Available online at www.sciencedirect.com
SCIENCE DIRECT®
Environmental and Experimental Botany 55 (2006) 29–40
Environmental and Experimental Botany
www.elsevier.com/locate/envexpbot

Response of miscanthus to toxic cadmium applications during the period of maximum growth

I. Arduini[a], L. Ercoli[b], M. Mariotti[c], A. Masoni[c,*]

[a] *Dipartimento di Scienze Botaniche, Università di Pisa, Via Luca Ghini 5, I-56126 Pisa, Italy*
[b] *Scuola Superiore Sant'Anna di Studi, Universitari e di Perfezionamento, Piazza Martiri della Libertà 33, I-56127 Pisa, Italy*
[c] *Dipartimento di Agronomia e Gestione dell'Agroecosistema, Università di Pisa, Via S. Michele degli Scalzi 2, I-56124 Pisa, Italy*

Accepted 15 September 2004

Abstract

Plants of miscanthus were grown in a Cd-free solution up to 1 month before heading and then were exposed to 0, 0.75, 1.5, 2.25 and 3 mg l^{-1} cadmium for 36 days. All cadmium levels were toxic to miscanthus. Growth response was not dose-dependent and two toxicity thresholds were identified: one between 0 and 0.75 mg l^{-1} Cd, the other between 2.25 and 3 mg l^{-1} Cd. The former caused a biomass decrease by about 50%, whereas the latter completely inhibited growth and disrupted the mechanisms that restricted Cd translocation to the shoot. Growth of the aerial part was affected by cadmium more than that of the hypogeal one. Cadmium did not change the N concentration of different plant parts, but markedly reduced the N uptake of the plant, the N net uptake rate (NUR) and the N net translocation rate (NTR) from the rhizome to the aerial part. These two indexes equalled zero when plants ceased to grow. Otherwise, the Cd-NUR increased with Cd supply and the Cd-NTR from rhizome to aerial part showed the highest increment when plants did not grow at all. This suggests different uptake pathways for the two elements, active for nitrogen and passive for cadmium. The Cd concentration and the Cd content markedly increased with all Cd levels, following the order roots $\gg$ rhizome > culms > leaves. The Cd concentration and the Cd content of aerial organs increased with Cd supply, but increments were highest between 2.25 and 3 mg l^{-1} Cd. The highest Cd concentrations were recorded in plants grown with 3 mg l^{-1} Cd and were 41 and 122 mg kg^{-1}, respectively, for the aerial and the hypogeal plant parts. The hypogeal plant part retained most of the cadmium taken up from solution, accounting for approximately 87% of total plant cadmium with the three lower Cd levels, and for 73% with the highest one. The maximum Cd content of the entire plant was achieved with the two higher Cd levels and was approximately 4.7 mg, while the Cd content of the aerial part was highest with 3 mg l^{-1} Cd (1.2 mg Cd per plant) and that of the hypogeal one with 2.25 mg l^{-1} Cd (4 mg Cd per plant). The highest aerial content achieved in this experiment was 10-fold that obtained in a previous research when small-sized plants were exposed to the same Cd level.

Keywords: Cadmium; Miscanthus; Cd-uptake; Phytoremediation; Toxicity

* Corresponding author.
E-mail address: amasoni@agr.unipi.it (A. Masoni).

0098-8472/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2004.09.009

1. Introduction

Disposal of industrial waste materials has been estimated to contaminate thousands of hectares of productive agricultural land throughout the world causing an increase in the soil concentrations of most metals (Naidu et al., 2001). Cadmium is a contaminant of high concern and in USA it is ranked the second metal in importance as environmental pollutant (Salt et al., 1998). Cadmium has an estimated half-life in soil varying between 15 and 1100 years and a relative high transfer coefficient from soil to plant, which increases the risk of its accumulation in the environment and its entry into the food chain (Vig et al., 2003). In the United Kingdom a progressive increase in cadmium concentration of soils has been demonstrated over the past 130 years, with the highest increase in the last 20 years (Jensen and Bro-Rasmussen, 1992).

In the last decade the use of plants for remediating Cd-contaminated soils has emerged as a promising, cost-effective and ecological-friendly alternative to conventional methods (Ebbs et al., 1997). In order to select species able to grow on Cd-contaminated soils and to extract the metal, research has been carried out both on hyperaccumulator plants (Brown et al., 1995; Robinson et al., 1998) and on crops (Salt et al., 1995; Peralta-Videa et al., 2002). Most known hyperaccumulators, however, have low biomass and/or slow growth rates, and are difficult to be grown and harvested (Brown et al., 1995; Sanità di Toppi et al., 2001). On the other hand, crops are generally sensitive to high cadmium levels and retain this metal mostly in roots (Smith et al., 1985; Salt et al., 1995; Gouia et al., 2000). In particular, cadmium is toxic to several physiological processes and especially to transpiration, that is essential for the uptake and translocation to the shoot of nutrients and of cadmium itself (Haag-Kerwer et al., 1999; Varga et al., 1999).

There is general acceptance that the root uptake of cadmium is a non-metabolic energy-independent mechanism that occurres with the aid of membrane carriers or even aqueous pores and is, therefore, concurrent with water inflow (Wagner, 1993; Siedlecka et al., 2001). Thus, the amount of cadmium absorbed by roots is independent from the uptake rate of nutrients and is determined only by the external concentration of cadmium and by the saturation of binding sites within the root (Salt et al., 1995; Maine et al., 2001). Internal transport to shoot, in contrast, is considered a Cd-sensitive process regulated by root content, by the rate of transpiration and by physiological barriers (Haag-Kerwer et al., 1999; Varga et al., 1999). At non-toxic levels the degree of Cd accumulation in the shoot is positively related to the rate of transpiration and to nutrient flow and is usually maintained at low and constant levels by transport-restriction mechanisms (Gouia et al., 2000; Kim et al., 2003), whereas at toxic levels it becomes a passive process similar to root uptake (Salt et al., 1995) and is, therefore, only dependent from root concentration and shoot biomass.

At present, the major limitations to the effective application of Cd phytoextraction to soil remediation are the low availability of cadmium in soil, generally about 5% of total content at neutral pH, and the low Cd translocation to the aboveground part of most species, mainly less than 20% of total plant content (Jarvis et al., 1976; Wagner, 1993; Salt et al., 1995). The addition of chelating agents and organic acids to soils was found to increase both the Cd availability for root uptake and the Cd transport to shoots (Gao et al., 2003; Madrid et al., 2003) but contrasting results were also reported, since the addition of EDTA to soil was found to increase for at least one month losses of contaminants and macronutrients through leaching (Wu et al., 2004). Besides, the addition of chelates to metal-polluted soils was found to have an adverse effect on plant growth and even survival, which decreased the biomass and the Cd concentration in aerial tissues, thus reducing the phytoextraction efficiency of the plant (Chen and Cutright, 2001; Sanità di Toppi et al., 2001). According to Salt et al. (1998), these drawbacks could be partly overcome applying the contaminated substratum or the chelating agent only when plants are near the end of the exponential phase of growth and harvesting them after a short accumulation phase, generally few days or weeks.

The identification of a safe and economically-profitable destination for the metal-enriched plant residues is considered a priority in phytoremediation. Therefore, special attention was devoted to non-food crops, i.e. bioenergy and industrial crops, that have fast growth rate and high biomass, such as willow, poplar, indian mustard and hemp (Salt et al., 1995; Punshon, 2001; Klang-Westin and Perttu, 2002; Linger et al., 2002; Pulford and Watson, 2003).

In order to assess the feasibility of miscanthus crops for the phytoremediation of Cd-contaminated waters

and sludges, we investigated the growth and Cd-uptake performance of plants of *Miscanthus sinensis* L. var. Giganteus grown in hydroponics. Miscanthus is a vigorous, high-yielding, perennial, Gramineae species, which reproduces vegetatively by rhizomes and is cultivated in Europe since the 1990s as energy crop (Ercoli et al., 1999).

Though results obtained with hydroponics-grown plants can not be directly referred to field conditions, they are crucial to highlight the physiological response of plants in controlled conditions. In previous investigations young, small-sized (about 20 cm tall) plants of miscanthus were exposed to cadmium in nutrient solution during the entire vegetative season. Miscanthus growth was significantly reduced with cadmium concentrations equal or higher than 0.75 mg l^{-1} and the amount of cadmium accumulated in the aerial part increased at increasing supply till the toxicity threshold and then progressively decreased with increasing supply (Arduini et al., 2003, 2004).

Aim of this work was to assess wether the application of toxic levels of cadmium to large-sized plants of miscanthus could ameliorate the uptake performance of this species. Four cadmium concentrations, comprised between 0.75 and 3 mg l^{-1}, were used. Taking into account previous knowledge on the growth patterns and the phenological development of miscanthus in hydroponics, Cd application was started approximately 1 month before heading. At this time, plants were near the end of their exponential phase of growth and, therefore, showed an elevated sink and a high uptake rate for elements. Moreover, their transpiration surface was highest and the reallocation of resources from culms and leaves to panicles and rhizome had not yet started. Plant growth and cadmium uptake and distribution patterns were analyzed in response to cadmium level. In order to try to elucidate how cadmium moves into and within the plant, the Cd uptake and translocation rates were compared with those of nitrogen, an essential element for plant nutrition.

2. Materials and methods

2.1. Plant material and growth conditions

Rhizomes of *Miscanthus sinensis* L. var. Giganteus (Greef and Deuter, 1993) were collected in the field from a 9-year-old crop, cut into pieces of about 20 g fresh weight and placed in pots filled with sand. Pots were placed in a greenhouse and regularly watered. About one month later (4th May), plants that were about 20 cm tall were transferred to an open-air hydroponics installation equipped for the nutrient film technique. The circulating nutrient solution had a volume of 80 l for each treatment and was arranged following Clark (1982) with slight modifications. Ion concentrations in the final solution (all the following expressed in mg l^{-1}) were: NO_3–N, 351; Ca, 302.4; K, 283; S, 192; NH_4–N, 128; Cl, 65; Mg, 37.8; Na, 4.56; Fe, 4; P, 2; Mn, 0.974; B, 0.536; Zn, 0.3; Cu, 0.076 and Mo, 0.155. In order to keep the concentration of the nutrient solution constant, evaporated and transpired water was continuously replaced with tap water and, every 2 weeks, the nutrient solution was completely renewed. The pH and conductivity were monitored at 2-day interval and they were 7.5 and 3.8 mS cm^{-1} throughout the whole experiment with only slight variations.

Plants were grown in the Cd-free nutrient solution for 81 days (24th July), then the metal was added to the solution as nitrate salt to final concentrations of 0 (control), 0.75, 1.5, 2.25 and 3 mg l^{-1} Cd. Each treatment (Cd level) was comprised of 16 plants spaced at 50-cm intervals, thus simulating a field density, which is commonly used for miscanthus crops in Italy (Ercoli et al., 1999).

Four plants per treatment were randomly harvested just before cadmium addition (0 days after treatment, DAT), and when control plants had achieved the heading stage (36 DAT).

2.2. Growth parameters

Harvested plants were divided into culms, green and dead leaves, rhizome and roots. Leaves were considered dead when about 90% of their sheet was dried up. The number of culms and green leaves was recorded. Plant height was determined as the length of the highest culm deprived from leaves. Roots were generously rinsed with tap water to remove Cd deposits. For dry weight determination, all plant parts were dried at 75 °C to constant weight. Previously, a fresh root sample had been taken from each plant for the estimation of root length, surface, volume and average diameter. Each root sample was divided into

three subsamples of about 1 g fresh weight that were weighed, cut into pieces of less than 10 cm, and distributed on a glass Petri dish of 20 cm diameter avoiding overlapping. By means of a Leica Quantimet 500 image analyser, we measured the length and the area of each root subsample, the former as the sum of the lengths of single root pieces, and the latter as the sum of the projections of single pieces on the Petri-dish surface. Then, the average diameter of each root system was estimated dividing total area by total length.

The root surface was estimated, after Kokko et al. (1993), as:

$$\text{Root surface} = \pi \times \text{root area}$$

Assuming that root systems are equivalent to cylinders, which diameter (d) corresponds to the root average diameter and which height (h) corresponds to total root length, root volume was estimated as:

$$\text{Root volume} = \pi \times \left(\frac{d}{2}\right)^2 \times h$$

2.3. Chemical analyses

Total nitrogen was determined by the microKjeldahl method (Bremnar, 1965) on dried plant parts ground to pass through a 40-mesh stainless steel screen. Cadmium concentration was determined by atomic absorption spectrometry (GBC 903 Single Beam, Australia) on ground samples (0.5 g) that were overnight predigested in 5 ml concentrated HNO_3, and then digested adding 1 ml $HClO_4$ at 220 °C in an aluminium block digestor (Tecator ab, Sweden).

2.4. Calculation of indexes and statistics

Following Engels (1993), the specific dry weight increment (SDWI) was used to quantify the mineral nutrient demand created by shoot growth that is imposed on each unit weight of the root system. It was calculated as:

$$\text{SDWI} = \frac{S_2 - S_1}{d} \times \frac{\ln(\text{Rs}_2/\text{Rs}_1)}{\text{Rs}_2 - \text{Rs}_1}$$

where S is the dry weight of the shoot, Rs the dry weight of the root system at the beginning (1) and at the end (2) of the treatment period and d is the duration of the period.

The net uptake rates (NUR) of N and Cd were determined following Engels (1993), as:

$$\text{NUR} = \frac{\text{Pc}_2 - \text{Pc}_1}{d} \times \frac{\ln(\text{Rs}_2/\text{Rs}_1)}{\text{Rs}_2 - \text{Rs}_1}$$

where Pc is the N or Cd, content of the whole plant, Rs the dry weight of the root system at the beginning (1) and at the end (2) of the treatment period, and d is the duration of the period.

The net translocation rates (NTR) were determined for the N or Cd, translocated from roots to rhizome and from rhizome to aerial part, as:

$$\text{NTR} = \frac{\text{Sn}_2 - \text{Sn}_1}{d} \times \frac{\ln(\text{So}_2/\text{So}_1)}{\text{So}_2 - \text{So}_1}$$

In the formula Sn is the N or Cd content of the sink, So the N, or Cd, content of the source, at the beginning (Sn_1 and So_1) and at the end (Sn_2 and So_2) of the treatment period, and d is the duration of the period.

Data were analyzed with analysis of variance techniques (randomized block design) to test effects of Cd

Table 1
Plant height, number of culms and number of green leaves of miscanthus plants treated for 36 days with different cadmium concentrations

DAT	Cadmium supply (mg l^{-1})	Plant height (cm)	Culm number (n per plant)	Green leaf number (n per plant)
0	0	45.2 ± 4.1 a	16 ± 1.6 a	42 ± 4.4 a
36	0	79.6 ± 8.8 c	28 ± 2.9 c	85 ± 7.9 c
	0.75	51.0 ± 4.7 b	22 ± 2.2 b	49 ± 4.1 b
	1.50	50.0 ± 5.0 ab	16 ± 1.4 a	42 ± 4.6 a
	2.25	48.8 ± 4.3 ab	16 ± 1.6 a	45 ± 4.3 ab
	3.00	45.2 ± 4.9 a	16 ± 1.4 a	22 ± 2.4 d

Data are means ± standard errors of four replicates. Values in each column followed by the same letter are not significantly different at $P \leq 0.05$ as determined by the Duncan's test.

Table 2
Growth of miscanthus plants treated for 36 days with different cadmium concentrations

DAT	Cadmium supply (mg l^{-1})	Dry weight (g per plant)						
		Culms	Leaves	Aerial part	Rhizome	Roots	Hypogeal part	Entire plant
0	0	15.3 ± 1.7 a	14.9 ± 1.6 a	30.2 ± 3.0 a	17.6 ± 1.8 a	10.1 ± 1.0 a	27.7 ± 2.9 a	57.9 ± 5.2 a
36	0	46.8 ± 4.9 d	42.0 ± 3.9 d	88.8 ± 7.7 d	50.8 ± 5.4 c	23.1 ± 2.3 c	73.9 ± 7.1 c	162.7 ± 16.6 c
	0.75	24.7 ± 2.4 c	26.4 ± 2.3 c	51.1 ± 4.5 c	33.3 ± 3.1 b	19.4 ± 1.9 c	52.7 ± 5.5 b	103.8 ± 10.1 b
	1.50	22.5 ± 2.1 bc	19.0 ± 1.7 b	41.5 ± 4.3 b	35.5 ± 3.3 b	13.8 ± 1.4 b	49.3 ± 5.1 b	90.8 ± 9.4 b
	2.25	20.8 ± 2.1 b	21.7 ± 2.2 b	42.5 ± 4.3 b	37.7 ± 3.7 b	10.7 ± 1.0 a	48.4 ± 4.8 b	90.9 ± 8.9 b
	3.00	15.3 ± 1.5 a	15.0 ± 1.6 a	30.3 ± 3.2 a	19.5 ± 2.0 a	8.4 ± 0.9 a	27.9 ± 2.7 a	58.2 ± 6.4 a

Data are means ± standard errors of four replicates. Values in each column followed by the same letter are not significantly different at $P \leq 0.05$ as determined by the Duncan's test.

level. Significantly different means were separated at 0.05 probability level by the least significant difference test (Steel et al., 1997).

3. Results

3.1. Plant growth

During the 36-day period of Cd treatment the culm height of control plants had increased by 76%, the number of culms per plant by 75% and the number of green leaves by 102% (Table 1). All cadmium levels drastically reduced miscanthus growth, so that the emission of new culms and leaves was completely inhibited with 1.5 mg l^{-1} Cd and culm elongation with 3 mg l^{-1} Cd. Besides, a marked increase of leaf senescence occurred with the highest Cd concentration (Table 1). As a consequence, the biomass of Cd-treated plants was much lower than that of controls at the end of the treatment period and no biomass production was recorded at all in culms, leaves and rhizome treated with 3 mg l^{-1} Cd, and in roots treated with 2.25 mg l^{-1} Cd (Table 2). Compared to controls, the lowest Cd application, 0.75 mg l^{-1}, decreased the dry weight of all plant parts except roots, with decrements ranging from 34% (rhizome) to 47% (culms). With the further increase of Cd concentration, the dry weight of culms and rhizome did not change greatly up to 2.25 mg l^{-1} and then decreased again, that of leaves decreased between the two lower and the two higher Cd concentrations and that of roots decreased progressively with increasing Cd level. The specific dry weight increment (SDWI) was decreased approximately by 74% up to 2.25 mg l^{-1} Cd and was equal zero with the highest Cd level, indicating that shoot growth was affected by Cd more than root growth (Fig. 1).

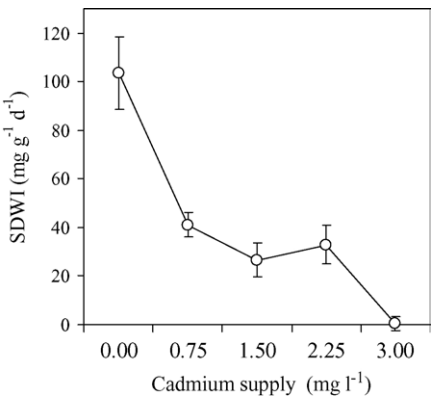

Fig. 1. Specific dry weight increment of miscanthus plants treated for 36 days with different cadmium concentrations. Bars represent standard errors of four replicates.

3.2. Root morphology

The development of the root system was greatly affected by all cadmium levels. During the treatment period, the length, the surface and the volume of roots increased only in control plants and in those treated with 0.75 mg l^{-1} Cd (Table 3). Thus, at the end of experiment, above root-size parameters were lower in Cd-treated roots than in controls. Decrements were by 46, 34 and 20% for root length, surface and volume with 0.75 mg l^{-1} Cd, and approximately by 70, 67 and 64% with the other Cd levels. The average diameter of roots

Table 3
Length, average diameter, surface and volume of the roots of miscanthus plants treated for 36 days with different cadmium concentrations

DAT	Cadmium supply ($mg\,l^{-1}$)	Root			
		Length (m per plant)	Diameter (mm)	Surface (dm^2 per plant)	Volume (cm^3 per plant)
0	0	209.5 ± 21.5 a	1.1 ± 0.10 a	72.4 ± 8.3 a	199.1 ± 15.7 a
36	0	557.5 ± 50.2 d	1.2 ± 0.11 a	203.4 ± 22.4 c	590.5 ± 52.6 b
	0.75	303.5 ± 30.0 c	1.2 ± 0.12 a	134.0 ± 12.7 b	471.0 ± 45.2 b
	1.50	212.1 ± 21.4 a	1.2 ± 0.12 a	79.4 ± 9.5 a	236.5 ± 24.4 a
	2.25	128.6 ± 13.2 b	1.3 ± 0.13 b	52.5 ± 5.1 a	170.9 ± 17.9 a
	3.00	169.7 ± 17.6 ab	1.3 ± 0.13 b	70.3 ± 6.5 a	231.9 ± 22.3 a

Data are means ± standard errors of four replicates. Values in each column followed by the same letter are not significantly different at $P \leq 0.05$ as determined by the Duncan's test.

was unchanged up to $1.5\,mg\,l^{-1}$ Cd and slightly increased with the two highest Cd levels.

3.3. Nitrogen uptake

The nitrogen concentration of all plant parts was not affected by cadmium and, averaged over all Cd levels, it was $25\,g\,kg^{-1}$ for the aerial part and $33\,g\,kg^{-1}$ for the hypogeal one. In contrast, we recorded marked decrements in the nitrogen content of different plant parts in response to cadmium, that were, therefore, exclusively due to the negative effect of cadmium on growth (data not reported).

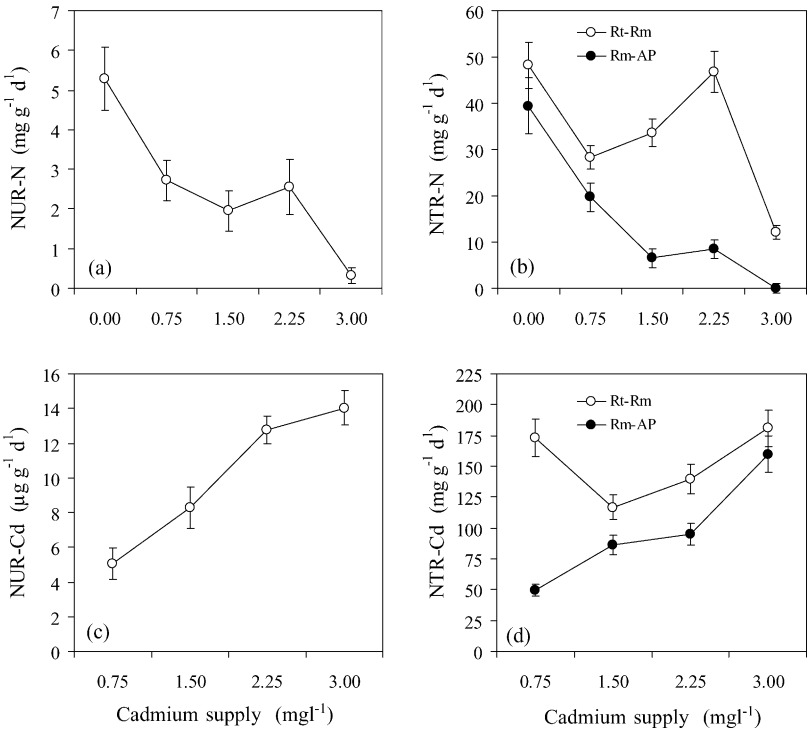

Fig. 2. Net uptake rates of nitrogen (a) and of cadmium (c) and net translocation rates of nitrogen (b) and of cadmium (d) from roots to rhizome (Rt–Rm) and from rhizome to aerial part (Rm–AP) of miscanthus plants treated for 36 days with different cadmium concentrations. Bars represent standard errors of four replicates.

Table 4
Nitrogen uptake of miscanthus plants during a 36-day treatment with different cadmium concentrations

Cadmium supply (mg l^{-1})	Nitrogen uptake (mg per plant)						
	Culms	Leaves	Aerial part	Rhizome	Roots	Hypogeal part	Entire plant
0	711.5 ± 83.0 a	624.2 ± 65.3 a	1336.0 ± 112.0 a	1044.0 ± 108.0 a	605.9 ± 71.6 a	1650.0 ± 153.0 a	2985.0 ± 272.0 a
0.75	265.8 ± 31.2 b	259.3 ± 30.9 b	525.1 ± 47.8 b	512.2 ± 49.0 b	353.6 ± 29.3 b	865.8 ± 91.2 b	1391.0 ± 115.0 b
1.50	158.8 ± 12.7 c	15.7 ± 5.1 d	174.5 ± 15.5 c	505.6 ± 52.4 b	150.2 ± 13.1 c	655.8 ± 77.8 b	830.3 ± 79.8 c
2.25	144.3 ± 15.1 c	96.4 ± 13.2 c	240.7 ± 17.1 c	644.9 ± 56.1 b	72.7 ± 14.5 d	717.6 ± 62.2 b	958.4 ± 81.3 c
3.00	24.2 ± 3.5 d	−22.8 ± 2.2 e	1.4 ± 1.0 d	142.9 ± 21.2 c	−38.0 ± 3.5 e	104.9 ± 12.7 c	106.3 ± 11.1 d

Data are means ± standard errors of four replicates. Values in each column followed by the same letter are not significantly different at $P \leq 0.05$ as determined by the Duncan's test.

The total N-uptake of miscanthus during the treatment period was 3 g per plant in controls, 1.4 g per plant with 0.75 mg l^{-1} Cd, approximately 0.9 g per plant with cadmium up to 2.25 mg l^{-1} and only 0.1 g per plant with 3 mg l^{-1} Cd (Table 4). At this Cd concentration the accumulation of nitrogen in the aerial part was negligible and that of roots and leaves was even lower than at the beginning of treatment.

Compared to control values, the net uptake rate of nitrogen decreased approximately by 55% with the Cd concentrations comprised between 0.75 and 2.25 mg l^{-1} and it was near zero with the highest Cd level (Fig. 2a). The net translocation rate of nitrogen from roots to rhizome decreased by 42% with the lowest Cd level, progressively increased up to control values with the increase of Cd concentration up to 2.25 mg l^{-1} and then drastically decreased again with the highest Cd level (Fig. 2b). In contrast, the NTR from rhizome to aerial part sharply decreased up to 1.5 mg l^{-1} Cd, was unchanged between this level and 2.25 mg l^{-1} Cd and then dropped to 0 with 3 mg l^{-1} Cd (Fig. 2b).

3.4. Cadmium uptake and distribution

No cadmium was recorded in any plant part before Cd application and in controls at the end of the treatment period (data not reported). With all Cd levels the Cd concentration markedly increased in miscanthus following the order roots ≫ rhizome > culms > leaves (Table 5). The Cd concentration of culms and leaves increased with the increase of cadmium in solution. In contrast, that of the rhizome did not increase between 0.75 and 1.5 mg l^{-1} Cd and then increased with Cd level, and that of roots increased according to supply up to 2.25 mg l^{-1} Cd and then was unchanged. The increase in the Cd concentration of culms, leaves and rhizome was not proportional to increased availability but was by far higher between 2.25 and 3 mg l^{-1} Cd than between the lower Cd levels. In consequence, the difference between the Cd concentration of roots and that of the other plant parts increased with Cd supply up to 2.25 mg l^{-1} Cd and then decreased markedly. The Cd concentration of the aerial and the hypogeal plant parts and that of the entire plant increased with supply and, again, increments were higher between the two highest Cd levels. Thus, in miscanthus, the highest Cd concentrations were recorded with 3 mg l^{-1} Cd and

Table 5
Cadmium concentration and content of miscanthus plants treated for 36 days with different cadmium concentrations

Cadmium supply (mg l^{-1})	Culms	Leaves	Aerial part	Rhizome	Roots	Hypogeal part	Entire plant
Cadmium concentration (mg kg^{-1})							
0.75	7.7 ± 0.9 a	3.2 ± 0.3 a	5.4 ± 0.6 a	32.4 ± 3.1 a	63.5 ± 6.4 a	43.8 ± 4.1 a	24.9 ± 2.5 a
1.50	16.3 ± 1.6 b	6.1 ± 0.6 ab	11.6 ± 1.4 b	30.5 ± 3.1 a	141.8 ± 14.9 b	61.6 ± 6.2 b	38.8 ± 3.6 b
2.25	27.5 ± 2.7 c	7.5 ± 0.8 b	17.3 ± 1.8 c	41.9 ± 4.6 b	229.4 ± 24.3 c	83.4 ± 10.0 c	52.5 ± 5.4 c
3.00	56.4 ± 5.8 d	24.9 ± 2.3 c	40.8 ± 4.1 d	81.0 ± 8.0 c	218.1 ± 23.1 c	122.1 ± 11.7 d	79.8 ± 7.8 d
Cadmium content (μg per plant)							
0.75	190.1 ± 20.3 a	85.0 ± 9.0 a	275.1 ± 28.6 a	1078 ± 109 a	1229 ± 129 a	2307 ± 213 a	2582 ± 274 a
1.50	366.6 ± 37.4 b	115.6 ± 10.3 a	482.3 ± 43.4 b	1084 ± 113 a	1956 ± 215 b	3040 ± 325 b	3522 ± 363 b
2.25	571.3 ± 52.0 c	162.8 ± 16.3 b	734.1 ± 74.9 c	1579 ± 152 b	2454 ± 260 c	4034 ± 355 c	4768 ± 491 c
3.00	862.9 ± 88.9 d	374.0 ± 35.5 c	1237.0 ± 123 d	1583 ± 165 b	1862 ± 179 b	3409 ± 348 b	4646 ± 511 c

Data are means ± standard errors of four replicates. For both measured parameters values in each column followed by the same letter are not significantly different at $P \leq 0.05$ as determined by the Duncan's test.

were 41 and 122 mg kg^{-1}, respectively, for the aerial and the hypogeal plant parts (Table 5).

The cadmium content of the different parts of the miscanthus plant was in the order roots > rhizome ≫ culms > leaves with all Cd levels (Table 5). In culms and leaves the Cd content increased progressively with increasing supply and the highest increments were recorded between 2.25 and 3 mg l^{-1} Cd. The cadmium content of the rhizome increased only between 0 and 0.75 mg l^{-1} Cd and between 1.5 and 2.25 mg l^{-1} Cd, whereas that of roots increased linearly up to 2.25 mg l^{-1} Cd and then decreased with 3 mg l^{-1} Cd to the value recorded with 1.5 mg l^{-1} Cd. The hypogeal plant part retained most of the cadmium taken up from solution, accounting for approximately 87% of total plant cadmium with the three lower Cd levels and for 73% with the highest one. Thus, the maximum Cd content of the entire plant was achieved with the two higher Cd levels and was approximately 4.7 mg, while the Cd content of the aerial part was highest with 3 mg l^{-1} Cd (1.2 mg Cd per plant) and that of the hypogeal one with 2.25 mg l^{-1} Cd (4 mg Cd per plant) (Table 5).

The net uptake rate of cadmium increased from 5 to 14 μg Cd g^{-1} d^{-1} with increasing Cd supply (Fig. 2c). The net translocation rate of cadmium drastically decreased when the Cd concentration in solution increased from 0.75 to 1.5 mg l^{-1} and then increased progressively up to the value recorded with the lowest supply (Fig. 2d). In contrast, the NTR from the rhizome to the aerial part increased with supply and the highest increment was recorded between 2.25 and 3 mg l^{-1} Cd.

4. Discussion

Growth patterns of miscanthus indicate that this species does not express a dose-dependent response to cadmium and suggest the existence of two distinct toxicity thresholds, the lower at 0.75 mg l^{-1} Cd and the higher at 3 mg l^{-1} Cd. Indeed, biomass production is decreased by about 50% with cadmium concentrations ranging from 0.75 to 2.25 mg l^{-1} and ceases completely with 3 mg l^{-1} Cd (Table 2). Considering also results obtained on miscanthus previously, the former toxicity threshold could be set more exactly between 0.5 and 0.75 mg l^{-1} Cd (Arduini et al., 2004), whereas the latter between 2.25 and 3 mg l^{-1} Cd. The complete inhibition of growth was also observed in plants of miscanthus exposed to 3 mg l^{-1} Cd for the entire vegetative season (Arduini et al., 2003), which confirms that this species is not able to effectively adapt to this level of cadmium. Although our results indicate that miscanthus is sensitive to cadmium concentrations higher than 0.5 mg l^{-1} in the nutrient solution, a similar degree of sensitivity was found by Landberg and Greger (1994) in willow clones and by Salt et al. (1995) in *Brassica juncea*, plants that are both considered suitable for the phytoextraction of cadmium. Moreover, in the present investigation, all Cd levels dramatically reduce root growth (Table 3), but do not cause the marked root thickenings and the changes in lateral root development that are commonly reported in response to cadmium stress (Punz and Sieghardt, 1993), and that we ourselves described in miscanthus plants exposed to cadmium for over three months (Arduini et al., 2003, 2004).

Growth of all plant parts was reduced with all cadmium levels and, similar to the findings of Gussarsson (1994) and Kim et al. (2003), the decrease was higher for the aerial part than for the hypogeal one (Tables 1 and 2). It is noteworthy, however, that the Cd concentrations we recorded in culms and leaves up to 2.25 mg l^{-1} Cd were consistent with those found in miscanthus and in other crop species that did not show any Cd injury (Table 5) (Jarvis et al., 1976; Arduini et al., 2004). In our opinion, at these Cd levels, the negative effect of Cd on shoot growth is probably caused indirectly by the reduced functioning of roots, which Cd concentration is more than 10-fold higher than that of the shoot. A reduced efficiency of the root system is also suggested by the SDWI, which values indicate that the production of each unit of shoot biomass is supported by a greater root biomass in Cd-treated plants than in controls (Fig. 1). Cadmium has been found to alter directly the trans-membrane potential of root cells, thus decreasing both the active uptake of nutrients and water and their transport into the xylem (Kennedy and Gonsalves, 1987; Aidid and Okamoto, 1992; Hernández et al., 1998). Indeed, the dramatic decrease of transpiration flow is widely recognized as a primar symptom of cadmium stress (Haag-Kerwer et al., 1999; Varga et al., 1999; Gouia et al., 2000) and several authors report that even low Cd concentrations decrease the root uptake and/or the translocation to the shoot of many essential nutrients such as calcium, iron, magnesium, manganese, potassium and zinc (Smith et al., 1985; Greger and Lindberg, 1987; Gussarsson, 1994; Brown et al., 1995; Arduini et al., 1998; Hernández et al., 1998; Varga et al., 1999; Kim et al., 2003). In the present work, we found that cadmium did not change significantly the N concentration of different plant parts, which values were consistent with those recorded in control plants and in field grown crops (Ercoli et al., 1999), but reduced dramatically the total uptake of nitrogen and, in particular, its allocation in the shoot (Fig. 2a, Table 4). Besides, with the highest Cd supply a re-translocation of nitrogen from leaves to culms probably occurred, which could be a consequence of the enhanced leaf senescence. Between 0.75 and 2.25 mg l^{-1} Cd the N-NTRs from roots to rhizome and from this to the aerial part show opposite trends suggesting that N accumulation occurs in the rhizome, and this could be either consequence of the reduced request of nitrogen by the shoot or of reduced transpiration (Fig. 2b). Both hypotheses are confirmed by Gouia et al. (2000) who found that cadmium drastically decreased the nitrate uptake of *Phaseolus vulgaris* plants and also reduced the transpiration rate and the nitrogen assimilation in leaves.

We observed that the N-NUR decreased accordingly to the decrease of biomass production and dropped to zero when plants ceased to grow, whereas the Cd-NUR was independent of growth patterns and increased almost linearly with the increase of cadmium supply (Fig. 2a,c). These contrasting patterns are consistent with the hypothesis that the uptake of the essential element nitrogen is governed by metabolic mechanisms and is therefore driven by plant growth, whereas that of cadmium, a non-essential element, mainly depends on its external concentration. Above trends also suggest that the two elements have different uptake pathways, active for nitrogen and passive for cadmium.

The cadmium distribution within miscanthus confirm the hypothesis of the two distinct toxicity thresholds already evidenced by growth patterns. Above the lower (0.75 mg l^{-1} Cd), root efficiency and, consequently, growth of all plant parts are reduced, but the mechanisms that retain cadmium in roots are not disrupted, so that the Cd concentration in roots remains more than ten times higher than that of the aerial part (Table 5). In agreement with Schneider et al. (1999) we suggest that the endodermis acts as an effective barrier to the diffusion of cadmium into the central cylinder and consequently to its loading into xylem vessels. The slight increase of the aerial Cd concentration between 0.75 and 2.25 mg l^{-1} Cd is proportional to that occurred in roots and may be attributed to cadmium leakage across non-suberized areas of the endodermis (Hardiman et al., 1984). The higher toxicity threshold (3 mg l^{-1} Cd), in contrast, causes the breakdown of the endodermis barrier so that the Cd concentration drastically increases in the rhizome, in culms and in leaves that show enhanced senescence (Table 5, Fig. 2d). In parallel, the cadmium concentration of roots slightly decreases suggesting that Cd translocation is not counterbalanced by new Cd uptake from solution, probably because roots are severely damaged and die, which was also observed by Maine et al. (2001) in *Pistia stratiotes*. Thus, between 0 and 3 mg l^{-1} Cd, the curve of the Cd concentration in the aerial part has a biphasic shape, similarly to those described by Salt et al. (1995) in *Brassica juncea* and by Kim et al. (2003) in *Pinus*

sylvestris. Indeed, the Cd concentration of the aerial part increases with the increase of cadmium in solution up to the first toxicity threshold, then it decreases slightly or keeps constant up to the second one, due to reduced shoot growth and transpiration, and then increases again for the brakedown of restriction barriers (Arduini et al., 2003, 2004). This last increment of both the rhizome and the aerial Cd-concentrations, is essentially due to the massive flow of cadmium into these tissues and probably ceases with the saturation of Cd-binding sites.

Taking into account both literature and our results, we suggest that the total amount of cadmium that miscanthus is able to accumulate in the aerial part results from several factors among which the most important are the aerial biomass, the transpiration flow and the endodermis barrier. These factors are both synergic and antagonistic and each one contributes differently to the whole Cd-uptake process at toxic and non-toxic conditions. Since the disruption of restriction mechanisms generally coincides with a severe inhibition of growth and transpiration, it is often reported that both the total amount of cadmium accumulated in aerial tissues and the Cd-partitioning between the aerial and the hypogeal plant parts decrease at extreme toxic conditions (Hardiman et al., 1984; Brown et al., 1995; Arduini et al., 2003). In the present work, in contrast, the Cd content progressively increases with supply, both in the rhizome and in the aerial part, and is highest with the Cd level that completely stops growth (Table 5). We think that, in our investigation, the high initial biomass of plants and the large transpiration surface allowed the rapid diffusion of cadmium just after the breakdown of restriction barriers.

5. Conclusion

In conclusion, we obtained a cadmium content of the aerial part of 1.2 mg per plant by exposing large-sized plants of miscanthus to 3 mg l^{-1} Cd for 36 days in the period between the end of the exponential stage of growth and heading. This value is consistent with that achieved with long-term applications of cadmium concentrations below the toxicity threshold (0.5 mg l^{-1} Cd) (Arduini et al., 2004) but is 10-fold that obtained when small-sized plants of miscanthus were exposed to 3 mg l^{-1} Cd for the entire vegetative season (Arduini et al., 2003). Considering to achieve similar results with field cultivations, it would be possible to remove approximately 50 g ha^{-1} Cd by harvesting only the aerial plant part and 100 g ha^{-1} Cd when rhizomes are harvested too. Above values are consistent with those achieved by Linger et al. (2002) with *Cannabis sativa* and by far exceed those reported in Pulford and Watson (2003) with *Salix*.

We are fully aware that results obtained in culture solution can not be directly referred to field conditions, first of all because there is no correspondence between the Cd levels that cause a given effect on plants in hydroponics and in soil. Nevertheless, we think it reasonable that the changes in plant physiological processes in response to toxic and non-toxic Cd levels are the same independent of substratum. Therefore, taking into account both present results and those previously obtained, we suggest that even Cd-sensitive species like miscanthus could effectively accumulate cadmium in harvestable plant parts when the Cd-contaminated substratum, or the chelates that mobilize the metal, are applied to large-sized plants. This would allow to widen considerably the range of species suitable for Cd phytoextraction, since Cd-sensitive crops could be effectively used besides Cd-tolerant hyperaccumulators.

References

Aidid, S.B., Okamoto, H., 1992. Effects of lead, cadmium and zinc on the electric membrane potential at the xylem/symplast interface and cell elongation of *Impatiens balsamina*. Environ. Exp. Bot. 32, 439–448.

Arduini, I., Godbold, D.L., Onnis, A., Stefani, A., 1998. Heavy metals influence mineral nutrition of tree seedlings. Chemosphere 36, 739–744.

Arduini, I., Masoni, A., Ercoli, L., Mariotti, M., 2003. Growth and cadmium uptake of *Miscanthus sinensis* as affected by cadmium. Agricoltura Mediterranea 133, 169–178.

Arduini, I., Masoni, A., Mariotti, M., Ercoli, L., 2004. Low cadmium application increase miscanthus growth and cadmium translocation. Environ. Exp. Bot. 52, 89–100.

Bremnar, T.M., et al., 1965. Total nitrogen. In: Black, C.A. (Ed.), Methods of Soil Analysis. Part 2. Agron. Monogr. 9, first ed. ASA and SSSA, Madison, WI, pp. 1149–1179.

Brown, S.L., Chaney, R.L., Angle, J.S., Baker, A.J.M., 1995. Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. Soil Sci. Soc. Am. J. 59, 125–133.

Chen, H., Cutright, T., 2001. EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. Chemosphere 45, 21–28.

Clark, R.B., 1982. Nutrient solution growth of sorghum and corn in mineral nutrition studies. J. Plant Nutr. 5, 1039–1057.

Ebbs, S.D., Lasat, M.M., Brady, D.J., Cornish, J., Gordon, R., Kochian, L.V., 1997. Phytoextraction of cadmium and zinc from a contaminated soil. J. Environ. Qual. 26, 1424–1430.

Engels, C., 1993. Differences between maize and wheat in growth-related nutrient demand and uptake of potassium and phosphorus at suboptimal root zone temperatures. Plant Soil 150, 129–138.

Ercoli, L., Mariotti, M., Masoni, A., Bonari, E., 1999. Effect of irrigation and nitrogen fertilization on biomass yield and efficiency of energy use in crop production of *Miscanthus*. Field Crops Res. 63, 3–11.

Gao, Y., He, J., Ling, W., Hu, H., Liu, F., 2003. Effects of organic acids on copper and cadmium desorption from contaminated soils. Environ. Int. 29, 613–618.

Gouia, H., Ghorbal, M.H., Meyer, C., 2000. Effects of cadmium on activity of nitrate reductase and on other enzymes of the nitrate assimilation pathway in bean. Plant Physiol. Biochem. 38, 629–638.

Greef, M.J., Deuter, M., 1993. Syntaxonomy of *Miscanthus* x *giganteus*. Angew. Bot. 67, 87–90.

Greger, M., Lindberg, S., 1987. Effects of Cd^{2+} and EDTA on young sugar beets (*Beta vulgaris*). II. Net uptake and distribution of Mg^{2+}, Ca^{2+} and Fe^{2+}/Fe^{3+}. Physiol. Plant 69, 81–86.

Gussarsson, M., 1994. Cadmium-induced alterations in nutrient composition and growth of *Betula pendula* seedlings: the significance of fine roots as a primary target for cadmium toxicity. J. Plant Nutr. 17, 2151–2163.

Haag-Kerwer, A., Schäfer, H.J., Heiss, S., Walter, C., Rausch, T., 1999. Cadmium exposure in *Brassica juncea* causes a decline in transpiration rate and leaf expansion without effect on photosynthesis. J. Exp. Bot. 50, 1827–1835.

Hardiman, R.T., Jacoby, B., Banin, A., 1984. Factors affecting the distribution of cadmium, copper and lead and their effect upon yield and zinc content in bush beans (*Phaseolus vulgaris* L.). Plant Soil 81, 17–27.

Hernández, L.E., Lozano-Rodríguez, E., Garáte, A., Carpena-Ruiz, R., 1998. Influence of cadmium on the uptake, tissue accumulation and subcellular distribution of manganese in pea seedlings. Plant Sci. 132, 139–151.

Jarvis, S.C., Jones, H.P., Hopper, M.J., 1976. Cadmium uptake from solution by plants and its transport from roots to shoots. Plant Soil 44, 179–191.

Jensen, A., Bro-Rasmussen, F., 1992. Environmental cadmium in Europe. Rev. Environ. Contam. Toxicol. 125, 101–181.

Kim, C.G., Bell, J.N.B., Power, S.A., 2003. Effects of soil cadmium on *Pinus sylvestris* L. seedlings. Plant Soil 257, 443–449.

Kennedy, C.D., Gonsalves, F.A.N., 1987. The action of divalent zinc, cadmium, mercury, copper and lead on the trans-root potential and H^+ efflux of excised roots. J. Exp. Bot. 38, 800–817.

Klang-Westin, E., Perttu, K., 2002. Effects of nutrient supply and soil cadmium concentration on cadmium removal by willow. Biomass Bioenerg. 23, 415–426.

Kokko, E.G., Volkmar, K.M., Gowen, B.E., Entz, T., 1993. Determination of total root surface area in soil core samples by image analysis. Soil Tillage Res. 26, 33–43.

Landberg, T., Greger, M., 1994. Can heavy metal tolerance clones of Salix be used as vegetation filters on heavy metal contaminated land? In: Aronsson, P., Perttu, K. (Eds.), Willow Vegetation Filters for Municipal Wastewaters and Sludges: A Biological Purification System. Uppsala, ISBN 91-576-4916-2, 5–10 June 1994, pp. 145–151.

Linger, P., Müssig, J., Fischer, H., Kobert, J., 2002. Industrial hemp (*Cannabis sativa* L.) growing on heavy metal contamined soil: fibre quality and phytoremediation potential. Ind. Crops Prod. 16, 33–42.

Madrid, F., Liphadzi, M.S., Kirkham, M.B., 2003. Heavy metal displacement in chelate-irrigated soil during phytoremediation. J. Hydrol. 272, 107–119.

Maine, M.A., Duarte, M.V., Suñe, N.L., 2001. Cadmium uptake by floating macrophytes. Wat. Res. 35, 2629–2634.

Naidu, R., Krishnamurti, G.S.R., Wenzel, W., Megharaj, M., Bolan, N.S., 2001. Heavy metal interactions in soils and implications for soil microbial biodiversity. In: Prasad, M.N.V. (Ed.), Metals in the Environment: Analysis by Biodiversity. Marcel Dekker Inc., New York, pp. 401–431.

Peralta-Videa, J.R., Gardea-Torresdey, J.L., Gomez, E., Tiemann, K.J., Parsons, J.G., Carrillo, G., 2002. Effect of mixed cadmium, copper, nickel, and zinc at different pHs upon alfalfa growth and heavy metal uptake. Environ. Pollut. 119, 291–301.

Pulford, I.D., Watson, C., 2003. Phytoremediation of heavy metal-contaminated land by trees—a review. Eviron. Int. 29, 529–540.

Punshon, T., 2001. Tree crops. In: Prasad, M.N.V. (Ed.), Metals in the Environment: Analysis by Biodiversity. Marcel Dekker Inc., New York, pp. 321–351.

Punz, W.F., Sieghardt, H., 1993. The response of roots of herbaceous plant species to heavy metals. Environ. Exp. Bot. 33, 85–98.

Robinson, B.H., Leblanc, M., Petit, D., Brooks, R.R., Kirkman, J.H., Gregg, P.E.H., 1998. The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. Plant Soil 203, 47–56.

Salt, D.E., Prince, R.C., Pickering, I.J., Raskin, I., 1995. Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol. 109, 1427–1433.

Salt, D.E., Smith, R.D., Raskin, I., 1998. Phytoremediation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 643–668.

Sanità di Toppi, L., Favali, M.A., Gabbrielli, R., Gremigni, P., 2001. Brassicaceae. In: Prasad, M.N.V. (Ed.), Metals in the Environment: Analysis by Biodiversity. Marcel Dekker Inc., New York, pp. 219–257.

Schneider, T., Haag-Kerwer, A., Maetz, M., Niecke, M., Povh, B., Rausch, T., Schüßler, A., 1999. Micro-PIXE studies of elemental distribution in Cd-accumulating *Brassica juncea* L. Nucl. Instrum. Methods Phys. Res. B 158, 329–334.

Siedlecka, A., Tuckendorf, A., Skórzynska-Polit, E., Maskymiec, W., Wójcik, M., Baszynski, T., Krupa, Z., 2001. Angiosperms (Asteraceae, Convolvulaceae, Fabaceae and Poaceae; other than Brassicaceae). In: Prasad, M.N.V. (Ed.), Metals in the Environment: Analysis by Biodiversity. Marcel Dekker Inc., New York, pp. 171–218.

Smith, G.C., Brennan, E.G., Greenhalgh, B.J., 1985. Cadmium sensitivity of soybean related to efficiency in iron utilization. Environ. Exp. Bot. 25, 99–106.

Steel, R.G.D., Torrie, J.H., Dickey, D.A., 1997. Principles and Procedure of Statistics: a Biometrical Approach. McGraw-Hill, New York.

Varga, A., Garcinuño Martinez, R.M., Záray, G., Fodor, F., 1999. Investigation of effects of cadmium, lead, nickel and vanadium contamination on the uptake and transport processes in cucumber plants by TXRF spectrometry. Spectrochim. Acta B 54, 1455–1462.

Vig, K., Megharaj, M., Sethunathan, N., Naidu, R., 2003. Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review. Adv. Environ. Res. 8, 121–135.

Wagner, G.J., 1993. Accumulation of cadmium in crop plants and its consequences to human health. Adv. Agron. 51, 173–212.

Wu, L.H., Luo, Y.M., Xing, X.R., Christie, P., 2004. EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. Agric. Ecosyst. Environ. 102, 307–318.