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

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#### 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  $100 \text{ mg } L^{-1}$  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  $100 \text{ mg } L^{-1}$  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  $100 \text{ mg } L^{-1}$  Cr, but that of the aerial part was highest with 150 and  $200 \text{ mg } L^{-1}$  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

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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  $2000-5000 \,\mathrm{mg} \,\mathrm{L}^{-1}$  (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  $(0.1 \text{ mg L}^{-1})$  and drinking waters  $(0.05 \,\mathrm{mg}\,\mathrm{L}^{-1})$  (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*, *Hydro-*

dictyon 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  $38 \,\mathrm{mg}\,\mathrm{L}^{-1}$  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  $\rm L^{-1}$ , 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 openair hydroponics installation equipped for the nutrient film

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