

Cadmium uptake and translocation in durum wheat varieties differing in grain-Cd accumulation

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ABSTRACT

Wheat grain products are the primary source of dietary Cd-intake for humans. Since varieties differ markedly in grain-Cd accumulation, efforts are needed to find traits associated with low, or high, accumulation. Two durum wheat varieties, selected in a field screening as low (Creso) and high (Svevo) grain-Cd accumulating, were grown on soil spiked with 1.5, 3 and 4.5 mg Cd/kg. Growth patterns, Cd uptake and translocation were investigated at heading and maturity. Cadmium did not affect plant growth and grain yield, but grain-Cd concentration always exceeded the permissible limit of 0.2 mg Cd/kg, and was approximately double in Svevo than in Creso. At maturity, total Cd-uptake increased linearly with supply in Creso, from 13 to 23 µg/plant, and was approximately 18 µg/plant, irrespective of Cd level, in Svevo. Partitioning to shoot was higher in Svevo than in Creso, both at heading and maturity. We suggest that reduced plant height, high root to shoot biomass ratio during vegetative growth and elevated post-heading dry matter accumulation promoted Cd accumulation into grain. Since these traits are common to modern wheat varieties, risks of grain Cd-contamination can increase in the future.

Keywords: food safety; genotype; cadmium contaminated soil; *Triticum durum*

Cadmium contamination of agricultural soils is expected to increase in the future, due to the prolonged application of Cd-containing phosphate fertilisers, and the rising use of waste waters and sewage sludge for irrigation and fertilisation (Kubo et al. 2008, Čásová et al. 2009).

Cadmium is rather mobile in soil and contaminates food and fodder crops even at low soil levels (Quinn et al. 2011). Since, in humans, prolonged exposure to Cd causes renal dysfunction, bone demineralisation, and increases the risk of lung cancer (Bernard 2008), the European food safety authority reduced the tolerable weekly intake of Cd from 7 to 2.5 µg Cd/kg body weight (Singh et al. 2011). Cereal products are estimated to account for approximately 40% of alimentary Cd in Europe (Greger and Landberg 2008), and figures will increase due to rising pasta consumption. Indeed, durum wheat, from which pasta is prepared, accumulates more Cd in grain than bread

wheat (Stolt et al. 2003, Greger and Löfsted 2004). The Codex alimentarius commission proposed a limit of 0.2 mg Cd/kg for wheat grain products, but higher Cd-concentrations were recorded in grain of wheat crops grown worldwide (Kubo et al. 2008, Adeniji et al. 2010).

Both in bread and durum wheat marked varietal differences were found in grain-Cd accumulation, suggesting a genetic control (Clarke et al. 1997, Stolt et al. 2003, Kubo et al. 2008). Since even small differences in grain Cd can lead to large differences in cumulative dietary intake, it is of high concern to individuate low accumulating varieties and to recognise varietal traits determining Cd uptake and translocation patterns. Physiological processes contributing to grain-Cd accumulation are: (i) uptake from soil; (ii) translocation to shoot, and (iii) remobilisation from culms and leaves to grain (Kubo et al. 2011). High and low grain-Cd accumulating lines were selected both in

bread and durum wheat, but a unique mechanism responsible for high or low accumulation has not been identified, yet. Greger and Landberg (2008) found a relationship between high accumulation and the exudation of organic acids that release Cd from soil particles, while Shenker et al. (2001) found that phytosiderophores ameliorated Cd mobilisation in soil, but not plant uptake. It was also hypothesised that low and high grain-Cd accumulation depends on differential Cd binding to organic acids in roots (Stolt et al. 2003, Adeniji et al. 2010). In bread wheat, high grain-Cd was associated with elevated Cd uptake during early growth, and with high root to shoot translocation, while in durum wheat it was associated with high translocation to the shoot, but not with high uptake (Zhang et al. 2002, Greger and Löfsted 2004, Kubo et al. 2011).

In a field screening carried out on Italian durum wheat varieties, Masoni et al. (2005) found that Cd concentration in grain was always higher in modern varieties than in older ones, though the values never exceeded permissible levels. Starting from this, we hypothesised that characters selected by breeders to increase yield, quality and mineral use efficiency (Guarda et al. 2004) can promote Cd accumulation into grain.

In the present research the varieties of *Triticum turgidum* L. var *durum* Creso and Svevo, selected from the field as the lowest and highest grain-Cd accumulating, were grown on Cd-spiked soil in order to assess (i) if varietal differences in grain-Cd accumulation are confirmed on Cd contaminated soils, and (ii) if Cd uptake and translocation patterns can be related to morphological and physiological traits of varieties.

MATERIAL AND METHODS

Growth conditions. The experiment was carried out at the Department of Agriculture, Food and Environment of the University of Pisa (Italy, 43°41'N, 10°23'E, 4 m a.s.l.). Cylindrical plastic pots (16 cm diameter, 40 cm height) were filled with 9 kg of loam soil, and placed outdoor. Physical and chemical properties of soil were 34% sand (2 mm > ϕ > 0.05 mm), 21% silt (0.05 mm > ϕ > 0.002 mm), 45% clay (ϕ < 0.002 mm), 7.2 pH, 2.2% organic matter (Walkley and Black method), 1.2 g/kg total N (Kjeldahl method), 33 mg/kg available P (Olsen method), 22 mg/kg available K (Dirks-

Sheffer method). Total Cd concentration in soil was measured by flame atomic absorption spectrometry (FAAS, GBC 903 Single Beam, Braeside, Australia) and was below detection threshold (< 0.05 mg/kg). Soil was spiked adding a distilled water solution of Cd(NO₃)₂, mixing thoroughly and allowing to age 15 days up to field capacity. Cadmium rates were calculated on soil dry weight basis. Pots were seeded on 1st February to obtain five seedlings per pot, corresponding to a field density of approximately 250 plants/m². NPK fertilizer was applied on soil dry weight basis at rates of 40.2 mg N/kg, 19 mg P/kg and 27.8 mg K/kg, corresponding to those conventionally used for durum wheat in central Italy (Arduini et al. 2006). In order to avoid water stress, after anthesis soil was watered regularly with tap water utilising a micro-irrigation system until field capacity was reached.

Treatments were set up with two durum wheat (*Triticum turgidum* L. var *durum*) varieties (Creso, released in 1974, and Svevo, released in 1997), four levels of Cd contamination (0 – control, 1.5, 3 and 4.5 mg Cd/kg soil dry weight), two harvest stages (heading and maturity), and two replicates (pots).

Measurements. Heading and maturity were achieved, respectively, on 5th May and 23th June in the variety Svevo (95 and 144 days after sowing); on 17th May and 30th June in Creso (107 and 151 days after sowing). Total rainfall during the growth period was 435 mm, 98% of which was distributed in 43 rainy days. Temperatures ranged between –4.8°C and 30.3°C, with average minimum and maximum temperatures of 6.6°C and 18.3°C, respectively.

At each harvest plants were cut at soil surface and aerial parts were separated into culms, leaves and spikes. Main culm height, the number of culms and spikes per pot, and the number of spikelets per spike were determined. At maturity, spikes were separated into grain and chaff, and the number of kernels per plant, per spike and per spikelet, and mean kernel weight were calculated. To gain roots, soil was gently washed under a continuous flow of tap water. All plant parts were oven dried at 65°C to constant weight for dry matter determination.

Cadmium analysis. Ground samples were digested in concentrated HNO₃ and HClO₄ at 220°C in an aluminium block digester (Tecator AB, Höganäs, Sweden). Cadmium concentration in roots, culms and leaves was determined by FAAS; that of spikes at heading, and grain and chaff at maturity, by graphite furnace AAS, because values were below the FAAS detection limit. Cadmium content was

Table 1. Plant height, biomass of separate parts, and partitioning to roots of durum wheat varieties at heading and maturity. Variety mean effect, $n = 8$

Variety	Plant height (cm)	Roots (g/plant)	Straw (g/plant)	Spikes (g/plant)	Plant (g/plant)	Root/plant (%)
Heading						
Creso	57.9 ^a	1.4 ^a	3.2 ^a	0.6 ^a	5.2 ^a	26.4 ^a
Svevo	45.6 ^b	1.4 ^a	1.9 ^b	0.4 ^a	3.7 ^b	37.8 ^b
Maturity						
Creso	65.1 ^a	0.7 ^a	4.0 ^a	3.1 ^a	7.8 ^a	9.1 ^a
Svevo	52.5 ^b	0.6 ^a	4.4 ^b	3.4 ^b	8.4 ^a	6.8 ^a

For each harvest stage, values in a column followed by the same letter are not significantly different at $P \leq 0.05$, Duncan's test

obtained by multiplying Cd concentration with dry matter. Cadmium partitioning was calculated as the Cd content of a given part in percentage of total Cd content.

Statistical analysis. Data collected at heading and maturity were analysed separately by means of ANOVA, arranging treatments in a complete randomised block design. Significantly different means were separated by the Duncan's multiple range test.

RESULTS AND DISCUSSION

Plant growth. According to ANOVA, the interaction between variety \times Cd supply was not significant for all growth parameters. Varieties differed in dry matter accumulation and parti-

Table 2. Grain yield and yield components of durum wheat varieties. Variety mean effect, $n = 8$

Variety	Grain (g/plant)	Spikelets (number/spike)	Kernels (number/spike)	Kernels (number/spikelet)	Mean kernel weight (mg)
Creso	2.0 ^a	15.1 ^b	17.6 ^a	2.1 ^a	45.0 ^b
Svevo	2.5 ^b	12.7 ^a	23.8 ^b	3.3 ^b	39.2 ^a

Values in a column followed by the same letter are not significantly different at $P \leq 0.05$, Duncan's test

tioning during life cycle, in grain yield and yield components, consistently with known varietal traits (Arduini et al. 2006). At heading plant dry weight was by 40% higher in Creso, due to higher biomass of straw and spikes, while at maturity, it was slightly higher in Svevo (Table 1). At both harvests, main culm height was approximately by 25% higher in the variety Creso. Post-heading increment of shoot biomass was 240% in Svevo and 87% in Creso, while root biomass approximately halved in both varieties. Accordingly, at heading roots represented 26% of total plant biomass in Creso and 38% in Svevo, while at maturity they accounted for approximately 8% in both. Grain yield was by 25% higher in Svevo, owing to higher number of kernels per spike and per spikelet (Table 2). Conversely, Creso showed higher number of spikelets per spike and mean kernel weight. Number of culms and spikes did not differ between varieties and were 3.5 and 2.7 per plant, respectively.

Varieties did not show any visible symptom of toxicity when grown on soil spiked with Cd ranging from 1.5 to 4.5 mg Cd/kg, and their growth was similarly affected. At heading, root biomass

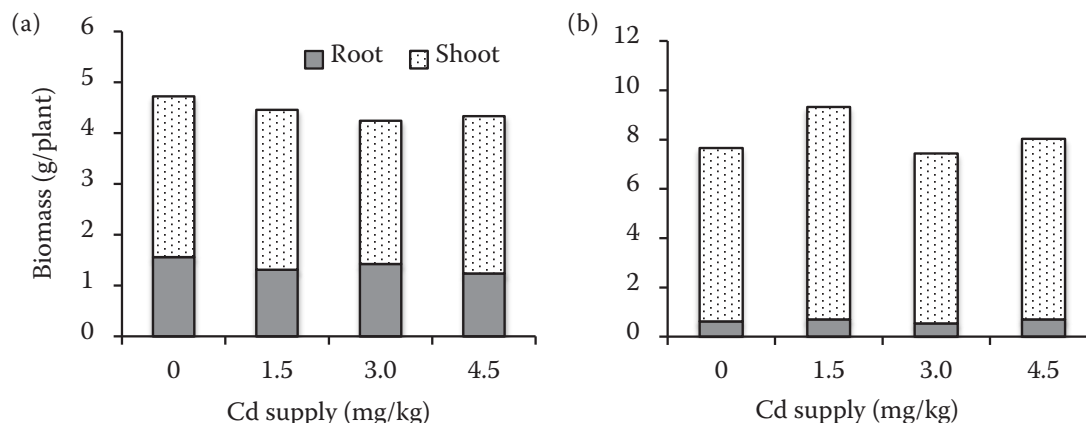


Figure 1. Shoot and root biomass of durum wheat at heading (a) and maturity (b) as affected by soil Cd. Variety mean effects, $n = 4$

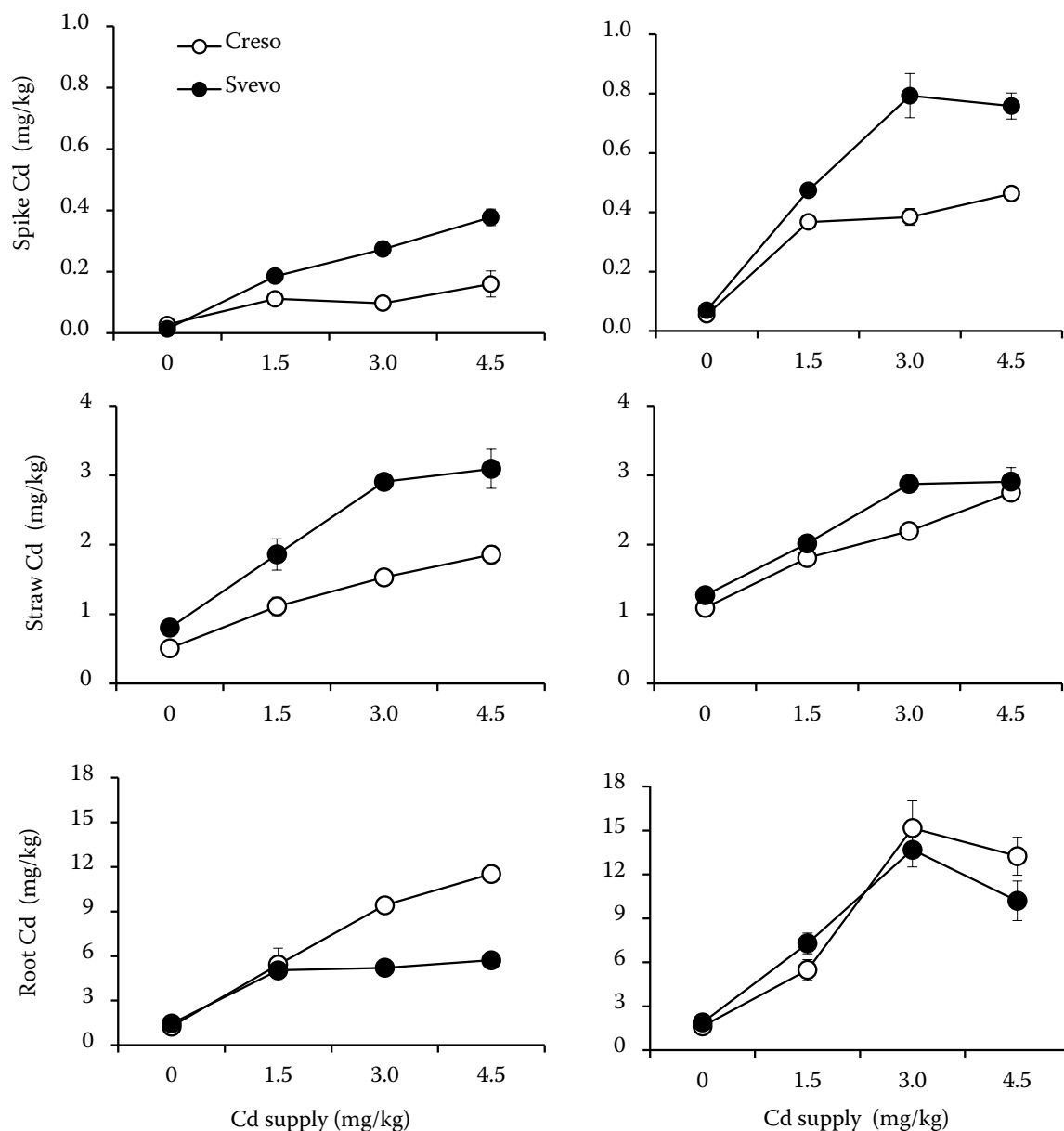


Figure 2. Cadmium concentration of separate plant parts in durum wheat varieties at heading (left) and maturity (right), as affected by soil Cd. Vertical bars denote standard error. Not visible bars lie within the symbol

was slightly decreased with all Cd supplies, while at maturity plant biomass was approximately by 20% higher with the lowest Cd level (Figure 1). Neither grain yield nor yield components were significantly affected.

Cadmium concentration. Plants grown on Cd spiked soil had a higher Cd concentration in all parts compared to controls, and values decreased in the order roots >> straw > spikes = grain (Figure 2). Cadmium concentration in grain always exceeded the regulatory limit (0.2 mg Cd/kg), and averaged over supplies it was by 58% higher in Svevo than in Creso (Table 3). Therefore, we classified the

variety Svevo as high grain-Cd accumulating and Creso as moderate accumulating.

Varieties responded differently to increasing Cd level, especially at heading. In Creso, Cd concentration increased almost linearly in roots and straw, but not in spikes, whereas in Svevo, it increased progressively in straw and spikes, while a plateau was observed in roots (Figure 2). In consequence at both harvests, the Cd concentration of roots did not differ between varieties with 1.5 mg Cd/kg and was higher in Creso with 3 and 4.5 mg Cd/kg. In contrast, the Cd-concentration of spikes was always higher in Svevo. In Creso, moderate grain-

Table 3. Grain Cd concentration and content, and partitioning of Cd to grain, in durum wheat varieties grown on Cd spiked soil. Partitioning of Cd to grain was calculated on plant basis, as the percentage ratio of grain Cd content on total plant Cd content. Variety and Cd supply mean effects, $n = 8$, $n = 4$, respectively

		Grain cadmium		Grain/plant (%)
		(mg/kg)	($\mu\text{g}/\text{plant}$)	
Variety	Creso	0.38 ^a	0.8 ^a	5.0 ^a
	Svevo	0.60 ^b	1.5 ^b	9.9 ^b
Cd supply (mg/kg)	0.0	0.05 ^a	0.1 ^a	1.9 ^a
	1.5	0.48 ^b	1.1 ^b	6.7 ^b
	3.0	0.73 ^c	1.6 ^c	8.8 ^b
	4.5	0.76 ^c	1.7 ^c	8.4 ^b

For each treatment, values in a column followed by the same letter are not significantly different at $P \leq 0.05$, Duncan's test

Cd accumulation seemed to be associated with high root retention (Adeniji et al. 2010), while, in Svevo, high accumulation could be due to active Cd translocation to shoot and grain, probably related to better mineral use efficiency (Pampana et al. 2007, Kubo et al. 2008).

Cadmium content and partitioning. Varieties differed in total Cd uptake: in Creso, Cd content rose from 10 to 21 $\mu\text{g}/\text{plant}$ at heading, and from 13 to 23 $\mu\text{g}/\text{plant}$ at maturity, while in Svevo values did not change significantly in response to Cd levels, being approximately 12 $\mu\text{g}/\text{plant}$ at heading and 18 at maturity (Figure 3). Conversely, Cd partitioning to grain was 5% in Creso and approximately 10% in Svevo, irrespective of supply (Table 3). Present results indicate that high grain-Cd accumulation of Svevo was not associated to high root uptake,

but to high translocation to the shoot, which is in agreement with Zhang et al. (2002) and Stolt et al. (2003). We suggest that also morphological traits of Creso, i.e. long culms and high spikelet number, could have reduced Cd partitioning to grain. Indeed, it was found that Cd translocation within the xylem is slowed down by binding to cell walls and by accumulation in the nodes, so that tall varieties with long spikes are less prone to accumulate Cd in grain (Kubo et al. 2008, Fujimaki et al. 2010, Quinn et al. 2011).

Similar to the findings of Rodda et al. (2011) in rice, most Cd uptake occurred before heading. At this stage, plants of Creso contained 80% of final Cd with the supply of 1.5 and 3 mg Cd/kg, and 90% with 4.5 mg Cd/kg, while those of Svevo approximately 70%. Post-heading Cd-uptake was markedly higher in Svevo than in Creso, 5.4 vs. 3.1 $\mu\text{g}/\text{plant}$ averaged over supplies, but corresponded to a rate of approximately 1 μg Cd per g of accumulated dry matter in both varieties. Post-heading Cd accumulation occurred in straw and spikes, while Cd content of roots decreased, maybe both because of Cd remobilization and decay (data not shown). As a consequence, Cd partitioning to shoots was markedly higher at maturity than at heading in both varieties (Table 4). With increasing Cd supply, partitioning to shoot was significantly higher in Svevo, which is consistent with a direct transport of Cd from root to grain during post-heading growth (Rodda et al. 2011). Since root is the primary sink for Cd, we suggest that high biomass partitioning to roots before heading could increase root to grain Cd remobilization (Chan and Hale 2004).

All summarized, high grain-Cd accumulation of Svevo can be related to the high allocation of biomass in roots during vegetative growth coupled

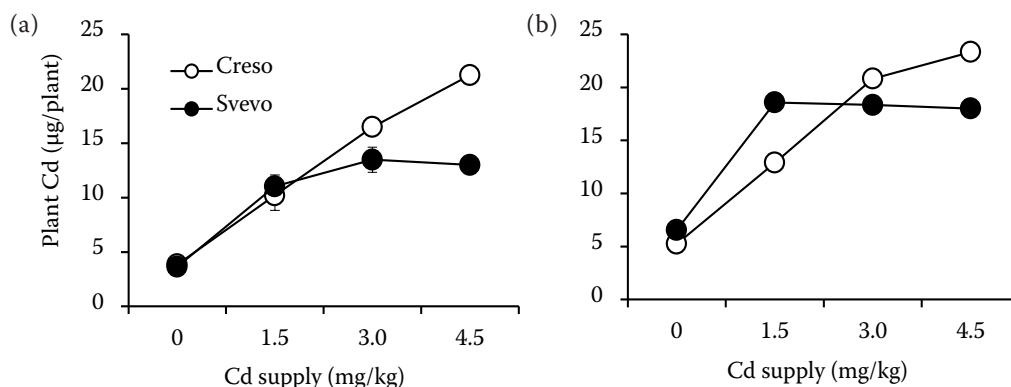


Figure 3. Cadmium uptake of durum wheat varieties at heading (a) and maturity (b) as affected by soil Cd. Vertical bars denote standard error. Not visible bars lie within the symbol

Table 4. Partitioning of Cd in roots, straw and spikes of durum wheat varieties at heading and maturity, as affected by soil Cd, $n = 2$

Variety	Cd supply (mg/kg)	Heading			Maturity		
		roots	straw	spikes	roots	straw	spikes
		(%)					
Creso	1.5	64.0 ^b	35.3 ^b	0.7 ^a	24.8 ^a	56.8 ^c	18.3 ^b
	3.0	72.6 ^c	27.0 ^a	0.4 ^a	52.1 ^c	35.4 ^a	12.5 ^a
	4.5	72.9 ^c	26.6 ^a	0.5 ^a	49.1 ^c	38.4 ^a	12.5 ^a
Svevo	1.5	64.6 ^b	34.7 ^b	0.6 ^a	31.1 ^b	55.1 ^c	13.9 ^a
	3.0	61.2 ^b	38.1 ^b	0.7 ^a	28.9 ^b	50.2 ^b	20.9 ^b
	4.5	49.7 ^a	49.0 ^c	1.2 ^b	30.8 ^b	47.7 ^b	21.5 ^b

Values in a column followed by the same letter are not significantly different at $P \leq 0.05$, Duncan's test

to high post-heading dry matter accumulation and root to grain remobilisation. High efficiency in mineral uptake during the entire crop cycle could also have contributed (Arduini et al. 2006, Pampana et al. 2007). Since morphological and physiological traits of Svevo are goals of breeding programs (Guarda et al. 2004, Uzik and Zofajova 2007), modern wheat varieties display higher tendency to accumulate Cd in grain.

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