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Abstract

Lignocellulosic energy crops matter due to their ability to grow on contaminated and degraded soils, producing biomass for biofuels and thereby reducing the pressure on the limited arable lands. *Sorghum bicolor* (L.) Moench can potentially produce a high biomass suitable for bioethanol, renewable gasoline, diesel, and sustainable aircraft fuel production on contaminated soils, despite adverse environmental conditions (e.g. drought) while phytoextracting relevant amounts of Cd, Pb and Zn. This field study aimed at assessing the *S. bicolor* growth on a metal-contaminated (11 mg Cd, 536 mg Pb and 955 mg Zn kg^{-1}) agricultural soil amended with humic/fulvic acid alone (HFA) and paired with arbuscular mycorrhizae fungi (HFAxAMF). The 2-year field trial consisted of three treatments in triplicates: control (C), HFA and HFAxAMF. After harvest, the shoot dry weight (DW) yield, ionome, and metal uptake of *S. bicolor* and the 0.01M $Ca(NO_3)_2$-extractable soil Cd, Pb and Zn concentrations were annually determined. The HFA and HFAxAMF treatments did not significantly affect the shoot DW yield and metal uptake. Sorghum produced an average of 12.4 t DW ha^{-1} $year^{-1}$ despite experiencing a severe drought season in year 1. Its growth contributed to decrease 0.01 M $Ca(NO_3)_2$-extractable soil Cd, Pb and Zn concentrations by 95%, 73% and 95%, respectively, in year 2. The annual shoot Cd, Pb and Zn removals averaged 0.14, 0.20 and 1.97 kg ha^{-1}, respectively. This evidenced *S. bicolor* as a relevant plant species for phytomanaging a large area with metal-contaminated soil, such as those near the former Pb/Zn Metaleurop Nord smelter, amidst ongoing climate change.

Keywords: Climate change, phytoremediation, biofuel, humic/fulvic acids, arbuscular mycorrhizae fungi

1. Introduction

Industrial activities, including mining and smelting, and agriculture are among the main drivers of anthropogenic soil metal(loid) contamination. Areas near the contaminant sources are prone to soil and/or air pollution. This poses potential health risks not only to humans but also to plants, microorganisms and animals. In particular, arable land around the former Pb/Zn Metaleurop Nord smelter (http://fr.safir-network.com/) are contaminated with various metal(loid) concentrations, this contamination mainly occurring in the ploughed soil horizon (0-25 cm) (Sterckeman *et al*. 2002). This was mainly due to the metal(loid)-enriched dust emissions and atmospheric fallouts that occurred during the operation of the Pb/Zn smelter until its closure in 2003. Some food crops are now restricted in this agricultural area as the topsoil Cd, Pb and Zn concentrations are 20-50 times higher than the regional background levels for agricultural soils (Sterckeman et al. 2002).

Phytomanagement strategies have been employed to manage the contaminated soils in this vast area (around 750 ha) since conventional soil remediation techniques such as the “dig and dump” are not feasible in this context. The main phytomanagement option implemented in this area was based on phytostabilization, i.e. the use of metal(loid) excluder and tolerant plant species potentially able to reduce both the bioavailability of metal(loid)s in the root zone and their transfer by natural agents. Accordingly, several plant species have been cultivated, including woody species, i.e. *Robinia pseudoacacia* L., *Alnus glutinosa* L., *Quercus robur* L., *Acer pseudoplatanus* L., *Ostrya carpinifolia* Scop., 1772, *Pterocarya stenoptera* C.DC., 1862, *Salix alba* L. and *Populus sp.* (regarding Cu and Pb for Salicaceae) (Lopareva-Pohu et al. 2011; Pourrut et al. 2011; Berthelot et al. 2016; Ciadamidaro et al. 2019), *Miscanthus* x *giganteus* J.M.Greef & Deuter ex Hodk. & Renvoize, 2001 (Nsanganwimana et al. 2015, 2016; Al Souki et al. 2017), *Cannabis sativa* L. (Bidar et al. 2019), and aromatic plant species i.e. *Coriandrum sativum* L. and *Salvia sclarea* L. (Raveau et al. 2020; Lounès-Hadj Saharoui and Fontaine, 2021). Another phytomanagement strategy for metal(loid)-contaminated soil is phytoextraction, i.e. the use of metal-tolerant plant species to uptake metal(loid)s from the soil into their shoots (Burges et al. 2018; Reeves et al. 2021). Several metal hyperaccumulators (e.g. for Cd: *Arabidopsis halleri* (L.) O'Kane & Al-Shehbaz, Grignet et al. 2022) and accumulators (e.g. regarding Cd and Zn: poplar clones, Skado (*Populus trichocarpa* x *P. maximowiczii*) and I-214 (*P. deltoides* x *P. nigra*), Phanthavongsa et al. 2017) were *in situ* cultivated in the Hauts-de-France. Hyperaccumulators generally have low biomass with high shoot metal(loid) concentrations while accumulators display the reverse (Moreira et al. 2021; Mench et al. 2018; Kidd et al. 2015; van der Ent and Rylott 2024). The high shoot DW yield of accumulators, such as sorghum regarding Cd and Zn, offers an economic value for the landowner hence making phytoextraction an attractive management strategy for metal-contaminated soils (Suman et al. 2018). The valorization pathways for these plant biomasses include bioenergy, biochar, fibers, essential oils, and bio-based construction materials.

Among the plant species used for phytomanagement, lignocellulosic energy crops are gaining an increased interest. This can be attributed to the shift from fossil fuels to biofuels as part of efforts to combat climate change and address global warming (European Commission, 2015). Lignocellulosic energy crops can grow on contaminated and marginal land thereby freeing up arable soils for agricultural purposes and reducing the competition between food and energy crops for the limited arable land (Mellor et al. 2021). This phytomanagement option is relevant as the potential exposure pathways through soil solution, soil erosion and leaching are managed while producing high biomass that can be locally processed for the bioenergy sector. Such lignocellulosic energy crops include miscanthus, industrial hemp, giant reed grass, poplar, willow, sorghum and switchgrass. *Phalaris arundinacea* L., bamboo, sugar cane, etc. (Courchesne et al. 2017; Moreira et al. 2021).

Sorghum bicolor is gaining attention as a relevant lignocellulosic crop for phytomanagement because it requires low agricultural input and is tolerant to high soil metal(loid) concentrations and harsh environmental conditions such as drought (Al Chami et al. 2015). Sorghum can produce a yield of roughly 30 tons DW ha^{-1} $year^{-1}$ on low quality soils with limited water supply (Renewable Energy World, 2000). It can accumulate Cd, Pb and Zn in its shoots (up to 8 Cd mg, 150 Pb mg and 320 Zn mg kg^{-1}) (Al Chami et al. 2015; Zhuang et al. 2009; Liu et al. 2020; Perlein et al. 2021). The possible valorization for its biomass includes bioethanol and biogas production, biochar, pulp and pyrolysis oil (Al Chami et al. 2015). This makes sorghum a relevant candidate for phytomanagement of contaminated soils in the context of climate change. Perlein et al. (2021) used *S. bicolor* to phytomanage contaminated dredged-sediments in the North of France (Hauts-de-France), reporting it was adapted to the current climatic conditions in this region. There are in this latter many metal(loid)-contaminated land, which include agricultural soils around the former Pb/Zn Metaleurop Nord smelter (Sterckeman et al. 2002). However, to our knowledge, no field study has been carried out with *S. bicolor* for phytomanaging metal(loid) contaminated agricultural soils in the Hauts-de-France region. Given the benefits of cultivating *S. bicolor* and its adaptation to the edaphic conditions in this region, it would be relevant to assess its use for phytomanaging metal(loid)-contaminated agricultural soil in a long-term field trial.

Addition of biostimulants such as humic/fulvic acids (HFA) and arbuscular mycorrhizae fungi (AMF) to metal-contaminated soil can improve plant growth, increase shoot yield and reduce pollutant linkages by either favouring metal(loid) uptake from the topsoil or immobilizing them (Bartucca et al. 2022; Wiszniewska et al. 2016; Phanthavongsa et al. 2017; Lounés-Hadj Sahraoui et al. 2022). In a previous pot experiment, HFA and its combination with AMF (HFAxAMF) decreased Cd and Zn concentrations in the soil pore water (SPW) and increased shoot DW yields of hemp and miscanthus growing on the metal-contaminated soil collected near the former Pb/Zn Metaleurop Nord smelter (11 mg Cd, 536 mg and 955 mg Zn kg^{-1}) (Ofori-Agyemang et al. 2024). This previous study was however limited to a 3-month period in a controlled environment, highlighting the need for further testing in a field trial.

This current 2-year field trial was carried out on the same metal(loid)-contaminated agricultural soil, near the former Pb/Zn Metaleurop Nord smelter (Hauts-de-France). It aimed to assess the phytomanagement of this soil by *S. bicolor* and evaluate the effects of humic/fulvic acids, alone and paired with arbuscular mycorrhizae fungi, on the production of shoot biomass, the shoot ionome, changes in the bioavailability of Cd, Pb and Zn in the soil and their uptake by shoots.

2. Materials and methods

2.1. Site description and experimental setup

A field trial of 0.07 ha was set up on a contaminated agricultural site located in Evin-Malmaison, approximately 700 m North of the former Metaleurop Pb/Zn smelter (France, Hauts-de-France, 50°26'17.3"N 3°01'05.8"E). The soil is contaminated with high concentrations of Cd, Pb and Zn due to the smelter past activities. The soil physico-chemical properties and metal concentrations are presented in Table 1. The area is characterized by a temperate climate, a mean temperature of 18.4°C and 18.5°C between May and September for both the first and second growing seasons in 2022 (year 1) and 2023 (year 2), respectively. A cumulative precipitation of 7.4 mm and 62.5 mm was recorded for the year 1 and year 2, respectively (InfoClimat, 2023). The plot tillage was carried out in early spring 2022 for the year 1. The trial was designed as a randomized split-plot arrangement comprising nine plots, with three plots for each treatment. Each plot measured 81 m^2 (9 m x 9 m). To mitigate herbivory and prevent encroachment by unauthorized individuals, the site was enclosed by a 2m-high fence (Figure 1). The experimental setup for the year 1 was replicated in the year 2. Prior to the field trial, this land has been cultivated with sugar beets, rapeseed and cereal crops such as wheat and barley.

2.2. Plant and treatments

The *S. bicolor* cv. Bulldozer seeds were obtained from the University of Bologna, Italy through Dr. Walter Zegada-Lizarazu. Sowing was conducted using a manual seeder in May 2022 for year 1 and May 2023 for year 2, with seeds roughly placed 5 cm deep. For each plot, 14 rows spaced 55 cm apart were implemented. Eighty sorghum seeds distanced 10 cm apart were sown to reach a plant density of 140

000 plants ha^{-1}. Manual weeding was performed at the early stages of the plant development to ensure the plantlet establishment.

Three soil treatments were assessed based on the previous pot experiment (Ofori-Agyemang et al. 2024): an unamended soil to serve as control plots (C), humic/fulvic acids (HFA) and the combination of humic/fulvic acids and mycorrhizae fungi (HFAxAMF). The humic/fulvic acids used was Lonite 80SP® (a water-soluble powder-based humic product) produced by Alba Milagro (Italy). The HFA product comprised both humic acid (75% w/w) and fulvic acid (5%w/w). A commercial arbuscular mycorrhizal fungi mix, Symbivit® obtained from Symbiom, Czech Republic was used in the trial. The mix was in granular form containing five AMF fungi (*Rhizophagus irregularis, Funneliformis geosporum BEG199, Funneliformis mosseae, Claroideoglomus lamellosum* and *Septoglomus deserticola*). The minimum number of spores was 200 per g. Application of mycorrhizae fungi involved applying 20 g m^{-1} of the inoculum along the planting furrow before seeding. The HFA was applied twice by diluting the product to 5 g per m^2 of soil. The first application was made on the topsoil when the plant reached the 4-6 leaf stage of development and the second one was four weeks after the first application.

2.3. Plant and soil sampling

Plants (shoots) and soil samples were collected in early October 2022 for year 1 (T1) and early October 2023 for year 2 (T2). Five sampling points were randomly selected for each plot following a quincunx pattern (Figure 2). To minimize foliar contamination from dust particles, sampling avoided the edges of plot. At each sampling point, one linear meter was measured along the planting furrow. The maximum shoot length of plants along the linear meter was measured before their harvest. Shoots were cut 10 cm above the ground level. The shoot was then divided into stems (consisting of the leaves and stem) and inflorescences. Onsite, the fresh weight (FW) of the plant samples was determined and samples were placed in paper bags for transportation. These samples were washed with tap water, rinsed in distilled water and divided into two batches; one was oven dried at 40°C for three days to determine metal (Cd, Pb and Zn) concentrations in the plant parts whereas the other one was dried at 105°C to determine the dry weight (DW) and moisture content of the plant samples. For each sampling point, a 2 kg composite soil sample comprising 4 cores of 500 g soil was taken from the first 25 cm soil layer using an auger.

2.4. Soil and plant analysis

Soil samples (sieved at 2 mm) were dried at 40°C and ground using an ultracentrifuge mill (ZM200, Retsch) to obtain a 250 µm particle size. Soil pH (H_2O) was determined according to ISO 10390. Some soil physicochemical properties were performed in an external laboratory (SADEF, Aspach le Bas, France). They include total carbonates (NF ISO 10 693), organic matter (NF ISO 14235), total organic carbon (NF ISO 14235), total nitrogen (NF ISO 13878), Cation Exchange Capacity (CEC) Metson (NFX 31-130), available P (P_2O_5) (NF ISO 11263-Olsen) and exchangeable Ca (CaO), Mg (MgO), K (K_2O), Na (Na_2O) were determined according to NFX 31-108. The pseudo-total soil Cd, Pb and Zn concentrations were determined after acid digestion in *aqua regia* ($HCl:HNO_3$, 3:1 v/v, 6 mL) of 300 mg of soil (ground and sieved at 250 µm) using a digestion block at 95°C for 75 min. After cooling, the volume was adjusted to 25 mL with double-distilled water and the solution was filtered (ash-free 0.45 mm cellulose acetate filters). The quality control of the extraction and analysis was provided by the introduction of two internal reference samples and a certified soil reference (CRM 141, IRMM, Belgium). The $Ca(NO_3)_2$-extractable soil Cd, Pb and Zn were determined according to a modified version of Sappin-Didier et al. (1997). The dry soil (sieved at 2 mm) was mixed with a 0.01 M $Ca(NO_3)_2$ solution in a ratio of 1:5 m/v for 2 h, and then filtered.

Plant samples were grounded (<1 mm particle size, Retsch MM200). For each plant sample, a 300 mg aliquot was instantly weighed into a digestion tube. Concentrated nitric acid (HNO_3, 70%, 5 mL) was added to the sample and manually shaken. Afterwards, the aliquot was heated at 95°C for 75 min using a digestion plate (HotBlock − 36-Position, 50 mL, Environmental Express, USA). After cooling, hydrogen peroxide (H_2O_2, 30%, 5 mL) was added and the mixture was heated again at 95°C for 3 h. Certified reference material (Polish Virginia Tobacco Leaves, INCT-PVTL-6, Poland) was used to check the accuracy and precision of the analytical determination of metals (Cd, Pb, and Zn). Each digested aliquot was adjusted to 25 mL using osmotic water and filtered using a 0.45 µm cellulose acetate membrane.

The Cd, Pb and Zn concentrations in both *aqua regia* and $Ca(NO_3)_2$ soil extracts and plant sample digests were measured by atomic absorption spectrometry (AAS) (AA-6800, Shimadzu, Japan).

The bioconcentration factor (BCF) of sorghum was calculated using the shoot metal concentration and either the pseudo-total (BCF_{tot}) or $Ca(NO_3)_2$-extractable (BCF_{ext}) metal concentration in the soil.

$$BCF_{tot} = \frac{shoot\ metal\ concentration}{total\ metal\ concentration\ in\ soil}$$

$$BCF_{ext} = \frac{shoot\ metal\ concentration}{extractable\ metal\ concentration\ in\ soil}$$

2.5. Determination of root mycorrhizal colonization

Fresh root samples of *S. bicolor* were collected with a spade a day after the harvest of the aboveground biomass and stored at 4°C for analyzing the root mycorrhizal colonization. The roots were washed with tap water to remove all soil particles. The roots were then cleaned in KOH (10%) and stained with Trypan blue (0.05%) as described by Phillips and Hayman (1970) and modified by Koske and Gemma (1989). The root mycorrhizal colonization was then determined by the method of McGonigle et al. (1990).

2.6. Statistical analysis

The influence of soil treatments on the $Ca(NO_3)_2$-extractable soil Cd, Pb and Zn concentrations, shoot DW yields, shoot ionome, metal uptake by shoots and root mycorrhizal colonization rates were tested using a one-way analysis of variance (ANOVAs). The influence of the growing year and the treatments used on metal uptake was tested using a two-way ANOVA. Normality and homoscedasticity of residuals were met for all tests. Multiple comparisons of mean values were performed using post-hoc Tukey HSD tests when significant differences occurred between treatments. The relationship between the shoot DW yield, shoot ionome and the metal uptake was analyzed using a principal component analysis (PCA). All statistical analyses were performed using R software (version 4.1.2, Foundation for Statistical computing, Vienna, Austria).

3. Results

3.1. $Ca(NO_3)_2$-extractable soil Cd, Pb and Zn concentrations

The $Ca(NO_3)_2$-extractable soil Cd, Pb and Zn concentrations significantly decreased for all treatments after the 2nd growing season (year 2). Roughly a 95%, 73% and 95% decrease in Cd, Pb and Zn concentrations was determined across all treatments (Table 2). There were no significant differences for $Ca(NO_3)_2$-extractable Cd, Pb and Zn concentrations across all treatments.

3.2. Root mycorrhizal colonization rate

For year 1, the mycorrhizal colonization rate data could not be assessed due to inadequate preservation of the samples. Therefore, only the results for year 2 are provided. The total root mycorrhizal rates ranged from 54%, 60% to 73% for the C, HFA and HFAxAMF treatments, respectively (Figure 3). The sorghum roots displayed a good mycorrhizal colonization for both inoculated (HFAxAMF) and non-inoculated plots (C and HFA). The inoculated plot showed a numerically increased mycorrhization as compared to non-inoculated plots, albeit not significant.

3.3. Shoot DW yield and maximum shoot length

The shoot DW yield of sorghum in year 1 did not differ from that in year 2 across all treatments. However, an increasing trend was observed for the shoot yield recorded in year 2 compared to year 1 (Table 3). The shoot DW yield (t ha^{-1}) ranged from 11.5 ± 4.2 to 15.9 ± 2.7. The average height of the plants ranged from 2.5 to 2.8 m in year 1 and reached 3.3 – 3.4 m in year 2. There was an average increase in plant height of 0.7 m in year 2 (Table 3). The plant height was strongly positively correlated with the shoot yield (0.82, $p < 0.05$).

3.4. Shoot ionome

On average, the shoot Cd, Pb and Zn concentrations ranged between 6.26 – 10.57 mg kg^{-1}, 6.33 – 12.58 mg kg^{-1} and 88.45 – 124.28 mg kg^{-1}, respectively for both growing seasons. A decreasing trend was found in year 2 for the shoot Cd concentration while an increasing one occurred for the shoot Pb and Zn concentrations for all the treatments, even though the differences were not significant (Table 4). The highest BCF_{tot} values were recorded for Cd (0.95 for T1 and 0.70 for T2), followed by Zn (0.09 for T1 and 0.13 for T2) and the least was Pb (0.01 and 0.02 for T1 and T2, respectively) (Table 6). The BCF_{ext} calculated in this study were all above 1. The BCF_{ext} for Pb was low (1.23 – 2.45) compared to Cd (8.81 – 14.64) and Zn (22 – 30.27) (Table 6).

3.5. Metal (Cd, Pb and Zn) uptake in shoots

Shoot metal uptakes ranged between 0.10 – 0.14 kg Cd ha^{-1}, 0.07 – 0.20 kg Pb ha^{-1} and 1.05 – 1.97 kg Zn ha^{-1} for both years (Figure 4). The treatments did not significantly change the uptake of Cd, Pb and Zn in shoots while the growing season significantly influenced shoot Pb and Zn uptake and not shoot Cd uptake (Supplemental material 1). Shoot Cd uptake numerically decreased in year 2 for the unamended plots while an increasing trend was found for the HFAxAMF plots. An increase in shoot Pb and Zn uptake occurred for all treatments in year 2 mainly due to a higher shoot DW yield (Table 3).

4. Discussion

4.1. Changes in Ca(NO3)2-extractable soil metals

In year 2, precipitation levels exceeded the average values, which could have potentially increased $Ca(NO_3)_2$-extractable soil Cd, Pb, and Zn concentrations. Contrary to this expectation, a decrease in these was determined. Specifically, while $Ca(NO_3)_2$-extractable soil Cd and Pb concentrations remained high in year 2, those of Zn remained comparable to typical background values usually found in soils following the standard ISO 19730:2008 (E) with NH_4NO_3, i.e. (in mg kg^{-1}) Cd: 0.005, Pb: 0.02, and Zn: 0.25 (Perlein et al. 2021).

Soil pH is one crucial factor influencing the mobility of metal(loid)s in soil (Kabata-Pendias and Szteke, 2015). Throughout the two-year trial, the soil maintained an alkaline pH consistent across both amended (HFA and HFAxAMF) and unamended soils (C) (Supplemental material 2). This result was consistent with our previous findings in a pot trial where the soil pore water (SPW) pH remained slightly alkaline after the application of HFA and HFAxAMF (Ofori-Agyemang et al. 2024). In alkaline soils, the Cd, Pb and Zn mobility is reduced compared to neutral soil pH, which may limit the metal resupply from the soil bearing phases. Hence the decrease in $Ca(NO_3)_2$-extractable soil Cd, Pb and Zn could be partly attributed to the uptake of Cd, Pb and Zn by sorghum roots from the labile Cd, Pb and Zn pool in the soil. Sorghum has been shown to accumulate up to 8 Cd mg, 150 Pb mg and 320 Zn mg kg^{-1} from soils thereby depleting the available pool of these three metals. This phenomenon underscored the complex interplay between soil properties, plant physiology, and environmental factors in shaping metal dynamics in contaminated soils (Al Chami et al. 2015; Jia et al. 2016; Zhuang et al. 2009; Perlein et al. 2021).

4.2. Shoot DW yield of *S. bicolor*

The shoot DW yield was consistent across all treatments and years (Table 3) aligning with the findings of Perlein et al. (2021) who reported that *S. bicolor* is adapted to the climatic conditions of the Hauts-de-France region and is not adversely affected by diffuse metal(loid) contamination in soils. This suggested that the planted sorghum may have reached their maximum biomass production potential under the given conditions, despite experiencing severe drought conditions in year 1 (with only 7.4 mm cumulative rainfall from May to October 2022). This corroborated sorghum is a drought-resistant crop, a trait increasingly valuable in the current context of climate change and global warming (Steduto et al. 1997; Martinez-Uribe et al. 2020). Similar or even higher shoot DW yields were reported in other metal-contaminated areas with similar soil treatments (in t DW ha^{-1}), e.g. 10 – 30 at the Lavrion site and 24-34 at the Kozani site in Greece, 15 – 20 at the UMCS site in Poland, 10 – 15 at the Chiarini site in Italy (Papazoglou et al. 2024), and 29 in Brazil (Martinez-Uribe et al. 2020). Other field trials with contaminated soils evidenced similar high shoot DW yields (t DW ha^{-1}): e.g. 22.1 (Italy, Marchiol et al. 2007), 12.4 – 32.7 (China, Wang et al. 2023).

Sorghum resilience could be attributed to its extensive root system (1.5 – 2.5 m deep into the soil) enabling access to water and nutrients in deeper soil horizons (Anami et al. 2015). The extraradical mycelium developed around roots may also enhance the uptake of nutrients and water (Lounès-Hadj Sahraoui et al. 2022). While an increase in shoot biomass was not observed in the plots amended with mycorrhizae, the colonization of *S. bicolor* roots (Figure 3) by the arbuscular mycorrhizae fungi could also contribute to the improved shoot yield and water uptake in the face of drought. Moreover, this symbiotic relationship may prove particularly beneficial in conditions of even greater biotic or abiotic stress than those experienced in this study.

The application of humic/fulvic acids did not enhance the shoot yield of *S. bicolor* as compared to the unamended soil. Bulgari et al. (2015) posited that biostimulants only impact the plants if they penetrate the plant tissue. Based on Pecha et al. (2012) and Kolomaznik et al. (2012), the absorbability of biostimulants depends on the prevailing field conditions. The HFA may have not penetrated the plant tissues as it was added into the soil using the irrigation water and the biostimulants would have remained on the topsoil. This may prevent the roots from absorbing the biostimulants. In Brazil, biomass sorghum reached 4.3 m at hard grain stage (average overall diameter of stem: 18.5 mm), common range being 3.5 m to 4.30 m at flowering time (Martinez-Uribe et al. 2020), which matched with our year 2 data (Table 3).

4.3. Phytoextraction potential of *S. bicolor*

Visible symptoms, i.e. reddish to brown spots, in linear punctuation, on the outer parts of the leaf blades, especially on the lower (aged) leaves, were observed in year 1 and not in year 2. (Supplemental material 3). In a pot experiment with Cd-spiked soils and two *S. bicolor* cultivars, the Cd stress started to reduce the growth as the stem and leaf Cd concentrations exceeded 2 mg Cd kg^{-1} (Jawad Hassan et al. 2020). It increased the electrolyte leakage, hydrogen peroxide concentration and malondialdehyde content in both cultivars. For *Zea mays* L. (cv. Volga), another C4 plant species, the relationship between the peroxidase (POD; E.C. 1.11.1.7) activity and the Cd concentration evidenced 3 and 5 mg Cd kg^{-1} DW as upper critical threshold values respectively in the 3^{rd} and 4^{th} leaves (Lagriffoul et al. 1998). Here, the shoot Cd concentrations of sorghum averaged 10.4 (year 1) and 7.7 (year 2) mg Cd kg^{-1} DW at harvest across the treatments (Table 4) and exceeded the upper critical threshold values reported above for *S. bicolor* and *Z. mays*. Nevertheless, shoot DW yields were in the common range (Table 3).

The metal(loid) phytoextraction by a plant species depends on the harvested biomass yield and its metal(loid) concentrations (Eben et al. 2024). *Sorghum bicolor* is a relevant candidate for both biomass production and phytoextraction of metal(loid)s from contaminated topsoils as it produces a relevant shoot DW yield on contaminated soils and accumulates metals, i.e. Cd and Zn, to some extent in its shoots. Here, the decreasing trend observed for the shoot Cd uptake in year 2 in the unamended and HFA plots mainly resulted from the decreased shoot Cd concentrations (Table 4). The increase in shoot Pb and Zn concentrations of sorghum in contrast induced an increased shoot Pb and Zn uptake for year 2. The shoot Pb and Zn uptakes in our experiment were similar to the values recorded in field trials testing the phytoextraction potential of *S. bicolor* (Table 5). The shoot Cd uptake was 95%, 57% and 50% higher than those reported by Marchiol et al. (2007), Zhuang et al. (2009) and Perlein et al. (2021), respectively (Table 5). Marchiol et al. (2007) posited that the extent exposure to metal(loid) contamination is a key factor in phytoextraction. The total soil Cd in our experiment (Table 1) was twice that for Marchiol et al. (2007) and Zhuang et al. (2009), i.e. 4.9 and 4.3 mg Cd kg^{-1}, respectively. However, it would be more important to account for bioavailable soil Cd in these soils. A principal component analysis (PCA) performed on the shoot DW yields, shoot Cd, Pb and Zn concentrations and the phytoextracted Cd, Pb and Zn amounts showed a positive correlation between the shoot DW yield and both shoot Pb and Zn concentrations and uptake (Supplemental material 4). The shoot Cd and Zn uptake for sorghum in our study was slightly higher than the Cd uptake for tobacco and willow (0.11 and 0.12 kg Cd ha^{-1}, respectively) and slightly lower for Zn uptake (1.9 and 3.1 kg Zn ha^{-1}, respectively) as reported by Thijs et al (2018) for metal-contaminated soils in the Kempen, Belgium.

Humic/fulvic acids alone and paired with AMF did not significantly impact the shoot metal uptake compared to the unamended soils. This could be explained by the inability of the HFA to penetrate the plant tissue due to the application mode (addition into the soil). Previous studies evidencing an impact of HFA on shoot metal uptake were undertaken in controlled conditions hence this would allow for HFA penetration into the plant tissues under optimal conditions (Ofori-Agyemang et al. 2024; Shahid et al. 2012). However, the HFA absorption in an uncontrolled field trial (as in our case) is likely to be affected by weather conditions such as drought and other extrinsic factors (Kolomaznik et al. 2012). Cadmium phytoextraction by biomass sorghum (BS) has been assessed in subtropical farmland in China (Wang et al. 2023). The agronomic traits and Cd accumulation of BS plants depend on varieties. Maximum shoot length, in line with the shoot DW yield, and stem Cd concentration were together crucial for the shoot Cd uptake.

The bioconcentration factors (BCF_{tot}) for Cd, Pb and Zn were below 1 (Table 6), which agreed that sorghum exhibited an excluder behaviour (Perlein et al. 2021). The highest BCF_{tot} value occurred for Cd (from 0.70 to 0.94 on average, and up to 0.96 (Table 6)), which showed that sorghum is relatively a Cd accumulator as compared to other lignocellulosic crops such as miscanthus and hemp. In a pot trial, sorghum showed higher bioaccumulation characteristics ($BCF_{tot} > 4$) for Cd in a spiked Cd (3 – 15 mg Cd kg^{-1}) contaminated soil (Wang et al. 2017). The BCF_{tot} values for Cd and Zn of tobacco and sunflower grown on a soil with similar climatic conditions (the Belgian Kempen) were higher for Cd (2.1 – 7.9 for tobacco and 0.5 – 2.1 for sunflower) and Zn (0.8 – 2.2 for tobacco and 1.1 – 2.8 for sunflower) (Thijs et al. 2018) as compared to sorghum in this study. The BCF_{tot} value for Pb was very low (<0.1) for both tobacco and sunflower, which was similar to that of sorghum. Given the BCF_{ext} values for Cd, Pb and Zn, all well exceeded 1, which corroborated Perlein et al. (2021). The greatest accumulation was observed for Zn followed by Cd, and Pb being the least. The BCF_{ext} values in this study were lower as compared to those reported by Perlein et al. (2021). However, the extractable soil Cd and Pb concentrations in this study were 24 and 86 times higher than those in Perlein et al. (2021). Our results showed that Pb was less bioavailable to sorghum as compared to Cd and Zn. In soils, Pb has a strong affinity to soil organic matter (SOM) (Kabata-Pendias and Szteke, 2015). Hence Pb would be bound to the SOM in our studied soil, which was rich in organic matter and displayed an alkaline soil pH.

High Cl concentration (0.18% DW) was reported in the sorghum shoots, which at high temperatures can promote boiler corrosion (Martinez-Uribe et al. 2020). It depended on the plant growth stage, flowering displaying the lowest Cl content. Shoot Cl concentration reached $1.55 \pm 0.03\%$ DW in sorghum samples harvested in the GOLD project (Panopoulos, 2024). The crucial Cl role in the shoot Cd uptake is well known notably for sunflower and Swiss chard (Li et al. 1994; Smolders and McLaughlin, 1996). The K fertilization sources do not affect sorghum productivity, but using KCl results in higher shoot Cl content and potassium sulfate or nitrate can be alternatives to reduce it (Martinez-Uribe et al. 2020). Accordingly, K fertilization source would be one potential factor for driving shoot Cd concentration in sorghum. Another option would be the intraspecific variability between sorghum cultivars. Bai et al. (2021) suggested nitrate might regulate Cd accumulation through expression of SbNRT1.1B rather than SbNramp1, SbNramp5 and SbHMA3, the well-documented genes related to Cd uptake and transport in sweet sorghum.

In comparison, biomass sorghum cultivated on a metal-contaminated dredged sediment in the Hauts-de-France displayed lower shoot Cd and Pb concentrations and similar shoot Zn concentrations (Table 5, Perlein et al. 2021), with a similar soil pH, lower total soil Cd, and higher total soil Pb and Zn. According to Perlein et al. (2021), these shoot metal concentrations would be suitable for biogas production. However, the shoot Cd concentration in our case exceeded the Flemish regulation setting threshold values for metal(loid) concentrations in the biomass used for bioenergy production (6 mg Cd, 300 mg Pb and 900 mg Zn kg^{-1} , Meers et al. 2010). Nevertheless, the sorghum biomass harvested at a metal-contaminated site could produce a metal-free bioethanol, as most metals, notably Cd and Zn, remained in the residual solid phase of the production process (Vintila et al. 2016).

4.4. Practical implications

In the current landscape of climate change and the increasing competition of energy and food production in the context of limited available arable soils, *S. bicolor* ability to produce a yield of 11.5 - 15.9 t DW ha^{-1} on a multi metal-contaminated soil is particularly noteworthy (Table 3). Sorghum biomass grown on metal(loid) contaminated soils can be processed by pyrolysis, liquefaction and gasification to produce biofuels (Vintila et al. 2016; Stamenković et al. 2020; Liu et al. 2020; Xiao et al. 2021; Perlein et al. 2021). However, the high moisture content of the shoots at harvest (67%) may necessitate drying prior to their processing, depending on factors such as facility availability, storage, and operational profitability throughout the year. The biomass produced in this study will be transformed into sustainable clean biofuels in the framework of the GOLD H2020 project whose main goal is to valorize shoot biomass harvested on contaminated soils into clean biofuels (Panopoulos 2024).

In our experiments, *S. bicolor* exported 0.12 kg Cd ha^{-1}, 0.14 kg Pb ha^{-1} and 1.47 kg Zn ha^{-1} in one growing season (Table 5). This highly decreased the extractable soil Cd, Pb and Zn concentrations (Table 2), even though the similar shoot Cd, Pb and Zn uptakes in year 2 suggested a re-supply of the labile pools of these metals by the soil bearing phases. Moreover, capture of fine soil particles (foliar exposure) cannot be ruled out (fine dust included into the stomatal chambers cannot be washed). Nevertheless, this represented a significant progressive bioavailable metal stripping within a comprehensive long-term (phyto)remediation strategy. This was particularly noteworthy given the absence of land pressure at this location. It will require multiple growing seasons to remediate the plot to meet acceptable levels for the labile metal fractions, which we could recall are the primary targets rather than the total soil metal concentrations according to the site-specific remediation approach in France. Hence at these phytoextraction rates of Cd, Pb and Zn (5.73%, 0.88%, and 12.16%, respectively (Supplemental material 5)) by sorghum, the phytoavailable metal concentrations would be reduced to acceptable concentrations in consecutive growing seasons. In comparison to miscanthus, which was cultivated in a field trial with the same metal-contaminated soil as in this study, sorghum shoots accumulated 5 times and 6 times more of Cd and Pb than miscanthus ones (1 – 1.7 mg Cd, 1.5 – 2 mg Pb kg^{-1}, Nsanganwimana et al. 2015). This suggested sorghum as a relevant lignocellulosic energy crop to be considered for phytomanaging metal(loid) contaminated soils.

As sorghum is an annual crop, integrating leguminous cover crops (e.g. alfalfa, white clover, common vetch, or fava bean) into a crop rotation system could enhance nitrogen and carbon sequestration, as well as improve soil moisture retention and fertility (Garcia-Gonzalez et al., 2018; Moreira et al., 2021). This practice would not only aid in managing soil contamination but also may enhance soil functionality and ecosystem health, thereby promoting ecosystem services.

5. Conclusion and perspectives

This 2-year field trial confirmed sorghum as a drought and metal-tolerant crop adapted to the pedoclimatic conditions of the Hauts-de-France region. Given that sorghum biomass production was already close to its maximum potential, the application of humic/fulvic acids alone and paired with arbuscular mycorrhizae fungi did not significantly improve the shoot DW yield and shoot metal uptakes compared to the unamended plots. However, it would be beneficial to continue testing these treatments, especially in the context of increasing abiotic stresses such as longer drought period due to climate change, which could increasingly impact biomass production. Also exploring alternative application methods for HFA, notably foliar application, could enhance the effectiveness of these treatments by facilitating better penetration into plant tissues. The sorghum cultivation significantly decreased the extractable soil Cd, Pb and Zn concentrations while been able to extract 0.12 kg Cd ha^{-1}, 0.14 kg Pb ha^{-1} and 1.47 kg Zn ha^{-1} in one growing season in our experiment. This result was particularly noteworthy, considering successive growing seasons would lead the labile metal fractions in the soil at this site to acceptable levels, which are the primary targets of phytoextraction rather than total soil metals. The harvested biomass will be processed into clean biofuels by the partners of the GOLD project, their results being pending. The influence of growing sorghum on metal-contaminated soils on ecosystem services other than provisioning must be investigated as well. Pathogen attacks on sorghum plants were not detected and fencing prevented herbivory damage. There are at least two limits to sorghum biomass production; there is the need for local facilities capable of processing it into valuable products, and herbicide application to control weeds during germination and the first few weeks of growth. In our case water supply and distribution along the growing period was enough, but it might however be a key point elsewhere.

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Table 1

Physico-chemical properties of the contaminated soil as compared to unpolluted regional agricultural soils (pedogeochemical background levels).

Parameters	Units	Contaminated soil	Uncontaminated agricultural soils
Clay		180.6 ± 8.8	180[1]
Fine silt		191.6 ± 10.2	245[1]
Coarse silt	g kg^{-1}	364.8 ± 7.1	447[1]
Fine sand		224.3 ± 11.3	93[1]
Coarse sand		39.0 ± 2.9	35[1]
pH		7.91 ± 0.21	6.8[1]
EC	µS cm^{-1}	122± 17	-
CEC Metson	$cmol^{+}$ kg^{-1}	13.6 ± 0.8	-
Organic carbon	g kg^{-1}	21.02 ± 3.97	18.6[1]
Organic matter	g kg^{-1}	36.38 ± 6.86	32.17[1]
Total carbonates	g kg^{-1}	4.18 ± 4.25	4[1]
Total nitrogen	g kg^{-1}	1.32 ± 0.18	2.02[1]
C/N		15.88 ± 0.88	9.2[1]
Exchangeable K_2O (NFX 31 -108)		0.36 ± 0.08	0.19[1]
Exchangeable MgO (NFX 31 -108)		0.14 ± 0.02	0.21[1]
Exchangeable CaO (NFX 31 -108)	g kg^{-1}	5.63 ± 0.95	4.04[1]
Exchangeable Na_2O (NFX 31 -108)		0.02 ± 0.01	0.03[1]
Extractable P_2O_5 (Olsen)		0.11 ± 0.03	0.22[1]
Total Cu		25 ± 3	16.7[2]
$Ca(NO_3)_2$-extractable Cu[3]		1.65 ± 0.77	-
Total Cd		11 ± 2	0.42[2]
$Ca(NO_3)_2$-extractable Cd[3]	mg kg^{-1}	0.71 ± 0.16	-
Total Pb		536 ± 70	38.4[2]
$Ca(NO_3)_2$-extractable Pb[3]		5.13 ± 1.84	-
Total Zn		955 ± 151	73.7[2]
$Ca(NO_3)_2$-extractable Zn[3]		4.02 ± 3.22	-

[1]Aligon and Douay (2011); [2]Sterckeman et al. (2002); EC: electrical conductivity; CEC: cation exchange capacity. Results are expressed as mean ± standard deviation (*n*=4). [3] 0.01 M $Ca(NO_3)_2$.

Table 2. 0.01 M $Ca(NO_3)_2$-extractable soil Cd, Pb and Zn concentrations before (T0) and after (T2) cultivation of sorghum in year 2.

	Cd (mg kg^{-1}) DW	**Pb (mg kg^{-1}) DW**	**Zn (mg kg^{-1}) DW**
Before cultivation (T0)	0.71 ± 0.16b	5.13 ± 1.84b	4.02 ± 3.22b
After cultivation (T2)			
Control	0.033 ± 0.003a	1.35 ± 0.11a	0.18 ± 0.04a
Humic/fulvic acids (HFA)	0.034 ± 0.007a	1.52 ± 0.07a	0.18 ± 0.02a
Humic/fulvic acids x arbuscular mycorrhizae fungi (HFA x AMF)	0.036 ± 0.002a	1.33 ± 0.11a	0.24 ± 0.08a

Table 3. Shoot DW yield and maximum shoot length (height) of sorghum during two growing seasons (*n*=3). Significant differences between treatments are indicated by different letters at the level of α = 0.05.

	Yield (t DW ha^{-1})		**Height (m)**	
	Year 1	Year 2	Year 1	Year 2
Control	13.9 ± 3.1a	15.7 ± 3.1a	2.8 ± 0.2a	3.3 ± 0.3a
HFA	11.8 ± 1.9a	14.3 ± 1.3a	2.5 ± 0.2a	3.3 ± 0.2b
HFA x AMF	11.5 ± 4.2a	15.9 ± 2.7a	2.7 ± 0.4a	3.4 ± 0.1b

HFA = Humic/fulvic acids; HFA x AMF = Humic/fulvic acids x arbuscular mycorrhizae fungi

Table 4. Shoot ionome of sorghum in year 1 (T1) and year 2 (T2) (*n*=3).

Treatment	**Cd (mg kg^{-1} DW)**		**Pb (mg kg^{-1} DW)**		**Zn (mg kg^{-1} DW)**	
	T1	T2	T1	T2	T1	T2
Control	10.25 ± 2.62a	6.26 ± 1.13a	8.49 ± 1.42a	10.00 ± 2.31a	88.45 ± 11.38a	113.52 ± 18.92a
Humic/fulvic acids (HFA)	10.40 ± 1.86a	8.09 ± 0.57a	6.33 ± 1.52a	12.10 ± 2.78a	88.90 ± 9.87a	121.68 ± 4.00b
Humic/fulvic acids x arbuscular mycorrhizae fungi (HFA x AMF)	10.57 ± 3.73a	8.73 ± 1.15a	7.30 ± 1.69a	12.58 ± 2.53a	92.11 ± 12.41a	124.28 ± 2.57b
Perlein et al. 2021	1.25 – 3.25		0.5 – 0.8		140 - 200	
BCF						

Control

B2

MB2

Table 5. Phytoextraction of Cd, Pb and Zn by *S. bicolor* shoots in field trials

Reference	Biomass (t ha^{-1} DW)	Cd (kg ha^{-1})	Pb (kg ha^{-1})	Zn (kg ha^{-1})
Zhuang et al. 2009	25.8	0.052	0.35	1.44
Marchiol et al. 2007	22.1	0.006	0.38	1.22
Perlein et al. 2021	9.85 – 21.75	0.03– 0.09	0.02 – 0.06	1.71 – 5.40
Wang et al 2023	12.4 – 32.7	0.030 (0.005 – 0.092)		
This study	11.5 - 15.9	0.10 – 0.14	0.07 – 0.20	1.05 – 1.97

Table 6. BCF_{tot} and BCF_{ext} values of sorghum in year 1 (T1) and year 2 (T2) (*n*=3). Significant differences between treatments are indicated by different letters at the level of $\alpha = 0.05$(lower case for BCF_{tot} ; upper case for BCF_{ext}).

	BCF_{tot}						**BCF_{ext}**					
Treatment	Cd		Pb		Zn		Cd		Pb		Zn	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
Control	0.931 ± 0.238a	0.568 ± 0.103a	0.016 ± 0.003a	0.019 ± 0.004a	0.093 ± 0.012a	0.119 ± 0.020a	14.43 ± 3.68A	8.81 ± 1.60B	1.66 ± 0.28A	1.95 ± 0.45A	22.00 ± 2.83A	28.24 ± 4.71B
HFA	0.945 ± 0.169a	0.735 ± 0.051a	0.012 ± 0.003a	0.023 ± 0.005b	0.093 ± 0.010a	0.127 ± 0.004b	14.64 ± 2.62A	11.39 ± 0.80B	1.23 ± 0.30A	2.36 ± 0.54B	22.12 ± 2.46A	30.27 ± 0.99B
HFA x AMF	0.961 ± 0.339a	0.794 ± 0.104a	0.014 ± 0.003a	0.023 ± 0.005b	0.096 ± 0.013a	0.130 ± 0.003b	14.89 ± 5.25A	12.30 ± 1.62B	1.42 ± 0.33A	2.45 ± 0.49B	22.91 ± 3.09A	30.92 ± 0.64B

BCF_{tot} = Bioconcentration factor based on (pseudo)total soil metal ; BCF_{ext} = Bioconcentration factor based on 0.01M $Ca(NO_3)_2$-extractable soil metal; HFA = Humic/fulvic acids; HFA x AMF = Humic/fulvic acids x arbuscular mycorrhizae fungi

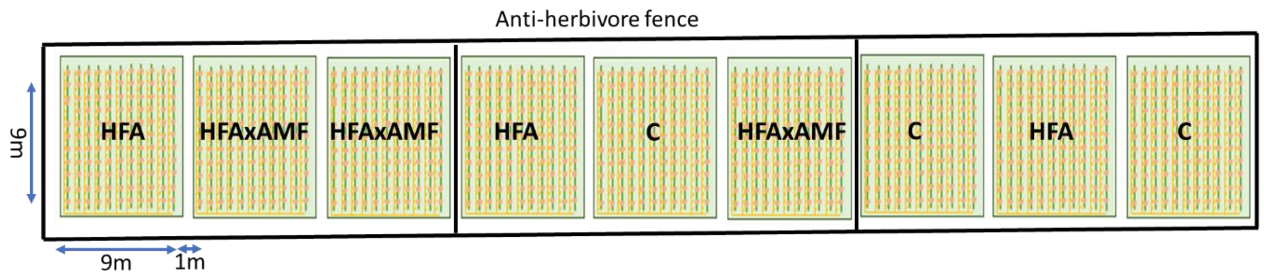

Figure 1: Design of the experiment showing dimensions of plots

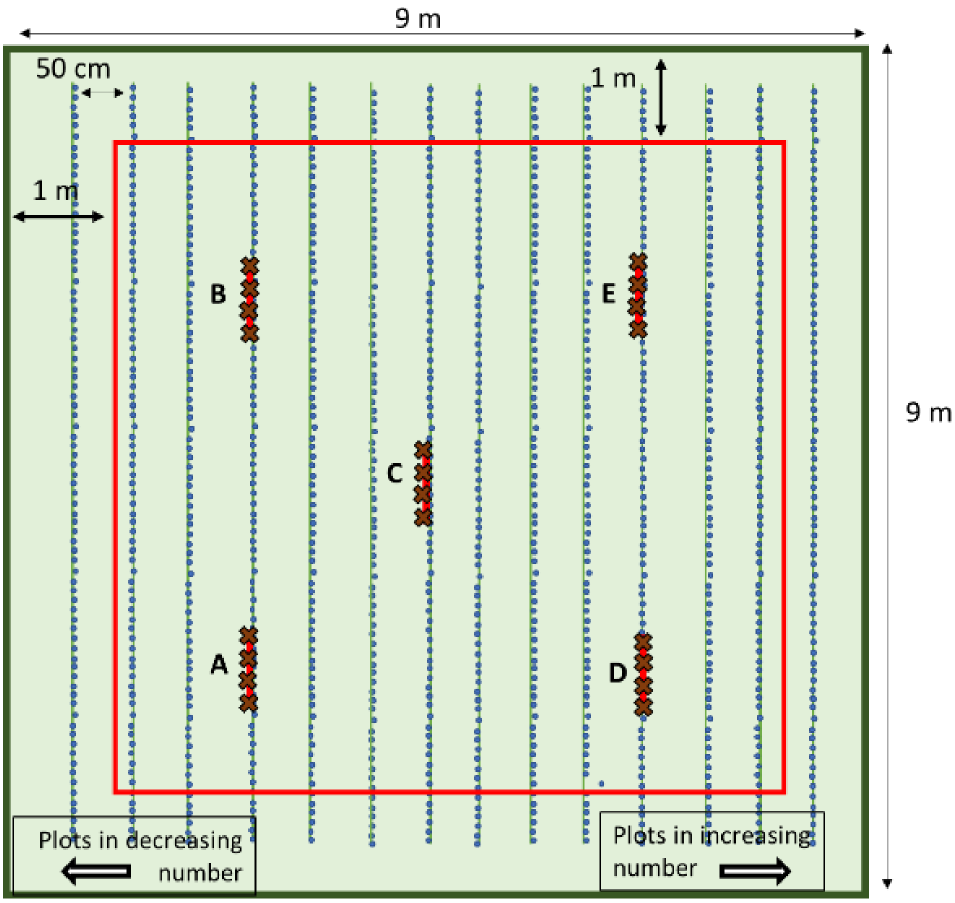

Figure 2. Sampling design adapted per plot

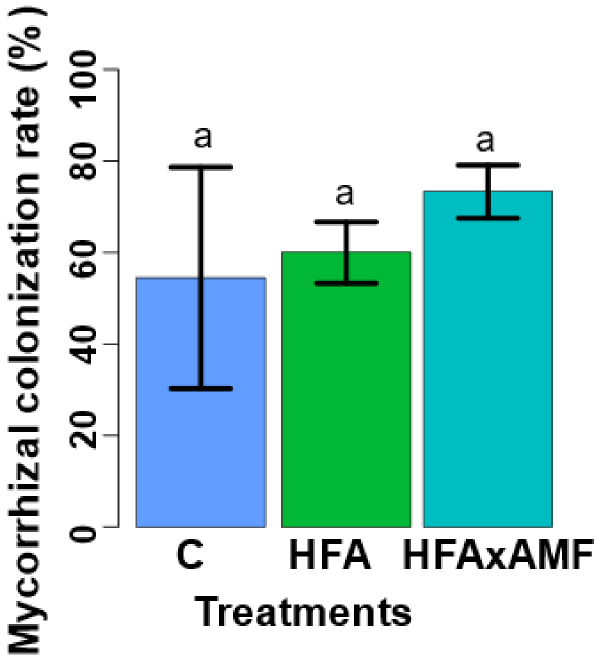

Figure 3. Mycorrhizal colonization rates of the sorghum roots during the 2nd growing season. Untreated soils (C), humic/fulvic acids treatment only (HFA) and humic/fulvic acids and mycorrhizae treatment (HFAxAMF). Mean values per treatment (n=3). Values with different letters differ significantly (one-way ANOVA, p-value < 0.05).

Supplemental material 6. Potential bioethanol yield per hectare for sorghum (*n*=3), calculated according to Johnston et al. (2009). Significant differences between treatments are indicated by different letters at the level of α = 0.05.

	Bioethanol yield (L ha^{-1})	
	Year 1	Year 2
Control	5641± 1335a	6327 ± 1232a
HFA	4800 ± 877a	5753 ± 536a
HFA x AMF	4627 ± 1674a	6388 ± 1093a

HFA = Humic/fulvic acids; HFA x AMF = Humic/fulvic acids x arbuscular mycorrhizal fungi