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BRIEF COMMUNICATION

Cadmium toxicity: the effect on macro- and micro-nutrient contents in soybean seedlings

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Abstract

The effect of Cd (10, 100, and 200 μM) on tissue contents of macronutrients (N, P, K, Ca, Mg) and micronutrients (Fe, Zn, Cu, Mn) was investigated in hydroponically grown soybean (*Glycine max*) seedlings. Concentration changes of analysed elements observed against increasing Cd accumulation indicated that acute Cd-phytotoxic effect monitored through chlorophyll content was not a consequence of nutrient deficiency.

Additional key words: *Glycine max*, growth parameters, leaves, roots.

General symptoms of Cd toxicity are leaf chlorosis, leaf and root necrosis and decrease of growth. One of the causes of Cd toxicity in plants is its interaction with essential elements (Sandalio *et al*. 2001, Mazen 2004). Available experimental reports address long-term effect of low Cd concentrations which induce a chronic stress, different from the one induced by short-term exposure to high concentrations of the pollutant (Larbi *et al.* 2002). The aim of the present study was to evaluate if the response to high Cd concentrations monitored through growth parameters and chlorophyll (Chl) content of soybean seedlings was directly correlated with a change in uptake and distribution of macro- and micro-nutrients.

Soybean plants (*Glycine maxima* L. cv. Nena) were germinated on wet filter paper in the dark (22 ºC, 97 % relative humidity) after surface desinfection (5 min in 3 % H_2O_2). From day 4 the seedlings were cultivated hydroponically in nutrition solution of Römheld and Marschner (1981) under greenhouse conditions (average temperature 22 °C, day length of 14 h, average irradiance of 90 μmol m^{-2} s^{-1}). After the plants reached the stage of fully developed leaves of the second node they were transferred into a fresh nutrition medium in which Cd was added at concentrations of 10, 100, and 200 μM. Control plants were grown in the same conditions without Cd. After 48 h the seedlings were harvested and fresh and dry masses were measured. Chl content was measured according to Moran (1982). Dry plant material was ground and wet mineralized in the presence of H_2SO_4 and H_2O_2 for N determination, and in the presence of HNO_3 and H_2O_2 for determination of other elements. Concentration of P was determined by a spectrophotometric method (Chen *et al*. 1956). Nitrogen content was determined by micro-Kjeldahl method. Determination of K, Ca, Mg, Fe, Zn, Cu, Mn, and Cd contents was performed by atomic absorption spectrophotometry (*Pye Unicam 192*, Cambridge, UK) using *Certipur* (*Merck*, Darmstadt, Germany) standards.

Impact of high Cd concentrations used was primarly reflected in a significant Chl content decrease, in the first leaf starting with 10 μM Cd, and in the second leaf starting with 100 μM Cd. Besides, impairment of leaf water balance occurred that led to a significant decrease of leaf fresh mass (Table 1). At long-term exposure to lower concentrations of this heavy metal root and shoot growth rates declined in correlation with external Cd concentration, duration of the treatment, plant species or genotype, and in extreme cases root necrosis appeared. The impaired water balance was a consequence of decreased water uptake as well as increased transpiration (Poschenrieder *et al*. 1989). Potassium content decreased in roots and increased in leaves (Table 2), was probably a consequence of disturbed water balance.

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Table 1. Fresh (FM) and dry (DM) mass of roots and leaves and chlorophyll *a*+*b* (Chl) content of soybean seedlings treated for 48 h with different Cd concentrations. Means of 5 samples ± SD, *t*-test: significant difference at * - 0.5 %, ** - 0.1 % and *** - 0.05 % level.

Cd [μM]	Root FM [mg plant^{-1}]	Root DM [mg plant^{-1}]	Leaf FM [mg plant^{-1}]	Leaf DM [mg plant^{-1}]	Chl, 1st leaf [g kg^{-1}(DM)]	Chl, 2nd leaf [g kg^{-1}(DM)]
0	19.62 ± 1.42	10.06 ± 0.64	138.74 ± 10.80	19.82 ± 1.44	45.23 ± 3.22	42.23 ± 3.88
10	20.94 ± 1.67	10.17 ± 0.84	124.16 ± 6.92	18.54 ± 1.38	21.43 ± 1.76**	44.36 ± 4.02
100	18.44 ± 2.74	8.69 ± 0.71	121.40 ± 8.37*	18.46 ± 1.82	14.05 ± 1.87***	13.29 ± 1.65***
200	17.64 ± 3.54	7.69 ± 1.98	102.72 ± 7.66**	18.20 ± 2.02	8.22 ± 1.34***	9.87 ± 1.12***

Table 2. Element contents in root and leaves of soybean seedlings Cd-treated for 48 h. Means of 3 biological and 3 chemical repetitions ± SD, *t*-test: significant difference at * - 0.5 %, ** - 0.1 % and *** - 0.05 % level. ND - not detected, detection limit was 1 mg(Cd) kg^{-1}(DM).

Element		Control	10 μM Cd	100 μM Cd	200 μM Cd
N [% DM]	root	4.12 ± 0.13	4.12 ± 0.27	4.13 ± 0.24	3.13 ± 0.51
	leaf	5.60 ± 0.24	5.63 ± 0.41	5.92 ± 0.36	6.22 ± 0.55
P [% DM]	root	0.40 ± 0.07	0.41 ± 0.06	0.43 ± 0.04	0.21 ± 0.07**
	leaf	0.41 ± 0.03	0.45 ± 0.04	0.43 ± 0.06	0.59 ± 0.07*
K [% DM]	root	2.21 ± 0.08	2.36 ± 0.06	1.41 ± 0.14**	1.45 ± 0.21**
	leaf	2.80 ± 0.09	2.30 ± 0.11	3.06 ± 0.36	3.95 ± 0.45***
Ca [g kg^{-1}(DM)]	root	3.60 ± 0.14	3.41 ± 0.17	3.15 ± 0.23	2.26 ± 0.27**
	leaf	3.78 ± 0.11	3.35 ± 0.17	3.27 ± 0.22	3.39 ± 0.28
Mg [g kg^{-1}(DM)]	root	1.40 ± 0.08	1.48 ± 0.14	3.39 ± 0.14***	3.06 ± 0.37***
	leaf	5.47 ± 0.67	4.72 ± 0.55	5.04 ± 0.74	5.13 ± 0.89
Fe [g kg^{-1}(DM)]	root	2.69 ± 0.47	2.56 ± 0.44	2.50 ± 0.46	1.06 ± 0.52***
	leaf	0.22 ± 0.02	0.23 ± 0.02	0.21 ± 0.03	0.23 ± 0.05
Zn [mg kg^{-1}(DM)]	root	65.69 ± 2.63	59.50 ± 3.22	44.04 ± 2.82**	38.50 ± 3.45**
	leaf	71.49 ± 7.24	57.68 ± 5.78	68.00 ± 6.88	63.59 ± 7.24
Cu [mg kg^{-1}(DM)]	root	242.18 ± 13.76	185.43 ± 23.26	104.24 ± 15.64**	75.56 ± 10.18***
	leaf	29.56 ± 3.82	23.45 ± 4.15	36.76 ± 3.72	62.68 ± 4.08***
Mn [mg kg^{-1}(DM)]	root	12.81 ± 0.67	8.24 ± 0.92*	9.22 ± 0.56*	6.60 ± 0.34***
	leaf	31.82 ± 2.26	23.07 ± 1.78	23.72 ± 2.68	28.34 ± 3.42
Cd [mg kg^{-1}(DM)]	root	ND	184.62 ± 26.44	785.14 ± 58.88	1445.26 ± 89.74
	leaf	ND	2.14 ± 0.78	113.16 ± 15.62	212.36 ± 18.78

Nitrogen and P contents decrease usually observed at long-term exposure to low Cd concentrations was not found after the short-term exposure to high concentrations of this toxic metal (Table 2). Contents of Ca and Fe in root were decreased under 200 μM Cd, but were still within the range optimal for plant growth (Marschner 1995), and in the leaf they remained unchanged. This is in contradiction to the reports on leaf chlorosis that was attributed to a Cd-induced Fe deficiency (Wallace *et al.* 1992). Also, there was no Mg content decrease in leaf, which would naturally be associated with Chl degradation. Magnesium content was increased in root in the presence of Cd which indicates that Mg participates in tolerance processes.

Contents of Cu, Zn, and Mn were decreased only in root (Table 2) which might be due to impaired uptake of these ions from the solution, their leaking from cells as a consequence of impaired membrane structure and function (Hernandez and Cooke 1997) or their redistribution within the plant because these elements are constituents of Cu,Zn superoxide dismutases that participate in protection against oxidative stress in leaf (Sandalio *et al.* 2001).

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