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Metal accumulation and shoot yield of *Miscanthus* × *giganteus* growing in contaminated agricultural soils: Insights into agronomic practices

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ABSTRACT

The choice of agronomic practices for phytomanagement of metal-contaminated soils is of crucial importance to optimize plant biomass yields and to mitigate both environmental and health risks due to metal exposure. The present study aimed to assess the effects of agronomic practices on shoot yield and on metal (Cd, Pb, and Zn) accumulation in the organs of the energy crop miscanthus (*Miscanthus* × *giganteus*) during the first three years since the plantation on metal-contaminated agricultural soils. Three miscanthus cultivars, hereafter named Mis-A, Mis-B and Mis-I, were planted at low and high density. An inoculum of arbuscular mycorrhizal fungi-AMF (*Glomus* LPA Val 1) was added during plantation, and nitrogen fertilization was applied during the third growing season. Metal accumulation in miscanthus organs was determined during the second growing season, whereas shoot yields and their metal concentrations were determined during both the second and the third growing seasons. Based on metal concentrations and bioconcentration factors, the three cultivars mainly accumulated metals in their roots. The shoot yields increased from 3.7 to 10.3 t DW ha^{-1} in the second growing season to 15.8–23.3 t DW ha^{-1} in third growing season. There were no or very few significant differences in metal concentrations and shoot yields within treatments comprising the same cultivar. The addition of AMF inoculum increased metal (mainly Cd and Zn) accumulation in miscanthus organs and in the shoot yields and this was more observed in both Mis-B and Mis-I which presented a higher root mycorrhization level than in Mis-A. Shoot yields in treatments comprising different cultivars depended not on fertilization but on the interactions between cultivar and planting density, and between cultivar, planting density and AMF inoculum. Whatever the treatment and the sampling period, Pb concentrations did not significantly differ in shoot yields. The interaction between cultivar and planting density resulted in higher Cd concentrations in the yields of Mis-B planted at low density during the third growing season. Zn concentrations increased with fertilization in all treatments, and with the addition of the AMF inoculum in Mis-B and in Mis-I. Overall, the results demonstrated that the three cultivars could be potential candidates for coupling phytostabilization and biomass production on metal-contaminated soils.

1. Introduction

Soil metal- and metalloid-contamination by industrial activities, mining wastes, and agricultural fertilizers, is of great concern worldwide (Su et al., 2014). Protecting inhabitants, especially by reducing their exposure to contaminated dusts and ingestion of contaminated foodstuff, are of prime concern. To this end, phytomanagement, which uses the phytoremediation techniques, is a very promising option (Conesa et al., 2012). Phytoremediation is a cost-effective, environmentally-friendly remediation using plants and associated microorganisms (bacteria and fungi) for either (1) phytoextraction, i.e. the uptake and accumulation of

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metals and metalloids from the soil to the aboveground plant parts; or (2) phytostabilization, i.e. metal/metalloid immobilization in the rhizosphere and the roots (Mench et al., 2010). The biomass from contaminated/marginal lands is expected to provide industrial feedstock for bioenergy and green chemicals, hence avoiding the nexus of food vs fuel controversy (Gopalakrishnan et al., 2011; Witters et al., 2012). The sustainability is well ensured when selected plants are able to tolerate on-site contaminants and other local environmental stresses whilst providing a high and economically valuable biomass production (Conesa et al., 2012).

Around the former lead smelter, Metaleurop Nord (http://fr.safir-network.com/), agricultural soils are contaminated at various levels and the contamination is mainly limited to the ploughed horizon (Sterckeman et al., 2002). This smelter has operated for up to 100 years until its closure in 2003, and has contaminated its surrounding environment via atmospheric fallouts and trace element (TE)-enriched dust emissions (Sterckeman et al., 2002). Within the site, cropland topsoils are contaminated mainly by Cd, Pb, and Zn at levels equivalent to 20–50-fold higher than regional agricultural topsoil concentrations (Sterckeman et al., 2002). Some food crops including wheat, barley, forage maize, and potato produced on such soils present Cd and Pb concentrations that often exceed legislation thresholds for food or feedstuff (Douay et al., 2013). Due to its high tolerance to abiotic stresses and low metal accumulation in aboveground biomass, *Miscanthus* species are among potential candidate crops for phytostabilization (Nsanganwimana et al., 2014). Consequently, the cultivation of the sterile hybrid *Miscanthus* × *giganteus* was proposed as a sustainable management option to maintain local farmers' activities on Metaleurop site contaminated agricultural soils.

In the last 10 years, many works have been done on *Miscanthus* species with a special emphasis on water and nutrient use efficiencies and on their ability to tolerate abiotic stresses such drought and chilling temperature (Zub and Brancourt Hulmel, 2010). In North America, Europe, and Asia, some intra- and inter-specific commercial cultivars have been identified for biomass production (Clifton-Brown and Lewandowski, 2002; Yan et al., 2012; Sacks et al., 2013). Much knowledge and progress have been gained in these fields in order to breed suitable genotypes depending on expected biomass uses and local environmental conditions (Gauder et al., 2012; Robson et al., 2013; Slavov et al., 2013). However, up to date, there are still very few works on the ability of *Miscanthus* species to grow on metal-contaminated soils. Moreover, there is a need to fully appraise the potential influence of different miscanthus cultivars and agronomic practices on metal mobility into the soils and accumulation into plant organs to maximize biomass production and to decrease pollutant linkages in soils (Tang et al., 2012).

Among the agronomic practices, plantation density and soil amendments allow mitigating negative effects of multiple stresses on plant performance, hence increasing the establishment rate and productivity (Singh et al., 2011). For trees and seeded crops, plantation density and fertilization increase the yields during the establishment phase in contaminated soils (Kidd et al., 2014). The mycorrhizal fungi play a key role in mineral uptake by plants, especially in nutrient (e.g., P-deficient conditions (Leung et al., 2013). Moreover, in metal-contaminated soils, these fungi can influence metal uptake but the extent to which this happens depends on plant species and cultivars, and on fungal species and/or strains (Göhre and Paszkowski, 2006).

The present study addresses two main questions: (1) Can metal accumulation and productivity in *M.* × *giganteus* differ among cultivars? (2) Do planting density, arbuscular mycorrhizal fungi (AMF) and nitrogen fertilization have effects on biomass production and metal (Cd, Pb, and Zn) accumulation in *M.* × *giganteus* grown in highly contaminated field conditions?

2. Materials and methods

2.1. Description of the experimental plot

The studied experimental agricultural plot is located at Evin-Malmaison (50°26′15.0″N, 3°01′05.7″E), at approximately 1 km from the former lead smelter, Metaleurop Nord. The site landscape presents a high degree of anthropization with residential suburbs, agricultural and woodlands, and transport networks. The soil physico-chemical parameters and metal concentrations are presented in Table 1. The soil is a clay sandy loam dominated by silt (53%), and with a slightly alkaline pH. The total carbonate, organic carbon, total nitrogen, and P_2O_5 contents are higher in top horizon than in deep horizons. The soil metal contamination is restricted to ploughed horizon (0–30 cm). Obviously, the soil is mainly contaminated by Cd, Pb, and Zn at concentrations of 14.1 ± 1.4 mg kg^{-1}, 731 ± 67 mg kg^{-1} and 1000 ± 88 mg kg^{-1}, respectively. These concentrations are 33, 23 and 15-fold (for Cd, Pb, and Zn, respectively) higher than their concentrations in regional uncontaminated agricultural topsoils (Sterckeman et al., 2002).

2.2. Miscanthus pre-growth in greenhouse

Three different miscanthus (*M.* × *giganteus*) cultivars, named Mis-A, Mis-B, and Mis-I were used in this experiment. Mis-B and Mis-A were respectively supplied by Bical France (currently NovaBiom, France) and Rhizosfer (Brienne sur Aisne, France). The Mis-I plants were supplied by a private farmer. Prior to field planting, the rhizomes of miscanthus cultivars were propagated in greenhouse. The rhizome fragments with 2–3 buds were placed in polyethylene pots containing potting soil. They were watered regularly.

2.3. Experimental design and miscanthus field growth

Soil tillage was conducted early in spring 2010. Thereafter, the plot was designed into a randomized split-plot comprising 6 blocks. Each block was divided into 12 subplots. Depending on treatments, a subplot (4 m × 10 m) incorporated four variables, namely the cultivar, the plantation density, the AMF inoculation and the

Table 1
Soil physico-chemical parameters and metal concentrations on the plot before miscanthus plantation. Values are means ± standard deviations of the analyzed composite soil samples, $n = 3$. The different letters represent significant differences (Tukey HSD test, $p \leq 0.05$).

	0–30 cm	30–60 cm	60–90 cm
Agronomic parameters			
Clay (%)	19.5	28.2	26.2
Silt (%)	53	39.1	29.4
Sand (%)	27.5	32.7	44.4
pH (H_2O)	8.2 ± 0.1	8.0 ± 0.1	8.1 ± 0.1
Carbonates (g kg^{-1})	10.2 ± 3.3	< 1	1.6 ± 0.4
Total C (g kg^{-1})	18.2 ± 0.4	3.6 ± 0.7	2.5 ± 0.6
Total N (g kg^{-1})	1.20 ± 0.03	0.43 ± 0.07	0.31 ± 0.06
C/N	15.2 ± 0.4	8.3 ± 0.3	7.7 ± 0.6
P_2O_5 (g kg^{-1})	0.16 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
CEC (cmol$^+$ kg^{-1})	14.9 ± 1.6	18.9 ± 1.9	18.0 ± 0.8
Metal concentrations (mg kg^{-1})			
Cd	14.1 ± 1.4^a	0.44 ± 0.06^{ab}	0.36 ± 0.08^b
Pb	731 ± 67^a	25.1 ± 2.4^b	24.6 ± 2.0^b
Zn	$1,000 \pm 88^a$	70.8 ± 9.2^b	65.4 ± 6.0^b
Co	9.3 ± 0.7^a	11.4 ± 0.3^c	11.1 ± 0.1^b
Cr	61.1 ± 3.1^a	63.6 ± 1.9^a	72.9 ± 16.9^a
Cu	36.8 ± 1.1^b	11.3 ± 1.8^{ab}	10.2 ± 2.0^a
Mo	0.54 ± 0.01^a	0.48 ± 0.07^a	0.38 ± 0.06^a
Ni	20.0 ± 1.9^a	26.5 ± 4.5^a	25.9 ± 5.8^a
Ti	0.72 ± 0.05^b	0.42 ± 0.08^{ab}	0.39 ± 0.07^a