006, Rodríguez-Serrano *et al*. 2006, Scebba *et al*. 2006) and Ni (L'Huillier *et al*. 1996, Seregin *et al*. 2003, Demchenko *et al*. 2005).

The effect of Cd on root anatomical structure was more pronounced than the effect of Ni. Plants treated with Cd had significantly thicker cortex and larger parenchyma cells (Table 1). It might be possible that enlargement of parenchyma cells was a consequence of the impairment of root elongation, but this issue remains to be studied in more details. The thicker root cortex most probably increased resistance to radial flow of water and mineral nutrients, which reduced root and shoot growth. However, Cd did not affect the size of exodermal cells, central cylinder and vascular tissue. Wójcik and Tukiendorf (2005) found that Cd induced serious damages to maize root cells, Vitória *et al*. (2003/4) reported that Cd altered root differentiation in radish and Lux *et al*. (2004) found significant genotypic specificity of *Salix* roots with respect to uptake, translocation and

Table 1. The effect of Cd and Ni on root and shoot growth and anatomical characteristics of maize seedlings (means ± SE, $n = 40$). The values marked with the same letter do not differ significantly at $P < 0.05$. (* - exodermal cells of the first layer under rhizodermis were measured, ** - parenchyma cells of the third layer from endodermis were measured).

Plant part	Measured parameter	Control	10^{-5}M Cd	10^{-5}M Ni
Shoot	height [cm]	33.3 ± 0.9^{a}	18.5 ± 0.6^{b}	32.2 ± 1.0^{a}
	total leaf area [cm^2 plant^{-1}]	48.8 ± 2.2^{a}	24.0 ± 2.1^{b}	45.3 ± 2.4^{a}
	fresh mass [g plant^{-1}]	2.8 ± 0.03^{a}	0.54 ± 0.02^{b}	0.38 ± 0.05^{c}
	dry mass [g plant^{-1}]	0.19 ± 0^{a}	0.07 ± 0^{b}	0.06 ± 0^{b}
	dry mass [%]	6.5 ± 0.2^{c}	12.7 ± 0.8^{b}	17.2 ± 0.8^{a}
Root	total root length [cm]	285.3 ± 26.5^{a}	94.9 ± 6.1^{b}	126.4 ± 19.5^{b}
	primary seminal root length [cm]	20.3 ± 0.8^{a}	6.4 ± 0.5^{c}	12.9 ± 0.7^{b}
	number of seminal roots	3.7 ± 0.2^{a}	3.4 ± 0.4^{a}	3.5 ± 0.2^{a}
	number of nodal roots	3.5 ± 0.3^{c}	6.8 ± 0.7^{a}	5.0 ± 0.3^{b}
	fresh mass [g plant^{-1}]	1.2 ± 0.1^{a}	0.56 ± 0.03^{b}	0.53 ± 0.53^{b}
	dry mass [g plant^{-1}]	0.09 ± 0^{a}	0.08 ± 0^{a}	0.09 ± 0^{a}
	dry mass [%]	5.8 ± 0.3^{c}	13.5 ± 0.6^{b}	16.2 ± 0.9^{a}
	shoot dry mass/root dry mass	2.0 ± 0.1^{a}	0.89 ± 0^{b}	0.74 ± 0^{b}
	diameter [μm]	1193.2 ± 41.3^{b}	1366.8 ± 56.1^{a}	1112.2 ± 29.1^{b}
	central cylinder diameter [μm]	526.2 ± 22.0^{ab}	575.3 ± 27^{a}	464.0 ± 21.1^{b}
	cortex thickness [μm]	303.5 ± 17.2^{b}	393.0 ± 23.1^{a}	309.2 ± 8.1^{b}
	number of cortex cell layers	8.5 ± 0.2^{b}	9.0 ± 0^{b}	9.8 ± 0.2^{a}
	root/cylinder diameter	2.2 ± 0.07^{a}	2.3 ± 0.05^{a}	2.3 ± 0.08^{a}
	root diameter/cortex thickness	3.9 ± 0.2^{a}	3.4 ± 0.1^{b}	3.5 ± 0.05^{b}
	cross section area of exodermal cells [μm^2]*	563.0 ± 56.3^{a}	583.4 ± 29.8^{a}	513.1 ± 38.5^{a}
	cross section area of parenchyma cells [μm^2]**	1485.6 ± 120.1^{b}	1750.0 ± 79.9^{a}	1433.8 ± 31.2^{b}
	number of late metaxylem elements	5.7 ± 0.5^{a}	5.3 ± 0.2^{a}	5.2 ± 0.5^{a}
	number of early metaxylem elements	14.5 ± 0.3^{a}	14.3 ± 0.2^{a}	14.7 ± 0.6^{a}
	diameter of late metaxylem elements [μm^2]	83.3 ± 2.6^{a}	83.5 ± 2.2^{a}	77.7 ± 1.4^{a}
	diameter of early metaxylem elements [μm^2]	35.0 ± 1.6^{a}	34.2 ± 1.4^{a}	32.9 ± 1.0^{a}

Table 2. Content of Cd, Ni, Ca, Mg, Zn, Fe, Cu and Mn in shoots and roots of maize seedlings [mg kg^{-1}(d.m.)] (means ± SE, $n = 15$). The values marked with the same letter do not differ significantly at $P < 0.05$.

Element	Shoot control	10^{-5}M Cd	10^{-5}M Ni	Root control	10^{-5}M Cd	10^{-5}M Ni
Cd	0.0	330.2 ± 5.3	0.0	0.0	948.5 ± 16.3	0.0
Ni	0.0	0.0	166.8 ± 4.3	0.0	0.0	1004.7 ± 0.2
Ca	1451.6 ± 15.0^b	1606.5 ± 16.3^a	1423.7 ± 6.1^b	1874.6 ± 130.9^a	1114.3 ± 15.0^c	1600.7 ± 37.9^b
Mg	496.2 ± 6.3^b	526.6 ± 4.2^a	523.4 ± 5.0^a	703.5 ± 6.0^a	555.4 ± 4.7^c	589.1 ± 5.1^b
Zn	51.0 ± 0.7^a	45.5 ± 0.8^b	42.3 ± 0.3^c	37.7 ± 0.5^b	29.8 ± 0.1^c	38.5 ± 0.4^a
Fe	88.1 ± 0.8^a	44.5 ± 0.7^c	52.3 ± 0.6^b	193.2 ± 8.6^c	513.4 ± 1.6^b	580.8 ± 13.6^a
Cu	12.1 ± 0.6^a	6.0 ± 0.2^c	7.1 ± 0.03^b	15.8 ± 0.2^a	14.6 ± 0.7^b	9.3 ± 0.2^c
Mn	63.0 ± 0.5^a	18.9 ± 0.3^c	28.4 ± 0.1^b	164.0 ± 0.2^a	7.9 ± 0.1^c	30.3 ± 0.4^b

development of root apoplastic barriers to Cd transport. These results confirm that Cd can also cause water deficit in the cells and tissues as a secondary stress effect (Barceló *et al*. 1986, Vitória *et al*. 2003/4).

In the roots of Ni treated plants the number of cortical cell layers increased, although the other measured parameters were not significantly affected. Therefore, the negative effect of Ni on root growth was not that prominent, compared to the effect of Cd. Our findings also agree with those of Yang *et al*. (1996), who explains maize tolerance to Ni toxicity by its extremely low transport, even though it had high influx into roots.

Cd caused strong alteration in essential metal concentrations and distributions (Table 2). Such effect of Cd according to Barceló *et al*. (1988) could be described very early; Cd decreased concentration of essential cations, with exception of Fe, for as much as 95 % for Mn, 60 % for Ca, 20 % for Mg and Zn and 9 % for Cu. Correspondingly to roots, Mn concentration in shoots was affected the most and reduced for about 70 %, Fe and Cu concentrations were reduced for 50 %, Zn for 10 % while Ca and Mg concentrations were not significantly affected. Similarly to Cd, Ni increased Fe concentration in roots but seriously reduced its content in shoots, for about 50 %. This might be explained by antagonism in transport processes in the plant between heavy metals and Fe. Ni also reduced Mn content in roots the most, for about 20 %, but it had stronger effect on Cu concentration in roots as compared to Cd (reduction for 40 %). Ni reduced Mg and especially Ca concentration in roots to a lesser extent than Cd (for about 15 % each). In shoots, Ni reduced for about 45 % Mn concentration, 40 % Fe and Cu concentration, 18 % Zn, but did not affect Ca and Mg concentrations. These results are in accordance with those of Gussarson *et al*. (1995) after which higher concentrations of Cd and Ni in the substrate inhibited metabolism of Mg, Fe, Mn and Zn. As a consequence, deficiency of those elements in young, newly-formed plant organs may appear as leaf chlorosis and necrosis, which is concomitant with an increase in concentration of these essential elements in roots. Our results show that in very young maize plants grown under conditions of constant presence of 0.1 mM Cd and Ni there were severe changes in root morphology and anatomy as well as in plant essential metal composition although so drastic symptoms did not yet appear on the shoots.

As Cd and Ni accumulated to important levels in shoots of young maize plants (0.3 and 0.15 mg kg^{-1}(d.m.), respectively) it may be worthwhile to consider some maize genotypes as suitable for phytoextraction of non-strongly contaminated sites.

The results described herein show that continuous intoxication of young maize plants with Cd and Ni at toxic but not lethal concentrations changes their morphology, anatomy of individual root tissues and accumulation and distribution of essential ions.

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