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Environmental and genetic effects on cadmium accumulation capacity and yield of maize

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Abstract: Maize (*Zea mays*) is an economic crop suitable for use in phytoremediation in low to moderately cadmium (Cd)-contaminated soils due to its ability to accumulate high concentration of Cd in parts of maize that are not used in human diet. The aim of this study was to test Cd content in nine female parents of the commercial maize hybrids (C1 = ♀2-48; C2 = ♀1767/99; C3 = ♀87-24; C4 = ♀135-88, C5 = ♀84-28; C6 = ♀84-44; C7 = ♀438-95; C8 = ♀30-8; C9 = ♀B-73) grown under field conditions in two soils (B1: eutric cambisol, B2: stagnosol) during three growing seasons (A1: 2006, A2: 2007, A3: 2008). The stationary trial was conducted in four replicates. The ear-leaves at flowering and grain at maturity were taken for chemical analysis. The average quantities of leaf-Cd were 0.081, 0.088 and 0.143 mg per kg of dry matter for A1, A2 and A3, 0.089 and 0.118, for B1 and B2, respectively. Grain-Cd was below the threshold (< 0.02 mg/kg). Five Cd-inefficient genotypes (C3, C5, C6, C7 and C9) had low leaf-Cd (average 0.049 mg/kg), while this content was about 6-times higher (average 0.299 mg/kg) in Cd-efficient genotype C4. The yield among the years ranged from 2.36 to 4.31 t/ha. Maize grown on B2 had about 26% lower yield than on B1. Five genotypes (C1, C2, C8 and C9) achieved yields less than 3.50 t/ha (mean 3.15 t/ha), while in two genotypes (C3 and C5) yields were above 4.00 t/ha (mean 4.14 t/ha). Very strong correlations (*r*) of leaf-Cd status among years (ranged from 0.52 to 0.77) confirmed high genetic effect on the capability of Cd accumulation in maize. However, correlations between Cd content and yield were low (ranged from –0.17 to 0.06). Cd-efficient C4 female parent could be used for development of maize hybrids suitable for phytoremediation, while Cd-inefficient female parents for hybrids could be suitable as forage maize crop contributing to the lower Cd input into food chain.

Keywords: genotype variation; leaves and grain;soil type; *Zea mays*

Cadmium (Cd) is regarded as one of the most toxic trace elements in the environment, thus the Cd uptake by crops is important from the aspect of human health. Vahter *et al*. (1991) reported that Cd concentrations in uncontaminated soils are usually below 0.5 mg/kg. Kabata-Pendias and Pendias (2001) found that mean concentration of Cd ranged in cereal grains from 0.013 to 0.220 mg/kg. According to European Food Safety Authority (EFSA) a tolerable weekly intake for Cd through food is 2.5 μg/kg body weight (EU No. 488/2014). Furthermore, the maximum level of Cd in cereals is 0.20 mg/kg wet weight (EC No. 1881/2006).

The uptake of Cd by roots from soil depends on the soil factors such as Cd concentration in soil, soil pH, level of organic matter and interactions between nutrients etc. (Bergmann 1992; Ivezić *et al*. 2015).

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Production of low-Cd crop cultivars can be used as a tool to reduce the risk of uptake of Cd into the human diet, but also into animal feed, through soil-plant-food system (Grant *et al.* 2008; Lončarić *et al.* 2012; Rebekić *et al.* 2015; Novoselec *et al.* 2018). On the other hand, high-Cd cultivar could be useful for phytoremediation in low to moderately Cd-contaminated soils (Xu *et al.* 2013). The aim of this study was to investigate the effect of growing season, soil type and genotype on Cd content in maize (*Zea mays* L.) grain and leaf and maize yield.

MATERIALS AND METHODS

The field experiments. Nine inbred lines (female parents of maize hybrids) developed by the Agricultural Institute Osijek (C1 = ♀2-48; C2 = ♀1767/99; C3 = ♀87-24; C4 = ♀135-88, C5 = ♀84-28; C6 = ♀84-44; C7 = ♀438-95; C8 = ♀30-8; C9 = ♀B-73) were grown under uncontaminated field conditions during three growing seasons (A1: 2006; A2: 2007; A3: 2008) in Osijek (B1: 45°32'N and 18°44'E) and Podgorac (B2: 45°29'N and 18°12'E). The experiments were conducted in four replicates (basic plots of 28.0 m^2 with four 10-m long rows) and fertilized uniformly with N, P and K (180 kg/ha N + 80 kg/ha P_2O_5 + 120 kg/ha K_2O). In autumn 400 kg/ha NPK (7 : 20 : 30) was applied and there were also 250 kg per ha urea (46% of N) and 150 kg/ha CAN (calcium ammonium nitrate with 27% of N) applied in spring. The maize was sown by pneumatic sowing machine at the end of April/beginning of May and harvested in the second half of October. No insecticide was used, while weed control was done properly.

Sampling, chemical and statistical analysis. Soil sampling was done by the auger up to 30 cm of depth before the experiment setup. Ear-leaves were collected at flowering stage (middle of July) and grain was collected at maturity stage for chemical analysis of plant material.

Soil pH, organic matter and mobile fraction of P and K (AL-method) determinations were according to Čosić *et al.* (2007), while Ca, Mg and Cd in the soil were extracted by acid solution (pH 4.65) of NH_4-acetate + EDTA (Lakanen & Erviö 1971). Total amounts of Cd in maize leaves and grain were determined using inductively coupled plasma (ICP-AES Jobin-Yvon Ultrace 238 spectrophotometer) after microwave digestion by concentrated HNO_3 + H_2O_2 in Research Institute for Soil Science and Agricultural Chemistry (RISSAC) in Budapest, Hungary. The same instrument was used to determine Ca, Mg and Cd contents in the soil solution.

The data were statistically analysed using ANOVA as three-factorial trial (A = year; B = soil type and C = maize genotype), with means comparisons at $P \leq 0.05$ and $P \leq 0.01$ probability levels, while correlation analysis (r) was done using the SAS software (Ver. 9.1.3, 2003).

Weather conditions. According to State Hydrometeorological Service (SHS 2008) precipitation and mean air temperature in Osijek were 210 mm and 19.7°C, 226 mm during May–September period and 20.2°C, 354 mm and 19.8°C, for 2006, 2007 and 2008, respectively. In two growing seasons precipitation was about 33% and 28% lower (2006 and 2007, respectively), while in 2008 it was 12% higher compared to 30-year average. Mean air temperature in analysed years 2006–2008 was higher for 0.9, 1.4 and 1.0°C, respectively. Water deficit and high mean air-temperature in July 2006 (15 mm precipitation and 23.5°C) were not favourable for maize development. The 2007 growing season was characterised by weather stress conditions, particularly in June and July because precipitation was 40% lower than usual and it was combined with 2.8°C higher temperature. Monthly precipitation and temperature regime in the 2008 growing season were more balanced than in the previous two years.

The soil properties. Soil was characterised as eutric cambisol (Osijek) and stagnosol (Podgorac) according to FAO/UNESCO soil classification (FAO 1990). The soil B1 is moderately acid and the soil B2 close to limit of acid/strong acid (below 4.5) soil. Mobile forms of K, Ca and Mg are high in both soils, although available Ca content in B2 soil is about 70% lower than in soil B1 (Table 1). The surface layer

Table 1. The main soil properties in layer 0–30 cm in spring 2006

	Soil pH		Organic matter (%)	AL-method (mg/100 g)		NH_4-acetate + EDTA (mg/kg)		
	H_2O	KCl		P_2O_5	K_2O	Ca	Mg	Cd
B1	6.77	5.96	1.84	15.4	26.7	14 622	1 016	0.10
B2	5.84	4.47	4.11	18.1	31.0	4 333	1 111	0.12

B1 – eutric cambisol; B2 – stagnosol

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Table 2. Soil physical properties in layer 0–30 cm in spring 2006

	Diameter of soil particle (mm)				Textural class	Soil porosity (% vol.)	Soil density (g/cm^3)	
	2.00–0.2 sand	0.063–0.02 cours silt	0.02–0.002 fine silt	< 0.002 clay			bulk	particle
	(%)							
B1	0	38.9	32.1	27.6	silt loam	45.9	1.51	2.70
B2	7.5	33.5	34.4	24.6	silt loam	43.4	1.40	2.51

B1 – eutric cambisol; B2 – stagnosol

of both soils matches in powder loam texture class with slight differences in soil porosity and density (Table 2).

RESULTS AND DISCUSSION

The average leaf-Cd quantities were 0.081 and 0.088 mg/kg Cd in 2006 and 2007, respectively, and this difference was non-significant (Table 3). In 2008, however, leaf-Cd was considerably higher (average 0.143 mg/kg Cd). Leaf-Cd in maize under eutric cambisol was considerably lower (average 0.089 mg per kg Cd) than under stagnosol (average 0.118 mg per kg Cd) conditions (impact of factor B). This difference could be explained by differences in soil pH. The two most important factors governing the uptake of Cd by crops are the soil pH and the concentration of Cd in the soil (Ivezić *et al*. 2013). Concentrations of Cd in tobacco leaves differed significantly among the growing regions of Hungary (Central part and North-east part) as follows: averages 0.50 and 1.89 mg/kg Cd (Gondola & Kadar 1995; Kadar *et al*. 2002). These findings are important for cigarette smokers, whose blood-Cd is typically more than

Table 3. Impacts of year, soil type and genotype on cadmium (Cd) accumulation in leaves and grains of maize

Genotype		Year (A)			Soil (B)		Average
		A1	A2	A3	B1	B2	C
The ear-leaf at silking stage (mg/kg Cd in dry matter)							
			AC interaction			BC interaction	
C1	♀2-48	0.096	0.104	0.218	0.136	0.143	0.139
C2	♀1767/99	0.094	0.106	0.219	0.140	0.139	0.140
C3	♀ 87-24	0.036	0.023	0.084	0.045	0.050	0.048
C4	♀135-88	0.260	0.360	0.278	0.184	0.414	0.299
C5	♀84-28	0.039	0.033	0.093	0.060	0.049	0.055
C6	♀84-44	0.040	0.024	0.071	0.036	0.054	0.045
C7	♀438-95	0.034	0.043	0.064	0.056	0.038	0.047
C8	♀30-8	0.090	0.068	0.169	0.098	0.120	0.109
C9	♀B-73	0.038	0.030	0.089	0.051	0.053	0.052
Average		0.081	0.088	0.143	0.089	0.118	0.104
			AB interaction				
		A1	A2	A3			
B1		0.032	0.082	0.154			
B2		0.129	0.093	0.131			
t-test		A	B	C	AB	AC	BC
$P \leq 0.05$		0.015	0.012	0.026	0.021	0.044	0.036
$P \leq 0.01$		0.027	0.022	0.047	0.038	0.081	0.066
Grain of maize at maturity (mg/kg Cd in dry matter)							
			A1–A3			B1 and B2	
C1–C9		below detection limit of ICP-OES method (< 0.02)					

A1 – 2006; A2 – 2007; A3 – 2008; B1 – eutric cambisol; B2 – stagnosol

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Table 4. The Pearson's correlation coefficients of leaf-Cd among years, soil type and yield

Genotype	Leaf-Cd				Leaf-Cd : yield
	A1 : A2	A1 : A3	A2 : A3	B1 : B2	(A1, A2, A3, B1, B2)
C (9 pairs)	0.77***	0.52***	0.71***		ns
C (27 pairs)				0.53***	–0.17 to 0.06

A1 – 2006; A2 – 2007; A3 – 2008; B1 – eutric cambisol; B2 – stagnosol; significant at *P < 0.05; **P < 0.01; ***P < 0.001; ns – not significant

double compared to nonsmokers' due to the exposure to Cd through inhalation (Waalkes 2003).

In our study, differences among nine genotypes varied from 0.045 to 0.299 mg/kg Cd and the highest value was more than 6-times higher compared to the lowest value. Contents of Cd in maize grains were considerably lower than in leaves and below threshold limit (Table 3). For this reason, possible differences in Cd uptake in plant parts above ground could be responsible for health status of animals which are mostly fed by maize as forage crop. The strong correlation of leaf-Cd among years (Table 4) in range from 0.52 to 0.77 is an indication that our study found higher genetic effect compared to environmental effects on Cd status in maize. Based on available data this phenomenon cannot be explained. It was also showed that the correlation between maize Leaf-Cd content and maize grain yield was not significant.

It is well known that maize genotypes have differential response to environmental conditions (Procházková *et al.* 2013; Antunović *et al.* 2014; Brkić *et al.* 2015; Galić *et al.* 2017). In this study, maize genotypes had significantly ($P \leq 0.01$) different Cd uptake in different years (Table 3). Five genotypes (C3, C5, C6, C7 and C9) had considerably lower leaf-Cd (average 0.049 and ranged from 0.045 to 0.055 mg per kg Cd), while three genotypes (C1, C2 and C8) were characterised by the range from 0.109 to 0.140 mg per kg Cd. However, C4 genotype had the highest leaf-Cd (average 0.299 mg/kg Cd) in three tested years (averages 0.260, 0.360 and 0.278 mg/kg Cd, in 2006, 2007 and 2008, respectively) and two soils (averages 0.184 and 0.414 mg/kg Cd, in eutric cambisol and stagnosol, respectively). For this reason, the first mentioned group of parents could be used for development of maize hybrids suitable for silage maize, while the second group for phytoremediation. This conclusion was confirmed by our earlier studies. Namely, the C2, the C4 and the C6 genotypes are parents of Tvrtko 303 hybrid, Os444 hybrid and OsSK552 hybrid, respectively (Jambrović *et al.* 2014). Differences of leaf-Cd status between their two parents (0.299 and 0.045 mg Cd/kg, for C4 and C6, respectively – Table 3) were in close connection with leaf-Cd status in their progeny as follows: 0.122 mg Cd kg in OsSK444 and 0.066 mg Cd kg in Os552 hybrids (Kovačević *et al.* 2002). Also, considerably higher leaf-Cd status (0.123 mg Cd/kg) in Tvrtko 303 hybrid (Kovačević *et al.* 2011) than in Os552 hybrid (0.040 mg Cd/kg) is closely connected with Cd status in their parents (Table 3).

Genotypic variations in Cd content under identical environmental conditions among varieties of the same plant species were found in numerous studies (Florijn & Van Beusichem 1993; Zhang & Song 2006; Gao *et al.* 2011).

Kovačević and Vragolović (2011) studied seven parents of maize and their 21 F_1 progenies under field conditions during two growing seasons on two soil types. Six hybrids of Bc707-1 had considerably higher leaf-Cd in comparison with six hybrids of Bc265-1 (means 0.100 and 0.050 mg/kg, respectively). Based on the study, the Cd-efficient parent Bc707-1 could be used in breeding strategies for creation maize hybrids suitable for phytoremediation while the Cd-inefficient Bc265-1 could be the a component of maize hybrids with low Cd uptake and for this reason suitable as forage maize.

Zhang *et al.* (2008) found significant correlation between Cd accumulation of male parents and their hybrids and some inbred lines (S37, 9782 and ES40), which were selected for cross-breeding with low Cd accumulation. Moreover, some inbred lines (178, R08, 48-2, and Mo17ht) could be used as soil phytoremediation species.

In our study, growing season characteristics, mainly weather conditions, were more significant factors for grain yield than soil and genotype (Table 5). Maize grown on albeluvisol had about 26% lower yield (impact of factor B) than on cambisol (3.01 and 4.09 t/ha, respectively). Maize yields ranged among genotypes (factor C) from 3.02 to 4.23 t/ha. Drought stress caused by higher temperatures was responsible for considerable lower yield in 2007 (mean 2.39 t/ha) which was

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Table 5. Impacts of year, soil type and genotype on grain yield of maize

Genotype (C)		Year (A) A1	A2	A3	Soil (B) B1	B2	Average C
Grain yield of maize (t/ha on 14% grain moisture basis)							
		AC interaction			BC interaction		
C1	♀2-48	2.64	2.73	4.11	3.67	2.66	3.16
C2	♀1767/99	3.14	3.04	4.01	4.22	2.58	3.40
C3	♀ 87-24	5.00	3.27	4.41	4.64	3.81	4.23
C4	♀135-88	3.77	2.35	4.38	4.29	2.71	3.50
C5	♀84-28	4.51	2.47	5.15	4.39	3.70	4.04
C6	♀84-44	3.80	2.92	4.56	4.62	2.90	3.76
C7	♀438-95	5.16	1.92	4.26	4.12	3.44	3.78
C8	♀30-8	3.89	1.18	4.00	3.62	2.43	3.02
C9	♀B-73	3.90	1.38	3.87	3.20	2.91	3.05
Average		3.98	2.36	4.31	4.09	3.01	3.55
		AB interaction					
		A1	A2	A3			
B1		3.65	3.29	5.32			
B2		4.31	1.44	3.29			
t-test		A	B	C	AB	AC	BC
$P \leq 0.05$		0.16	0.13	0.27	0.22	0.47	0.38
$P \leq 0.01$		0.29	0.23	0.50	0.41	0.87	0.71

A1 – 2006; A2 – 2007; A3 – 2008; B1 – eutric cambisol; B2 – stagnosol

about 40% and 45% lower compared to yields achieved under more favorable weather conditions in 2006 and 2008, respectively.

CONCLUSIONS

Genotype affected Cd status in leaves of maize (to a greater extent) compared to growing season and soil effects. Cd-inefficient C3, C5, C6, C7 and C9 female parents of the commercial maize hybrids are suitable as forage maize as they have lower Cd input in food chain. However, Cd-efficient C4 genotype could be used for development maize hybrids suitable for phytoremediation of low to moderately Cd-contaminated soils.

Leaf-Cd concentration in maize under neutral soil was about 25% lower compared to acid soil. Low quantities of Cd were found in maize leaves and grain, therefore, the production of healthy food is possible on the majority of arable land in Croatia.

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