PRELIMINARY ASSESSMENT OF THE USE OF BIOLOGICAL AGENTS TO ENHANCE SORGHUM BICOLOR BIOMASS PRODUCTION AND PHYTOREMEDIATION CAPACITY: GREENHOUSE AND FIELD EXPERIENCES

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ABSTRACT: The cultivation of lignocellulosic crops on lands contaminated by heavy metals can represent a double solution for the production of biomass avoiding ILUC effect and providing for the decontamination of the lands through phytoremediation processes. Biomass sorghum is one of the most promising crops for this dual purpose. In the present study field and greenhouse trials have been performed to evaluate the best biological agents to increase biomass productivity and consequently heavy metals uptake in a soil contaminated by Zn, Cu, Pb, Ni and Sn. In the greenhouse trial the following biostimulant treatments have been evaluated: mycorrhiza (M), foliar biostimulants (F), root biostimulants (R), combinations of MF and MR, and the untreated control (C). After 14 weeks MR and MF were found capable of improving the productivity of plants in fresh and dry weight and the total amount of Zn and Cu extracted, while no difference was found for the concentrations of metals in the biomass. These two treatments were then tested under field conditions (a former landfill) together with the untreated control. At the end of the first year of growth, their ability to improve sorghum productivity was confirmed, while only MR was able to determine an increase in the total Zn uptake.
Keywords: sorghum bicolor L. moench, biomass, phytoremediation, heavy metals.

1 INTRODUCTION

More than half of the millions of contaminated hectares around the world are mainly contaminated with heavy metals. Most of these soils cannot be used for food production because critical thresholds of metals, or their bioavailability, would lead to a contamination of the food products and consequently to serious threats to human and animal health. Therefore, they represent a considerable reserve of land potentially available for the cultivation of biomass crops avoiding competition for land and/or land use changes. Moreover, this would allow different lignocellulosic species to assume an important ecological function through phytoremediation processes. Indeed, it is known that several lignocellulosic crops can tolerate and accumulate discrete amounts of heavy metals in in their aerial biomass(3). Metals are then removed from the soil at harvesting, making possible their phytoextraction, and it is possible to precipitate and recover them, through appropriate pre-treatments, in biofuel production process (e.g. pyrolysis). In addition, biomass crops are preferable to hyper-accumulator species (e.g. Thlaspi caerulescens) traditionally used for phytoremediation, because, despite accumulating a lower concentration of heavy metals in their tissues, they produce a considerably greater quantity of biomass and, therefore, can determine a greater overall uptake of heavy metals (6).

Biomass sorghum is one of the most promising crops for biomass production due to its productivity and resilience to biotic and abiotic stresses (drought, high temperatures, diseases)(1, 2). It is also known for its ability to accumulate some heavy metals in its biomass, especially some micronutrients, such as Cu and Zn, normally used by plants, but that above certain thresholds can become toxic (3, 8).On the other hand, the use of biological inputs is considered an optimal strategy to increase the uptake and phytoremediation capacity of biomass crops, because, compared to the use of chelating agents (e.g DPTA), it avoids the risk of leaching and secondary pollution phenomena (6).To date, most studies on phytoremediation are still conducted in pots to test the tolerance of plants to the accumulation of heavy metals and the use of biological agents in improving their performance and physiological well-being. However, further confirmation under field conditions is needed to confirm the potential of these strategies.

2 MATERIALS AND METHODS

The soil used for the trials comes from a former illegal landfill located in the suburbs of Bologna city, Italy (44° 50’ N, 11° 28’ E). The presence of heavy metals in the soil 1 m depth was investigated (Table 1). The first analyses of their total concentration determined by ICP-MS revealed that five metals: Zn, Cu, Ni, Pb and Sn had values above the legal threshold set by Italian law. Subsequently, for these metals, the bioavailable fraction was also determined by DPTA extraction, which indicated that Zn and Cu were the most bioavailable.

Table I: Soil metal total concentrations, the threshold defined by Italian law and their bioavailable fraction.

Metal	Legal Threshold (mg/kg DM)	Total Concentration (mg/kg DM)	Bioavailable concentration (mg/kg DM)
Zn	150	455	62
Cu	120	137	45
Ni	120	209	9,9
Pb	100	159	33
Sn	1	8.8	Not detected

2.1 Greenhouse trial

The following biological agents have been evaluated: M a mixture of 6 species of mycorrhiza (SYMBIVIT, Symbiom, CZ); F foliar biostimulants consisting of protein hydrolysates (55%) and amino acids (10%) in aqueous solution (SIAPTON, Agrology, GR); R root biostimulants consisting of a mixture of humic acids

(75%), fulvic acids (5%) and organic carbon (20%), in the form of a water-soluble powder (LONITE 80 SP, Alba Milagro, IT); combinations of MF and MR; and the untreated control C. For each treatment, 3 pots of 12 L previously sterilized were filled with soil taken from the field area at 1m depth and thoroughly mixed. Pots were randomized into a single block and rotated periodically. The variety of sorghum used was Bulldozer. The plants were pre-germinated in 6 petri dishes containing 50 seeds each using a sandy substrate and kept for 5 days in growth chambers (photoperiod 16-8 hours; temperature 20-30 °C).For the plants involving the use of mycorrhizae (M, MF, MR), the application of the product was performed before the transplant by giving 15 g of product mixed with topsoil. The root biostimulants was given for the first time when the plants (R, MR) reached the state of 3-4 true leaves, vigorously mixed in the irrigation water once a week, in doses of 0.5 g per pot for the first 4 weeks and 0.7 g thereafter.The foliar biostimulant was given for the first time when the plants (F, MF) reached a height of 10 cm. The first application was performed by diluting 13.5 cL of product in the irrigation water. Subsequently, when the plants reached a sufficient height to be sprayed, foliar application was performed by diluting 3 mL of product per liter of water and completely spraying the leaves of the plants once every 10 days. The trial lasted 14 weeks during which pots have been maintained at 75% of their pot capacity.The photoperiod was 12 hours of light and 12 hours of darkness for the first month and subsequently of 14 hours of light and 10 hours of darkness for the remaining part of the trial. The temperature was maintained in a range of 18-26°C At the harvest, plants were cut for fresh and dry biomass determination. The concentration of heavy metals was determined by ICP-MS analysis of the whole plant biomass and then the uptake of Cu and Zn was calculated multiply the metal concentration by the dry biomass produced (8)

2.2 Field trial

MR and MF were chosen to be tested in the field trial where three plots of 10 m^2 for each treatment were arranged in randomized blocks with a plant density of 18 plants/m^2. The trial took place between May and October 2022. The agronomic management and the application of the treatments was done manually. M was applied in a dose of 20 g per linear metre, distributed in a uniform manner along the furrow where the seed was located, subsequently covered. F was applied with a dilution ratio: 250 cm3/100 L H2O in order to adequately wet all the biomass produced by the plants (Figure 1). The first application was made at the 6^{th} true leaf stage, the second one 15 days after the first one. R was applied with a dilution ratio of 50 g per 60 L of water per plot (dosage of 50 kg / ha). The first application was at the 6^{th} true leaf stage, the second application one month after the first one. A fertilization with urea N46 (YaraVera Eura 46, YARA, IT) was carried out to cover the inter-row (120 kg/ha). When needed, it was irrigated to improve plants establishment and the weeds were constantly controlled by hand. At harvest, the fresh and dry weight of the plants collected in a sample area (2 m^2) was determined. Heavy metal content was determined by ICP-MS analysis and Cu and Zn uptake calculated in a similar way as in the greenhouse experiment.

Figure 1: Foliar biostimulant application (July 2022)

3 RESULTS AND DISCUSSIONS

3.1 Greenhouse trial

The initial results of the greenhouse trial showed that R, MF and MR produced more fresh biomass than C and M, while F did not exhibit any difference from all the others treatments. As for the dry biomass it emerged that MR produced more than all the other treatments(34, 08 g/plant) apart of only MF, which produce more than C and M but was, not statistically different from R and F. Untreated plants produced on average, less than one third of MR and nearly one third of MF. No significant difference was found for the concentration of metals in aboveground tissues, while the estimation of the overall uptake of Zn and Cu (concentration determined on whole plant samples per dry weight) showed that the highest biomass yielding plants were able to phytoextract greater quantities of these metals, with MR showing statistically greater values than the other treatments including MF (+89% for Cu and + 117% for Zn) that, showed statistically greater differences than M and C (+ 249% Cu and +311% for Zn).

3.2 Field trial

MR and MF confirmed a greater fresh and dry biomass (17.63 Mg ha-1 DM and 13.97 Mg ha-1 DM) production than C (7,98 Mg ha-1 DM). Hence, the yield differences remained very high, with the control producing less than half of the MR yield, around 60% of the yield of MF, but still smaller than the greenhouse trial. The results obtained with the biological inputs can therefore be considered appreciable and in line with the yields that sorghum can obtain in marginal contexts (4), while the yield obtained with the control is instead low. This result cannot be attributed to the consistently above average temperatures and water scarcity recorded in the growing season in Northern Italy in 2022 as several irrigation interventions were provided as needed. The main causes can rather be considered the poor fertility conditions of the area never previously used for agricultural purposes and the lack of a suitable preparation of the seedbed, considered among the most important agronomic operations for the cultivation of sorghum (2). The application of biostimulants in the early stages of development was probably decisive in the development of a more extensive root system capable of dealing with adverse conditions. In this trial it was also confirmed that the concentration of metals in the tissues did not change between treatments.However, under field conditions considerably lower concentrations were observed both for Cu (with concentrations more than halved compared to those found in greenhouses) and for Zn (with concentrations even 4 times higher in greenhouses). However, greater differences in metal concentration in greenhouse studies are typical of studies performed in pots (6). Moreover, Cu total uptake in this case showed no significant difference, while in the MR treatment Zn extracted was higher than in MF and C. But also in this case the difference between MR and C was considerably more limited compared to what was detected in the greenhouse with an overall uptake by MR about double that of C and no significant difference between C and MF.

4 CONCLUSION

From these preliminary results it is evident that the use of biostimulants, especially at root level, such as humic and fulvic acids, in combination with mycorrhizae (MR) can determine a significant improvement in the biomass produced by sorghum and therefore its phytoremediation capacity. Also the use of foliar biostimulants, based on ammino acids and protein hydrolysates, in combination with mycorrhizae (MF) can improve sorghum performances. The use of the mycorrhizae alone, in the greenhouse trial of the present study did determine appreciable differences compared to the control, however, they can broaden the effectiveness of other treatments capable of acting on hormonal development (F) and on the absorption of nutrients (R). Research in this area require the use of trials in a controlled environment, but such results must necessarily be validated under real field conditions as contaminated areas often present additional marginal factors that limit crop growth and metal absorption or not allow their optimal agronomic management, Also, the heterogeneous distribution of metals in the soil must be taken into account when evaluating the uptake patterns.

5 REFERENCES

[1] Amaducci, S., Monti, A., & Venturi, G. (2004). Non-structural carbohydrates and fibre components in sweet and fibre sorghum as affected by low and normal input techniques. Industrial Crops and Products, 20(1), 111-118.

[2] Farré, I., & Faci, J. M. (2006). Comparative response of maize (Zea mays L.) and sorghum (Sorghum bicolor L. Moench) to deficit irrigation in a Mediterranean environment. Agricultural water management, 83(1-2), 135-143.

[3] Marchiol, L., Fellet, G., Perosa, D., & Zerbi, G. (2007). Removal of trace metals by Sorghum bicolor and Helianthus annuus in a site polluted by industrial wastes: a field experience. Plant Physiology and Biochemistry, 45(5), 379-387. G. Campolmi, Proceedings of the 3rd World Biomass Conference – Biomass for Energy, Industry and Climate Protection, III Vol. (2005), pag. 981.

[4] Reinhardt, J., Hilgert, P., & Von Cossel, M. (2021). A review of industrial crop yield performances on unfavorable soil types. Agronomy, 11(12), 2382. D. Reed, Evaluation of Biomass Resources in the southern regions in Nigeria, (2007), pag. 124.

[5] Silva, T. N., Thomas, J. B., Dahlberg, J., Rhee, S. Y., & Mortimer, J. C. (2022). Progress and challenges in sorghum biotechnology, a multipurpose feedstock for the bioeconomy. Journal of experimental botany, 73(3), 646-664.

[6] Vangronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., ... & Mench, M. (2009). Phytoremediation of contaminated soils and groundwater: lessons from the field. Environmental Science and Pollution Research, 16, 765-794.

[7] Zegada-Lizarazu, W., & Monti, A. (2012). Are we ready to cultivate sweet sorghum as a bioenergy feedstock? A review on field management practices. Biomass and Bioenergy, 40, 1-12.

[8] Zhuang, P., Wensheng, S. H. U., Zhian, L. I., Bin, L. I. A. O., Jintian, L. I., & Jingsong, S. H. A. O. (2009). Removal of metals by sorghum plants from contaminated land. Journal of Environmental Sciences, 21(10), 1432-1437.

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