Available online at www.sciencedirect.com
ScienceDirect
Environmental and Experimental Botany 58 (2006) 234–243
Environmental and Experimental Botany
www.elsevier.com/locate/envexpbot

Effects of high chromium applications on miscanthus during the period of maximum growth

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

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

Received 11 May 2005; received in revised form 5 August 2005; accepted 15 September 2005

Abstract

Chromium levels comprised between 50 and 200 mg L^{-1} Cr were toxic to miscanthus and growth was completely stopped with concentrations equal or higher than 150 mg L^{-1} Cr. Root growth was less affected than shoot growth, but root morphology changed drastically. Up to 100 mg L^{-1} Cr, total length of roots increased and their average diameter decreased, whereas the opposite occurred with higher Cr levels. The net uptake rate of nitrogen, its net translocation rate from the hypogeal to the aerial plant part, and the N content of all plant parts decreased in parallel to growth reduction. The Cr concentration of the hypogeal part was approximately 18 times higher than that of the aerial part up to 100 mg L^{-1} Cr, and only eight times higher with higher Cr levels. Green leaves always showed the lowest Cr concentration, but a consistent translocation of Cr to dead leaves was observed. These patterns suggest the existence of different active mechanisms restricting Cr accumulation in green leaves. The hypogeal plant part retained between 90% and 95% of the Cr accumulated by the plant. The highest Cr content of the entire plant was achieved with 100 mg L^{-1} Cr, but that of the aerial part was highest with 150 and 200 mg L^{-1} Cr. Thus, in our experiment, Cr accumulation in the aerial part of miscanthus was higher with extreme toxic levels, whereas the overall ability of this species to remove Cr from solution was higher with moderate toxicity.

Keywords: Chromium; Miscanthus; Cr-uptake; Phytoremediation; Toxicity

1. Introduction

Chromium is a heavy metal which release into the environment poses a potential risk to human health. Trivalent and hexavalent Cr are the major stable chemical forms, with Cr(VI) causing the greatest concern because of its carcinogenic properties (Mei et al., 2002). Generally, most Cr(VI) added to soil is promptly reduced to the inert form Cr(III) by several agents among which sulfides, humic compounds and plant and microbial activity. However, Sethunathan et al. (2005) found that soil microorganisms also contribute to the reoxidation of Cr(III) to Cr(VI) and, therefore, both Cr oxidation states should be regarded hazardous for the environment and for humans.

Anthropogenic Cr sources contribute greatly to current Cr pollution, and the global industrial-age cumulative Cr production has been estimated as 105.4 million tonnes, with a significant increase since the 1950s (Han et al., 2004). Though chromium salts are widely used in dyeing, tanning and plating (Dube et al., 2003), Cr pollution has deserved relatively little attention compared to other heavy metals, because this metal is poorly absorbed and translocated by plants, so that both Cr phytotoxicity and accumulation in the food chain rarely occur in field conditions (Barceló and Poschenrieder, 1997; Khan, 2001). Nevertheless, laboratory investigations have assessed that both Cr(III) and Cr(VI) are toxic to plant growth and cause disorders in mineral nutrition (Moral et al., 1995; Vajpayee et al., 1999; Dube et al., 2003).

Relevant Cr pollution phenomena are reported to occur in small areas where many leather manufactures concentrate, since the tanning process consumes huge quantities of

* Corresponding author. Tel.: +39 050 599208; fax: +39 050 540633.
E-mail address: iard@agr.unipi.it (I. Arduini).

0098-8472/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2005.09.004

Cr to form stable complexes with amino acids and proteins (Vajpayee et al., 1999; Khan, 2001). The uptake of chromium into the leather is not complete, so that large amounts escape into effluents, which Cr concentration can reach up to 2000–5000 mg L^{-1} (Vajpayee et al., 1999). In addition, about 10% of the handeled leather is lost as solid wastes that contain up to 2% Cr (Tomaselli and Ummarino, 1995; Ciavatta and Gessa, 1997). Thus, great economic efforts are requested to both the producers and the communities for the treatment, disposal and, eventually, transformation of Cr containing solid and liquid wastes in reusable products (Khan, 2001; Rengaraj et al., 2001). Nevertheless, these efforts are often not enough to make the Cr concentration of effluents from treatment facilities and landfill eluates comply with the water quality standards set by single countries (Tomaselli and Ummarino, 1995; Vajpayee et al., 1999; Rengaraj et al., 2001) or with the limits raccomended by the World Health Organization for irrigation (0.1 mg L^{-1}) and drinking waters (0.05 mg L^{-1}) (Enderlein et al., 1997). For instance, Imai and Gloyna (1996) found considerable amounts of organic Cr compounds in the effluents of wastewater treatment plants. Despite this, both liquid and solid tannery wastes could be beneficial for agriculture for their fertilizer potential, since they are rich in organic carbon and nitrogen, and in other mineral elements (Ciavatta and Gessa, 1997; Masoni et al., 2003). The world production of hydrolyzed leather fertilizers can be estimated to about 85–100,000 tonnes yearly and, in Italy alone, the production of untreated leather wastes is about 800,000 tonnes yearly, representing 1.5% of total industrial wastes (Scaccabarozzi, 1989; Ciavatta and Gessa, 1997).

The possibility to reduce the Cr concentration of wastewaters and sludges by means of phytoremediation would be of great interest for mantaining environmental quality standards and for improving agriculture with a relative low cost (Rai et al., 1995; Vajpayee et al., 1999). Phytoremediation is an emerging and promising technology that uses living plants and the associated microrganisms to remove contaminants from soils and waters by means of degradation (phytodegradation), adsorption (rhizofitration) and absorption (phytoextraction) (Arduini and Masoni, 2002).

In the last decade, the use of plants for the abatement of Cr contamination has been investigated for both soil (Chen and Cutright, 2001; Khan, 2001; Han et al., 2004) and water conditions (Rai et al., 1995; Vajpayee et al., 1999; Mei et al., 2002). The major limitations of Cr phytoremediation are Cr toxicity to plants and to associated microrganisms, which reduces the size of the potential sink for Cr and even plant survival (Khan, 2001; Mei et al., 2002; Han et al., 2004), and the scarce translocation of Cr to harvestable plant parts, which is not increased by the addition of chelators (Chen and Cutright, 2001). However, encouraging results were obtained by Mei et al. (2002), who found that *Glycine max* was Cr tolerant and had a significant translocation of Cr to the aboveground plant parts, and by Rai et al. (1995), who found that the aquatic macrophytes *Spirodela polyrrhiza*, *Hydrodictyon reticulatum* and *Ceratophyllum demersum* reduced by 90% the Cr concentration of waters within 15 days. Floating aquatic plants are widely investigated for the abatement of Cr in waters, since the entire plant can be harvested, increasing phytoremediation performances (Rai et al., 1995; Vajpayee et al., 1999). The contaminated biomass of these plants, however, has no commercial value, can not be burnt for bioenergy production because of its low fibre content, and must, therefore, be safely disposed of. The use of crop species and, in particular of energy plants, for phytoremediation has raising attraction, since it is possible to gain a profit from their biomass (Mei et al., 2002; Han et al., 2004).

Miscanthus sinensis L. var. Giganteus is a vigorous perennial Gramineae species that originates from East Asia, but is widely cultivated as energy crop throughout Europe. This species reproduces vegetatively by rhizomes and develops a dense net of fibrous roots that could be an effective filtering system. In a previous investigation, we exposed miscanthus plants to Cr for a period of over three months comprised between the beginnings of the vegetative growth and heading. We found that plant biomass was reduced with Cr concentrations higher than 38 mg L^{-1} and that about 99% of the Cr taken up by the plant was retained by the hypogeal part (Arduini et al., in press). In addition, we also found that the accumulation in the aerial part of miscanthus of another heavy metal, cadmium, was markedly enhanced when plants were exposed to toxic Cd concentrations during their exponential phase of growth (Arduini et al., 2006).

In the present work, we aimed to assess if the accumulation of Cr in the aerial part of miscanthus could be increased exposing plants to toxic concentrations of chromium nitrate during the period of maximum growth, when plants show an elevated sink and a high uptake rate for elements. Therefore, plants of miscanthus were exposed to four chromium concentrations, comprised between 50 and 200 mg L^{-1}, during the month before heading. The growth and Cr-uptake patterns of separate plant parts and root morphology were recorded. Besides, in order to try to elucidate how chromium moves into and within the plant, the Cr uptake and translocation rates were compared with those of the essential nutrient, nitrogen.

2. Materials and methods

2.1. Plant material and growth conditions

At the end of winter, rhizomes of *M. 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 to allow rhizome sprouting. About 1 month later (4th May), plants that were approximately 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 based on 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; 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, after two weeks, the nutrient solution was completely renewed. The conductivity and pH of the solution were measured at 2-day intervals. The former was 3.8 mS cm^{-1} with slight modifications throughout the whole experiment and, when necessary, the latter was adjusted to 7.5 by adding H_2SO_4.

Plants were grown in the Cr-free nutrient solution for 81 days (24th July), then the metal was added as nitrate salt to final concentrations of 0 (control), 50, 100, 150 and 200 mg L^{-1} Cr. The N concentration was equalled among treatments adding different amounts of ammonium nitrate, so that the effective concentrations in solution were 382 mg L^{-1} for NO_3-N and 159 mg L^{-1} for NH_4-N. Each treatment (Cr 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 chromium addition (0 days after treatment, DAT), and when control plants had achieved the heading stage, that corresponded to the complete emergence of the inflorescence (36 DAT).

2.2. Growth parameters

Harvested plants were carefully rinsed with tap water and then they 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. For dry weight determination, all plant parts were oven 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. Since two types of roots, very fine and very thick, were easily recognizable, for each subsample we separated roots that were approximately thicker or finer than 1 mm. These subsubsamples 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 sub-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. The average diameter of each sub-subsample was calculated separately by dividing total area by total length. Thereafter, the average diameter of the entire root system was estimated on the basis of the dry weight ratio of fine and thick roots. The root surface was estimated, after Kokko et al. (1993), by multiplying root area by π. 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 calculated as: $\pi \times (d/2)^2 \times h$.

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. Chromium 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

The net uptake rates (NUR) of Cr and N, that indicate the amount of Cr or N daily taken up by each unit of root dry weight, were determined following Engels (1993), as:

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

where Pc is the Cr or N content of the whole plant, Rw 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), that indicate the amount of N or Cr accumulated in the hypogeal part that is daily translocated to the aerial one, were determined as:

$$\mathrm{NTR} = \frac{\mathrm{Ap}_2 - \mathrm{Ap}_1}{d} \times \frac{\ln(\mathrm{Hp}_2/\mathrm{Hp}_1)}{\mathrm{Hp}_2 - \mathrm{Hp}_1}$$

where Ap is the Cr or N content of the aerial part, Hp the Cr or N content of the hypogeal part, at the beginning (Ap_1 and Hp_1) and at the end (Ap_2 and Hp_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 Cr 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

Plants harvested just before the addition of chromium nitrate (DAT 0) had an average of 16 culms and 42 green leaves, and the main culm measured 45.2 cm (Table 1). At the end of the treatment period, culm length was by approximately 17% lower than in control plants up to 100 mg L^{-1} Cr and by 42% lower with the two highest Cr levels. The

Table 1
Shoot growth parameters of miscanthus plants treated for 36 days with Cr concentrations ranging from 50 to 200 mg L^{-1}

DAT	Chromium level (mg L^{-1})	Plant height (cm)	Culms (n $plant^{-1}$)	Green leaves (n $plant^{-1}$)
0	0	45.2 ± 2.4a	16 ± 1a	42 ± 3a
36	0	79.6 ± 5.9c	28 ± 4c	85 ± 10c
	50	69.0 ± 5.3b	23 ± 1b	63 ± 7b
	100	63.2 ± 6.0b	16 ± 1a	60 ± 4b
	150	47.0 ± 2.7a	16 ± 4a	39 ± 7a
	200	45.8 ± 4.2a	16 ± 1a	38 ± 7a

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

number of culms was decreased by 18% with 50 mg L^{-1} Cr and by 43% with the other Cr levels, whereas the number of green leaves was decreased by 28% up to 100 mg L^{-1} Cr and then by 55%. Taking into account initial values, culm elongation was completely stopped with 200 mg L^{-1} Cr, and the emission of secondary culms ceased with Cr concentrations equal or higher than 100 mg L^{-1}. Finally, the number of green leaves was even lower than at the beginning of the treatment period with Cr levels equal or higher than 150 mg L^{-1}.

A 36-day exposure to 50 mg L^{-1} Cr decreased the dry weight of culms, leaves (green + dead) and rhizomes, compared to control plants, but slightly increased that of roots (Table 2). Between 50 and 150 mg L^{-1} Cr, the dry weight of all plant parts decreased according to increasing supply, and then it did not change further between 150 and 200 mg L^{-1} Cr. As a result, the dry weight of the entire miscanthus plant was decreased by 17% with 50 mg L^{-1} Cr, by 37% with 100 mg L^{-1} Cr and by approximately 59% with the two highest Cr levels. The comparison of dry weights at DAT 0 and 36 indicated that no significative biomass production occurred in all plant parts with Cr concentrations equal or higher than 150 mg L^{-1}.

The highest Cr level (200 mg L^{-1}) drastically changed the dry weight ratio between green and dead leaves (Table 2). Up to 150 mg L^{-1} Cr, the dry weight of both green and dead leaves decreased with increasing Cr supply, and the former weighed more than twice the latter. In contrast, with 200 mg L^{-1} Cr, the dry weight of dead leaves was higher than with 100 and 150 mg L^{-1} Cr and equalled that of green leaves.

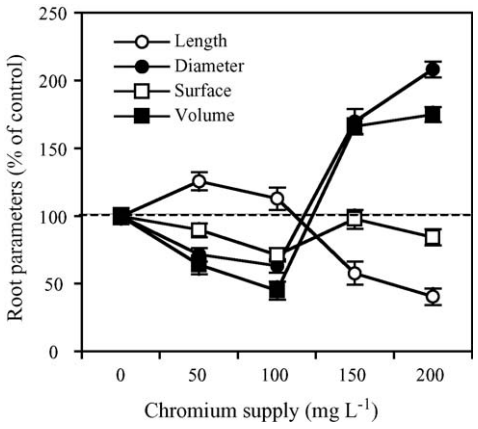

Fig. 1. Root length, average diameter, surface and volume (% of control) of miscanthus plants treated for 36 days with Cr concentrations ranging from 50 to 200 mg L^{-1}. Vertical bars denote S.E. $n = 4$. When not indicated, error bars lie within the symbol.

3.2. Root morphology

The morphology of the root system of miscanthus changed in response to chromium nitrate supply. Chromium levels up to 100 mg L^{-1}, increased root length, with the highest values recorded with 50 mg L^{-1} Cr, and decreased the other root parameters in the ascending order surface < average diameter < root volume (Fig. 1). The two highest Cr levels, 150 and 200 mg L^{-1} Cr, in contrast, drastically decreased root length both compared to the lower Cr levels and to the control, and increased the average diameter of roots up to 100% and their

Table 2
Dry weight of miscanthus plants before and after a 36-day treatment with Cr concentrations ranging from 50 to 200 mg L^{-1}

DAT	Chromium level (mg L^{-1})	Dry weight (g $plant^{-1}$)							
		Culms	Green leaves	Dead leaves	Aerial part	Roots	Rhizome	Hypogeal part	Entire plant
0	0	15.3 ± 1.4a	10.9 ± 1.1a	4.0 ± 0.7a	30.2 ± 2.7a	10.1 ± 2.3a	17.6 ± 2.4a	27.7 ± 4.2a	57.9 ± 6.4a
36	0	46.8 ± 5.1d	28.5 ± 3.1d	13.5 ± 1.6c	88.8 ± 9.5d	23.1 ± 2.2bc	50.8 ± 5.8d	73.9 ± 8.0c	162.7 ± 17.4e
	50	29.0 ± 1.2c	25.2 ± 0.9c	10.6 ± 1.0c	64.8 ± 2.6c	25.9 ± 0.9c	43.9 ± 2.8c	69.8 ± 3.4c	134.6 ± 6.0d
	100	21.8 ± 2.6b	16.8 ± 2.3c	5.5 ± 1.0a	44.2 ± 5.5b	20.6 ± 6.2b	37.4 ± 3.6b	58.0 ± 8.3b	102.2 ± 11.2c
	150	19.7 ± 2.5ab	10.7 ± 2.5a	4.2 ± 0.9a	34.6 ± 5.7a	13.1 ± 3.5a	22.8 ± 5.9a	35.9 ± 9.4a	70.5 ± 15.0b
	200	18.0 ± 4.1a	7.6 ± 1.3b	7.4 ± 1.8b	32.9 ± 6.9a	10.4 ± 2.1a	20.8 ± 4.4a	31.2 ± 6.1a	64.1 ± 12.5ab

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

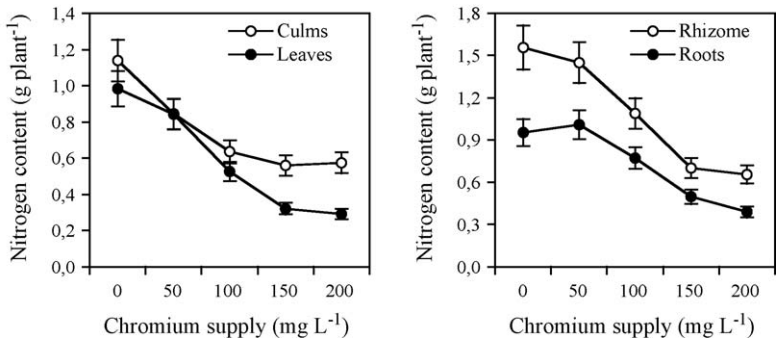

Fig. 2. Nitrogen content of culms, leaves, rhizome and roots of miscanthus plants treated for 36 days with Cr concentrations ranging from 50 to 200 mg L^{-1}. Vertical bars denote S.E. $n = 4$. When not indicated, error bars lie within the symbol.

volume up to 75%. Root surface was only slightly affected by these Cr levels. Visual observations showed that, up to 100 mg L^{-1}, chromium enhanced the development of fine, long and scarcely branched, white lateral roots. With higher Cr concentrations, these fine lateral roots did not develop, so that the root system consisted only of short and thick main roots. In addition, a light blue deposit formed on roots exposed to all chromium levels.

3.3. Nitrogen concentration and content

At the end of the treatment period, the N concentration of culms was always higher in chromium nitrate treated plants (approximately 30 g kg^{-1}) than in controls (24 g kg^{-1}). That of leaves, rhizome and roots was not affected significantly by the addition of Cr and, averaged over controls and all Cr levels, it was respectively 22, 31 and 39 g kg^{-1}.

At the beginning of the treatment period the N content of leaves was 360 mg $plant^{-1}$, that of culms 428 mg $plant^{-1}$, that of rhizomes 514 mg $plant^{-1}$ and that of roots 347 mg $plant^{-1}$. At the end, the N content of leaves, culms and of the rhizome drastically decreased with the increase of Cr in solution up to 150 mg L^{-1}, and did not change between this level and 200 mg L^{-1} Cr (Fig. 2). The N content of roots was slightly higher than in control with 50 mg L^{-1} Cr, but then markedly decreased according to increasing $Cr(NO_3)_3$ supply. In addition, the N content of culms, rhizomes and roots was higher at the end of the treatment than at the beginning with all Cr levels, while that of leaves was lower with the two highest Cr levels.

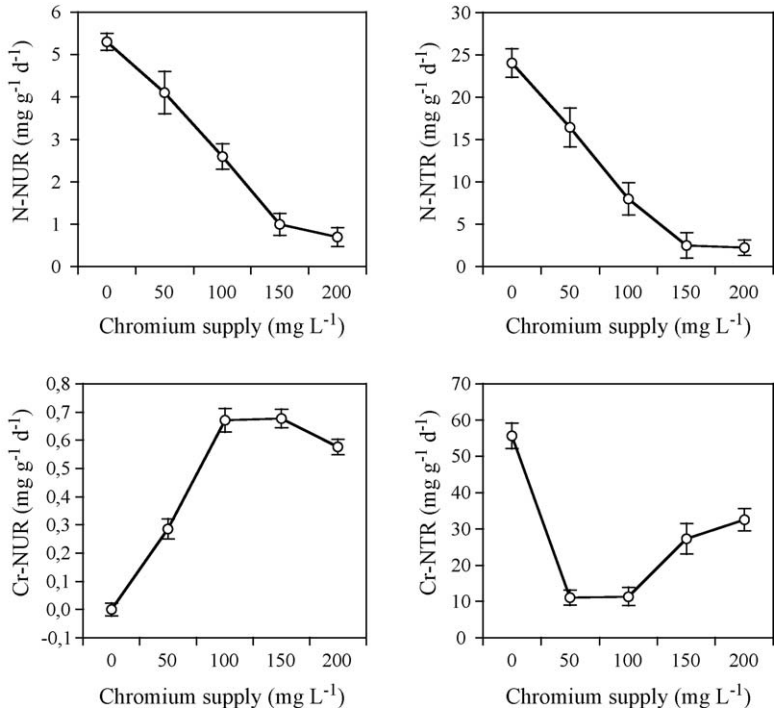

Fig. 3. Net uptake rates (NUR) of nitrogen and of chromium, and net translocation rates (NTR) of N and of Cr from the hypogeal to the aerial part of miscanthus plants treated for 36 days with Cr concentrations ranging from 50 to 200 mg L^{-1}. Vertical bars denote S.E. $n = 4$. When not indicated, error bars lie within the symbol.

Both the net uptake rate and the net translocation rate of nitrogen from the hypogeal to the aerial part decreased markedly and almost linearly with the increase of Cr level up to 150 mg L^{-1}, and only slightly decreased further between this level and the highest Cr supply (Fig. 3).

3.4. Chromium concentration and content

With the addition of chromium nitrate the Cr concentration increased markedly in miscanthus, but the increase showed different patterns among plant parts and was consistent to supply only in culms (Fig. 4, left). In green leaves, the Cr concentration was approximately 34 mg kg^{-1} between 50 and 150 mg L^{-1} Cr and then increased to 70 mg kg^{-1} with 200 mg L^{-1} Cr. In dead leaves, in contrast, it increased drastically up to 150 mg L^{-1} Cr and then markedly decreased with 200 mg L^{-1} Cr. In the rhizome, the Cr concentration increased up to 100 mg L^{-1} Cr, was unchanged between this level and 150 mg L^{-1} Cr and then decreased slightly with the highest supply. In roots, finally, it increased about twofold with each increase of Cr level up to 150 mg L^{-1} Cr and then it did not change further. With all Cr levels, the Cr concentrations recorded in the rhizome and in roots were by far higher than those of aerial organs, but up to 100 mg L^{-1} Cr the rhizome showed higher concentrations than roots, whereas the opposite occurred with the two highest Cr levels. Among aerial organs, green leaves always showed the lowest Cr concentration, whereas dead leaves showed the highest Cr concentration up to 150 mg L^{-1} Cr, but equalled culms with 200 mg L^{-1} Cr. The Cr concentration of the entire hypogeal

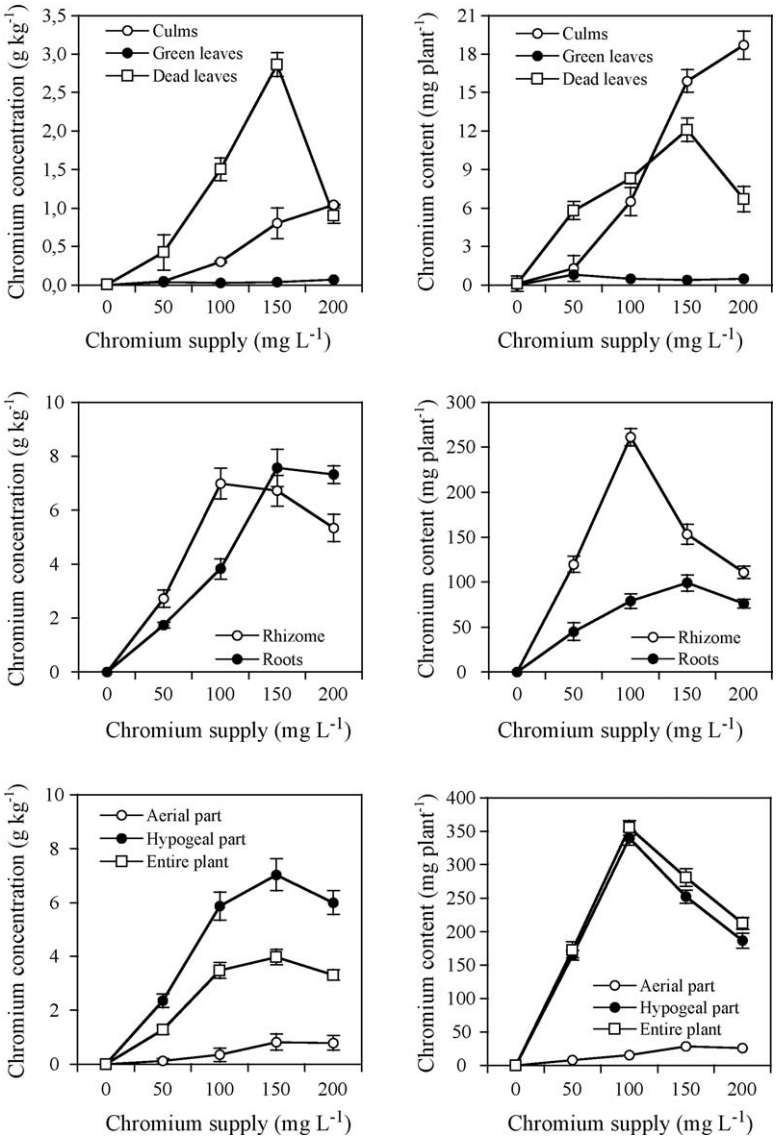

Fig. 4. Chromium concentration (figures left) and Cr content (figures right) of culms, green and dead leaves, rhizome, roots, aerial and hypogeal plant part, and entire plant of miscanthus after a 36-day treatment with Cr concentrations ranging from 50 to 200 mg L^{-1}. Vertical bars denote S.E. $n = 4$. When not indicated, error bars lie within the symbol.

part was 18 times higher than that of the aerial part with the two lower Cr levels and only eight times higher with the two higher levels (Fig. 4, left). The highest Cr concentration of the entire plant was recorded with treatments ranging from 100 to 200 mg L^{-1} Cr and was approximately 3.6 g Cr kg^{-1}.

With all $Cr(NO_3)_3$ supplies, the Cr content of the rhizome greatly exceeded that of other plant parts (Fig. 4, right). In descending order followed roots, then at a distance culms and dead leaves and, finally, green leaves. The highest Cr content was achieved by the rhizome with 100 mg L^{-1} Cr (261 mg Cr $plant^{-1}$), by roots and dead leaves with 150 mg L^{-1} Cr (99 and 12 mg Cr $plant^{-1}$, respectively), and by culms with 200 mg L^{-1} Cr (19 mg Cr $plant^{-1}$). Finally, the Cr content of green leaves was only 0.6 mg Cr $plant^{-1}$, independently of the applied Cr level. The Cr content of the entire miscanthus plant increased with the increase of supply up to 100 mg L^{-1} Cr and then decreased, so that the lowest content (173 mg Cr $plant^{-1}$) was achieved with 50 mg L^{-1} Cr and the highest (355 mg Cr $plant^{-1}$) with 100 mg L^{-1} Cr (Fig. 4, right). The hypogeal plant part retained approximately 95% of the accumulated Cr up to 100 mg L^{-1} Cr, and almost 90% with the two highest supplies.

The net uptake rate of Cr sharply increased with the increase of $Cr(NO_3)_3$ supply up to 100 mg L^{-1} Cr, was unchanged between this level and 150 mg L^{-1} Cr and then slightly decreased (Fig. 3). Compared to control values, the net translocation rate of Cr from the hypogeal to the aerial part drastically decreased between 0 and 50 mg L^{-1} Cr, did not change between this level and 100 mg L^{-1} Cr, and then increased according to supply (Fig. 3).

4. Discussion

All chromium nitrate concentrations used in this experiment negatively affected plants of miscanthus grown in hydroponics. Dry weights recorded at the end of the treatment period indicated that, for all plant parts, growth depression was greater with the increase of Cr level up to 150 mg L^{-1}, but was unchanged between this level and 200 mg L^{-1}. In addition, dry weights recorded with 150 and 200 mg L^{-1} Cr did not differ significantly from those measured at the beginning of the treatment period, which indicates that biomass production was completely inhibited.

Growth of separate plant parts was differently sensitive to the supply of chromium nitrate. Culms were by far the most affected, both in terms of elongation, biomass production and new emission, the last one being already completely inhibited with Cr levels equal or higher than 100 mg L^{-1} Cr. In the order, culms were followed by leaves, that also showed increased drying up with 200 mg L^{-1} Cr, and by the rhizome. Root biomass was the less affected by $Cr(NO_3)_3$ and it did not differ significantly from that of control plants up to 100 mg L^{-1} Cr. Despite this, all Cr levels changed dramatically root morphology. Compared to controls, roots exposed to Cr up to 100 mg L^{-1} showed a great development of white, very fine, long and only low branched lateral roots from short main roots. With Cr concentrations equal or higher than 150 mg L^{-1}, the production of these fine lateral roots was inhibited, so that total length and biomass of roots were severely decreased, while both the average diameter and the volume of roots increased dramatically. However, the higher diameter and volume did not correspond to increased tissue production since the specific dry weight of roots was only 30 mg cm^{-3} compared to 160 mg cm^{-3} of the other treatments. A severe growth reduction and the formation of stunted roots was also reported for Cr treated plants of *Phaseolus vulgaris* and for seedlings of *Triticum aestivum* (Karataglis, 1987; Vázquez et al., 1987), whereas the enhancement of lateral root growth was suggested to cause root biomass increase in Cr stressed plants of *Brassica oleracea* (Pandey and Sharma, 2003).

Results obtained in our research suggest that biomass production of the aerial part is more affected than that of the hypogeal one and that root elongation is stimulated with Cr levels below the upper toxic threshold. Literature on the response of distinct plant parts to chromium is scarce, but a higher decrease of shoot biomass compared to root biomass was found by Pandey and Sharma (2003) in *B. oleracea*, and a higher sensitivity of roots compared to shoots was reported for seedlings of *Sorghum bicolor*, *T. aestivum* and *Vigna radiata* (Karataglis, 1987; Shanker and Pathmanabhan, 2004; Shanker et al., 2004), and for plants of *Avena sativa* (McGrath, 1982). Long term exposures of *Lycopersicon esculentum* to 50 mg L^{-1} Cr even increased shoot dry weight, whereas 100 mg L^{-1} Cr reduced the dry weight of roots and only slightly affected that of the shoot (Moral et al., 1995).

The inhibition of ion transport is considered one of the major causes of Cr(III) toxicity in plants (Moral et al., 1995; Mei et al., 2002; Dube et al., 2003). Vajpayee et al. (1999) found that Cr greatly affected the nitrogen metabolism of *Nelumbo nucifera*, in that it decreased the nitrate reductase activity and the protein content, with a more severe effect on roots than on leaves. In plants of *L. esculentum*, Moral et al. (1995) found that Cr decreased the N concentration of roots and stems + branches, but did not affect that of leaves, and decreased the N content of all plant parts. In our research, we found that chromium nitrate decreased the nitrogen content of separate plant parts but affected only slightly the N concentration. The NUR for nitrogen indicated that the amount of N taken up by each unit of root biomass decreased dramatically with the increase of Cr level, which could be the consequence of either a lower uptake efficiency of roots or a reduced demand for nitrogen by the plant. As a consequence of both reduced root N uptake and reduced plant growth, the amount of N accumulated by the miscanthus plant during the treatment period decreased dramatically with the increase of $Cr(NO_3)_3$ supply, and was even lower than at the beginning in leaves treated with the two highest supplies.

All Cr levels caused a dramatic increase in the Cr concentration of miscanthus, but values differed consistently among plant parts. Rhizomes showed the highest Cr concentration up to 100 mg L^{-1} Cr, and were surpassed by roots with Cr levels equal or higher than 150 mg L^{-1}. Aerial organs always followed hypogeal ones in the order of dead leaves, culms and finally, at a distance, green leaves. The higher Cr concentration in hypogeal organs compared to aerial ones is widely reported (Zayed et al., 1998; Mei et al., 2002; Shanker and Pathmanabhan, 2004). The higher concentration of this metal in old leaves compared to both culms and young leaves was found in *P. vulgaris* (Barceló et al., 1986) and *Brassica juncea* (Han et al., 2004), and the opposit was reported for *Citrullus vulgaris* (Dube et al., 2003).

In culms and green leaves the highest Cr concentration was achieved with 200 mg L^{-1} Cr, whereas in dead leaves, rhizomes and roots it was reached with 150 mg L^{-1} Cr and then it decreased. Above patterns support the hypothesis that in miscanthus several restriction mechanisms concur to minimize Cr transport to the aerial part and, especially, to green leaves. As far as plant metabolism is more or less active, that is to say up to 100 mg L^{-1} Cr, chromium immobilization occurs primarly in the rhizome, maybe through precipitation as simple salt or complexed with proteins and carbohydrates within vacuoles and plastids (Vázquez et al., 1987; Han et al., 2004). Though to a lower extent, with Cr concentrations higher than 50 mg L^{-1}, immobilization of the metal occurs also in culms, which Cr concentration greatly exceeds that of green leaves. Finally, an additional mechanism of Cr detoxification in miscanthus, can be considered the huge accumulation of Cr in dead leaves, that is observed up to the 150 mg L^{-1} Cr treatment.

The patterns of Cr-NTR are consistent with the hypothesis that the mechanisms that hinder Cr translocation to aerial organs are linked to plant metabolism, since they seem to be active with 50 and 100 mg L^{-1} Cr and to be disrupted with Cr levels that completely inhibit growth. Similarly, the decrease of Cr concentration that we observed in dead leaves between the two highest Cr levels suggests that also the translocation to senescent organs is an active process that is impaired at extreme toxic conditions. We suggest that also the uptake of Cr into roots is driven by plant metabolism, since the Cr-NUR increased sharply up to 100 mg L^{-1} Cr, probably in consequence of the enhanced development of lateral roots, and it was unchanged and even decreased with $Cr(NO_3)_3$ supplies that completely stopped biomass production. As a result, the amount of the metal that could be translocated to the rhizome was reduced, which could explain the lower Cr concentration recorded in this organ compared to roots with the extreme toxic Cr levels.

The NURs and NTRs for Cr and N showed opposite trends in response to the increase of chromium nitrate in solution. We explain this difference suggesting that the uptake of nitrogen into roots and its translocation to the aerial part are driven by the demand of growing tissues and, therefore, both decrease in parallel to growth decrease. In contrast, Cr enters roots passively following the water flux, while its translocation to the aerial part is actively restricted. Restriction barriers are inactivated above 100 mg L^{-1} Cr in consequence of the severe reduction of plant metabolism, and Cr translocation to the aerial part increases with increasing supply. This, however, did not cause a massive flow of Cr to the aerial part, like that we recorded for cadmium in similar conditions (Arduini et al., 2006), and this may be considered a limit to the use of miscanthus for Cr phytoextraction. Indeed, with 200 mg L^{-1} Cr, only the Cr concentration of green leaves increased markedly, whereas that of the entire aerial part even decreased. These findings agree with those of Han et al. (2004), who reported that the transfer factor from roots to shoots decreased in *Brassica juncea* with Cr levels that reduced its growth.

In our research, the Cr concentration of the entire miscanthus plant did not change significantly between 100 and 200 mg L^{-1} Cr and was approximately 3.6 g Cr kg^{-1}, value that was by far lower than that we had obtained with longer exposures of small miscanthus plants to less toxic concentrations (Arduini et al., in press). In contrast, the maximum Cr concentrations achieved in the aerial part (approximately 0.8 g Cr kg^{-1} with 150 and 200 mg L^{-1} Cr) were much higher. All above summarized, this suggests that Cr uptake by the whole miscanthus plant increases with time of exposure, probably because of the higher deposition of Cr on the outer surfaces of roots and rhizomes, but decreases with increasing toxicity in consequence of the reduced metabolic activity of the plant. In contrast, the translocation of Cr to the aerial part is greatly enhanced in toxic conditions, because of the breakdown of restriction barriers. Similar trends were observed also by Moral et al. (1995) in *L. esculentum*. Though it is difficult to compare results obtained in different experiments, Cr concentration values obtained with miscanthus were similar to those reported by Moral et al. (1995) with *L. esculentum* and by Sharma et al. (1995) with *T. aestivum*, were lower than those found by Mei et al. (2002) with *G. max* and *Helianthus annuus* and higher than those obtained by Pandey and Sharma (2003) with *B. oleracea* and by Dube et al. (2003) with *C. vulgaris*.

The phytoremediation performance of a plant is not only determined by its ability to achieve high metal concentrations within its tissues, but also by its ability to translocate the metal to aerial organs and to produce a high biomass. In our research, the Cr content of the entire miscanthus plant increased according to supply only up to 100 mg L^{-1} Cr, and then decreased with increasing Cr level, probably as a result of the dramatic reduction of both biomass production and metabolic activity. The Cr content of the hypogeal plant part showed a similar pattern, whereas that of the aerial one increased linearly up to 150 mg L^{-1} Cr and then was unchanged. Thus, the highest Cr accumulation in the aerial part was achieved with extreme Cr toxicity, while the highest content of the entire plant was achieved with the treatment just below the upper toxicity threshold.

5. Conclusions

The exposure of miscanthus plants to Cr concentrations of 50 and 100 mg L^{-1} during the period of maximum growth decreased growth of the aerial part and of the rhizome, but did not change root growth. In spite of this, root morphology was greatly affected in consequence of the enhanced production of fine and long lateral roots, which increased total root length and decreased root average diameter, surface and volume. With Cr concentrations equal or higher than 150 mg L^{-1}, in contrast, growth of miscanthus was completely stopped and both the net uptake and the net translocation rate of nitrogen from the hypogeal to the aerial part were severely inhibited, suggesting that this Cr level represents the upper toxicity threshold for this species.

The overall ability of miscanthus to remove chromium from a nutrient solution supplied with $Cr(NO_3)_3$ increased up to the upper toxicity threshold and then decreased markedly. The metal was strongly retained in the hypogeal plant part and the application of toxic levels increased only slightly, from 5 to 10%, its partitioning to the aerial part. Similarly, the use of large sized plants was not effective in achieving high Cr contents in a short period, probably because both Cr uptake and translocation within the plant are slow processes.

References

Arduini, I., Masoni, A., 2002. Fitodepurazione. Riv. Agron. 36, 17–32.

Arduini, I., Ercoli, L., Mariotti, M., Masoni, A., 2006. Response of miscanthus to toxic cadmium applications during the period of maximum growth. Environ. Exp. Bot. 55, 29–40.

Arduini, I., Ercoli, L., Masoni, A., in press. Response of *Miscanthus sinensis* to long term application of low Cr levels in hydroponics, Agrochimica.

Barceló, J., Poschenrieder, C., 1997. Chromium in plants. In: Canali, S., Tittarelli, F., Sequi, P. (Eds.), Chromium Environmental Issues. FrancoAngeli, Milano, Italy, pp. 101–129.

Barceló, J., Poschenrieder, C., Gunsé, B., 1986. Water relations of chromium VI treated bush bean plants (*Phaseolus vulgaris* L. cv. Contender) under both normal and water stress conditions. J. Exp. Bot. 37, 178–187.

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

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

Ciavatta, C., Gessa, C., 1997. Chromium-containing fertilizers and their production. In: Canali, S., Tittarelli, F., Sequi, P. (Eds.), Chromium Environmental Issues. FrancoAngeli, Milano, Italy, pp. 61–82.

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

Dube, B.K., Kamlesh, T., Chatterjee, J., Chatterjee, C., 2003. Excess chromium alters uptake and translocation of certain nutrients in citrullus. Chemosphere 53, 1147–1153.

Enderlein, U.S., Enderlein, R.E., Williams, W.P., 1997. Water quality requirements. In: Helmer, R., Hespanhol, I. (Eds.), Water Pollution Control. A Guide to the Use of Water Quality Management Principles. WHO/UNEP, E&FN Spon, London, United Kingdom.

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.

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

Han, F., Sridhar, B.B.M., Monts, D.L., Su, Y., 2004. Phytoavailability and toxicity of trivalent and hexavalent chromium to *Brassica juncea*. New Phytol. 162, 489–499.

Imai, A., Gloyna, E.F., 1996. Speciation of chromium(III) in activated sludge. Water Environ. Res. 68, 301–310.

Karataglis, S., 1987. Estimation of the toxicity of different metals, using as criterion the degree of root elongation in *Triticum aestivum* seedlings. Phyton (Austria) 26, 209–217.

Khan, A.G., 2001. Relationships between chromium biomagnification ratio, accumulation factor, and mycorrhizae in plants growing on tannery effluent-polluted soil. Environ. Intern. 26, 417–423.

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.

Masoni, A., Arduini, I., Ercoli, L., 2003. Fertilizzazione del miscanto con fanghi conciari. In: Mori, M., Fagnano, M., (Eds.), Obiettivo qualità integrale: il ruolo della ricerca agronomica. Atti XXXV Convegno della Società Italiana di Agronomia, Napoli, 16–18 settembre 2003. Imago Media, Caserta, pp. 167–168.

McGrath, S.P., 1982. The uptake and translocation of tri- and hexa-valent chromium and effects on the growth of oat in flowing nutrient solution and in soil. New Phytol. 92, 381–390.

Mei, B., Puryear, J.D., Newton, R.J., 2002. Assessment of Cr tolerance and accumulation in selected plant species. Plant Soil 247, 223–231.

Moral, R., Navarro Pedreno, J., Gomez, I., Mataix, J., 1995. Effects of chromium on the nutrient element content and morphology of tomato. J. Plant Nutr. 18, 815–822.

Pandey, N., Sharma, C.P., 2003. Chromium interference in iron nutrition and water relations of cabbage. Environ. Exp. Bot. 49, 195–200.

Rai, U.N., Sinha, S., Triphati, R.D., Chandra, P., 1995. Wastewater treatability potential of some aquatic macrophytes: removal of heavy metals. Ecol. Eng. 5, 5–12.

Rengaraj, S., Yeon, K.-H., Moon, S.-H., 2001. Removal of chromium from water and wastewater by ion exchange resins. J. Hazard. Mater. B87, 273–287.

Scaccabarozzi, A., 1989. Lo smaltimento in agricoltura dei fanghi di conceria. Inquinamento 11, 82–83.

Sethunathan, N., Megharaj, M., Smith, L., Kamaludeen, S.P.B., Avudainayagam, S., Naidu, R., 2005. Microbial role in the failure of natural attenuation of chromium(VI) in long-term tannery waste contaminated soil. Agric. Ecosys. Environ. 105, 657–661.

Shanker, A.K., Pathmanabhan, G., 2004. Speciation dependant antioxidative response in roots and leaves of sorghum (*Sorghum bicolor* (L.) Moench cv CO 27) under Cr(III) and Cr(VI) stress. Plant Soil 265, 141–151.

Shanker, A.K., Djanaguiraman, M., Sudhagar, R., Chandrashekar, C.N., Pathmanabhan, G., 2004. Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (*Vigna radiata* (L.) R. Wilczek. Cv CO 4) roots. Plant Sci. 166, 1035–1043.

Sharma, D.C., Chatterjee, C., Sharma, C.P., 1995. Chromium accumulation and its effects on wheat (*Triticum aestivum* L. cv. HD 2204) metabolism. Plant Sci. 111, 145–151.

Steel, R.G.D., Torrie, J.H., Dickey, D.A., 1997. Principles and procedure of statistics: a biometrical approach. McGraw-Hill, New York.

Tomaselli, M., Ummarino, G., 1995. Caratteristiche e possibilità di smaltimento di fanghi da impianti di depurazione di acque conciarie. Cuoio Pelli Mat. Concianti 71, 71–81.

Vajpayee, P., Sharma, S.C., Tripathi, R.D., Rai, U.N., Yunus, M., 1999. Bioaccumulation of chromium and toxicity to photosynthetic pigments, nitrate reductase activity and protein content of *Nelumbo nucifera* Gaertn. Chemosphere 39, 2159–2169.

Vázquez, M.D., Poschenrieder, C.H., Barceló, J., 1987. Chromium VI induced structural and ultrastructural changes in bush bean plants (*Phaseolus vulgaris* L.). Ann. Bot. 59, 427–438.

Zayed, A., Lytle, C.M., Qian, J-H., Terry, N., 1998. Chromium accumulation, translocation and chemical speciation in vegetable crops. Planta 206, 293–299.