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RESEARCH ARTICLE

Beneficial effects of commercially available preparations of humic substances and mycorrhiza on growth and photosynthesis of sorghum and hemp cultivated on a metal(loid)-polluted field

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Abstract
Background and Aims Metal pollution in agricultural soils threatens global food security and reduces both the yield and quality of crops cultivated for non-food purposes. Biostimulants can support plant growth in such soils by mitigating the effects of pollution and enhancing biomass production. However, the mechanisms underlying the beneficial effects of biostimulants remain poorly understood.
Methods The effects of humic substances (HS) alone or in combination with mycorrhiza (HS + M) on the growth, metal accumulation, photosynthesis, and selected stress markers in hemp (*Cannabis sativa* L.) and sorghum (*Sorghum sudanense x bicolor*) grown in a field polluted with Zn, Cd, Pb, and As was investigated.
Results Application of HS significantly increased the shoot fresh weight of both crops. However, only in sorghum was this increase correlated with higher CO_2 assimilation rates, water use efficiency, and

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chlorophyll content. In general, chlorophyll fluorescence parameters remained unchanged in plants treated with biostimulants, indicating that the light phase of photosynthesis was unaffected. Similarly, no significant effects were found on the mineral profile, including pollutant concentrations, or lipid peroxidation levels (as a stress marker). PCA analysis revealed a higher level of lipid peroxidation in hemp, which was positively correlated with the contents of flavonols, anthocyanins, and sugars – components likely involved in oxidative stress mitigation.

Conclusion The application of biostimulants, specifically HS, represents a promising approach for improving crop yield and quality on metal(loid)-polluted agricultural soils, with potential implications for more sustainable agriculture and ecosystem services.

Keywords Biostimulants · Chlorophyll fluorescence · Metal pollution · Photosynthetic rate · Stress metabolites

Introduction

The growing human population and economic development lead to severe environmental pollution and soil degradation (Luo 2024). It is estimated that the number of polluted areas in the world exceeds 2,5 million, of which 25% are located in Europe (Mench et al. 2018). The major problem concerns pollution with metals and metalloids (over 35% of the areas), followed by mineral oils (over 24% of the areas) and polycyclic aromatic hydrocarbons (PAHs, over 11% of the areas) (Panagos et al. 2013). Excess of metals (*e.g.* cadmium, Cd; copper, Cu; lead, Pb; zinc, Zn; nickel, Ni; manganese, Mn; molybdenum, Mo; cobalt, Co; chromium, Cr; and mercury, Hg) and metalloids (*e.g.* arsenic, As) in the soil can cause deleterious effects in plants, including morphological, physiological, and biochemical changes, as well as disturbances in soil–plant interactions (El-Sayed and Kamel 2020; Jorjani and Pehlivan Karakaş 2024; Omar et al. 2019). Some of these elements, including Zn, Cu, Mn, Co, Mo, and Ni are essential for plant growth and development, but become toxic if present in excess. On the other hand, elements like Cd, Pb, Hg, and As do not have any known beneficial role and are severely harmful to plants even in relatively low concentrations. Soil pollution with metals and metalloids can result in disturbed water balance, mineral availability and homeostasis, inhibition of photosynthesis and respiration, carbohydrate and lipid metabolism, etc., which contributes to significant inhibitions of plant growth and quantity and quality of the produced biomass (Paunov et al. 2018; Rizwan et al. 2016; Salari et al. 2024; Wang et al. 2024).

Photosynthesis, as a fundamental process underlying plant productivity, is particularly sensitive to metal(loid) toxicity. This toxicity arises from both direct and indirect effects on the structure and functionality of the photosynthetic apparatus. The primary target of metal(loid)s is the electron transport chain, particularly photosystem II (PSII). However, several enzymes involved in photosynthesis, including those participating in the Calvin cycle or synthesis of chlorophyll, are also adversely affected (Guidi et al. 2019). In addition, metal(loid) induced stress triggers secondary oxidative stress, resulting, among other effects, in peroxidation of phospholipid membranes and decreased activity of numerous photosynthesis-related enzymes (Omar et al. 2019; Srivastava et al. 2017). Excess metal(loid)s also diminishes the rate of transpiration and stomatal conductance, further limiting photosynthetic efficiency. Consequently, chlorophyll fluorescence increases, making its analysis one of the most widely used techniques to assess stress-induced changes in photosynthetic performance and overall plant condition (Park et al. 2024; Paunov et al. 2018).

Plants intended for human or animal consumption should not be cultivated in metal(loid)-polluted areas due to the risk of pollutants entering the food chain (Csikós and Tóth 2023). Therefore, in such

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areas, increasing attention is being given to the cultivation of plants for alternative purposes, such as energy production, textiles, chemicals, etc. If, in addition, such plants possess some potential for phytoextraction, their cultivation can offer both economic benefits and gradual remediation of polluted soils, potentially restoring them for all-purpose agricultural use in the future. Selected species should be characterized by high metal tolerance, high biomass yield, low environmental requirements, limited need for fertilizers and crop protection products, and low labour intensity (Wang and Aghajani Delavar 2023). Such criteria are met, among others, by two annual lignocellulosic crops: hemp (*Cannabis sativa* L.) and sorghum (*Sorghum sudanense x bicolor*). Both crops are characterized by high yields (up to 30 t ha^{-1} in the case of hemp and up to 50 t ha^{-1} for sorghum), and their biomass has a broad and diverse range of applications (Crini et al. 2020; Fagundes et al. 2021; Huang et al. 2023; Kraszkiewicz et al. 2019; Wu et al. 2024). Hemp is one of the species with the most versatile uses. Its fibres have already long been used in the textile and paper industries. Currently, hemp is also employed in the construction, chemical, cosmetics, pharmaceutical, and biofuel sectors, and even in car production (Kaminski et al. 2024; Murkara et al. 2021). Sorghum, on the other hand, is characterized by high concentrations of lignin and sugar in its stems. Its biomass is used in the paper and energy industries, as well as for biofuel production (Appiah-Nkansah et al. 2019). Both species possess high energy values, and their cultivation for energy purposes does not exert pressure on the global food market unlike other cereals or sugar beets (Velmurugan et al. 2020). Therefore, identifying efficient and sustainable agrotechnical approaches to enhance the biomass production of such industrial crops on metal(loid)-polluted areas is of critical importance.

Biostimulants are substances or microorganisms that stimulate plant growth independently of their nutritional value, both under normal and abiotic and biotic stress conditions (Boutahiri et al. 2024; Du Jardin 2015). They proved to be effective in mitigating adverse effects of drought, salt stress, extreme temperatures, and metal(loid) pollution. Application of biostimulants is both cost-effective and environmentally friendly, as it reduces the use of synthetic fertilizers and pesticides. Consequently, they are attracting increasing attention in modern and sustainable agriculture, particularly on marginal soils. Despite numerous studies, their mode of action is not always fully understood (Baltazar et al. 2021; Garbisu et al. 2020). Based on their nature, two main groups of biostimulants can be distinguished: (i) non-microbial biostimulants (including humic substances, protein hydrolysates, and other nitrogen-containing compounds of plant and animal origin, as well as beneficial elements), and (ii) microbial-derived biostimulants, namely plant growth-promoting microorganisms (PGPM), including rhizospheric and endophytic bacteria and fungi (Castiglione et al. 2021). Two types of biostimulants were selected for this study: humic substances (HS) and arbuscular mycorrhizal fungi (AMF).

Humic substances play a key role in maintaining the chemical, physical and biological properties of soil and stimulating plant growth. They improve soil fertility by supporting beneficial microorganisms in the rhizosphere and enhancing nutrient availability. Humic substances promote root development and nutrient uptake, and influence the expression of genes related to nutrient metabolism, photosynthesis, and phytohormone synthesis (Olivares et al. 2017; Nardi et al. 2021; Savarese et al. 2022). Structurally, HS are composed of humic acids, fulvic acids, and humins, which differ in their solubility and chemical properties.

Arbuscular mycorrhizal fungi are beneficial microorganisms able to enter into symbiotic associations with approximately 80% of terrestrial plant species, including the most important agricultural crops (Bernardo et al. 2019). The fungal hyphae penetrate root cortical cells and form branched structures known as arbuscules. AMF exert their effects at multiple levels: (i) by supporting the acquisition of essential minerals and water, while diminishing the uptake and translocation of potentially harmful metal(loid)s through their retention in fungal hyphae; (ii) by releasing plant hormones that are promoting root development; and (iii) by modulating gene expression (Ofori-Agyemang et al. 2024a).

Although several studies have investigated the effects of HS or AFM on the growth of hemp and sorghum under controlled greenhouse or field conditions (Ofori-Agyemang et al. 2024a,b; Peroni et al. 2024), a significant knowledge gap remains regarding their mechanisms of action and effectiveness when applied to crops grown in real field conditions of metal(loid)-polluted soils. We hypothesize that application of these biostimulants will enhance photosynthetic performance and mitigate stress, thereby promoting increased growth and biomass production of both crops. Therefore, the aim of the present study was to evaluate the effects of specific commercially available biostimulants – HS alone or combined with AFM – on growth, photosynthetic efficiency, and selected metabolic responses in hemp and sorghum cultivated on metal(loid)-polluted agricultural soil.

Materials and methods

Experimental site

The study area is located in the administrative region of the city of Piekary Śląskie, in the Upper Silesia Industrial Region, southern Poland (50°21′19″ N; 19°00′17″ E). On its northern side, the experimental field partially borders an old metalliferous waste dump, which is the main source of the metal(loid) pollution in the surrounding areas. The waste dump was created from the end of the nineteenth century until the years 1915–1930 and contains wastes from the gravitational enrichment of Zn-Pb ore – zinc blende – by the mining and metallurgy company "Orzeł Biały" (Wójcik et al. 2014). The soil is a silty loam with a slightly alkaline pH (H_2O) ranging from 7.37 to 7.6 (KCl pH range 6.59 to 6.75). The total nitrogen (N) content ranges from 0.15 to 0.23%. The average total carbon content is 3.2% (range 2.35–4.74%), of which organic carbon comprises 2.31–3.26%. Available forms of phosphorus (P) and potassium (K) range from 5.81 to 18.38 mg P_2O_5 per 100 g soil, and from 11.64 to 46.48 mg K_2O per 100 g soil, respectively. The soil is characterised by high concentrations of metals Zn, Pb, and Cd, as well the metalloid As, which significantly exceed the permissible threshold values for agricultural soils of this soil type, established by the Polish Ministry of Climate and Environment (Regulation 2024) (1000, 500, 5, and 50 mg kg^{-1}, respectively). The average total concentrations are 8057.15 mg Zn kg^{-1}, 2939.7 mg Pb kg^{-1}, 51.56 mg Cd kg^{-1}, and 94.13 mg As kg^{-1} soil. The 0.01 M $CaCl_2$ extractable concentrations (often considered potentially bioavailable) of these elements are (mg kg^{-1}): 2.856 for Zn, 0.295 for Pb, and 0.195 for Cd.

The experimental field is located in the transition zone between oceanic and continental climates, with a dominance of air masses from the Atlantic Ocean (approx. 60%), a large share of continental air masses from the east (approx. 30%), and only a marginal influence of tropical and Arctic air masses. The average annual air temperature is 9 °C; the warmest month of the year is July (average temperature 19.3 °C), and the coldest is January (average temperature −2.1 °C). The average annual rainfall is approx. 817 mm, with the highest value in July (average 109 mm) and the lowest in February (average 38 mm). The duration of snow cover ranges from 60 to 90 days, and the growing season lasts from 200 to 210 days (Climate-Data.org).

Experimental design, plant material and treatments

The experiment was conducted from the end of May to the end of September 2022. Eighteen 8×8 m plots were established, nine for hemp and nine for sorghum, with three plots per treatment. Seeds of industrial hemp (*Cannabis sativa* L. var. Futura 75) and sorghum (*Sorghum sudanense x bicolor* var. Bulldozer) were sown manually in the last week of May. The distance between rows was 50 cm, and the distance between seeds/plants in each row was 10 cm (for hemp) and 15 cm (for sorghum). The plots were weeded manually twice (June and July) during the growth season, and no herbicides or pesticides were applied during the course of the experiment.

Three treatments were set up in triplicates for each crop: (i) control (C) – no biostimulant application; (ii) humic substances (HS); (iii) HS combined with mycorrhiza (HS + M). Biostimulants used:

- humic substances: a commercial product Lonite (a liquid product comprising both humic and fulvic acids; produced by Alba Milagro, Italy). It was applied via root irrigation according to the protocol provided by the producer twice during plant growth: (i) when the plants had 4–6 leaves, and (ii) four weeks after the first application.
- mycorrhiza: a commercial mix of AMF, Symbivit® (provided by Symbiom, Czech Republic). The mix was in granular form containing five AMF fungi (*Rhizophagus irregularis*, *Funneliformis geosporum* BEG199, *Funneliformis mosseae*, *Claroideoglomus lamellosum*, and *Septoglomus deserticola*). It was applied to the soil at the time of sowing, in the amount of 20 g of the inoculum per linear meter, at the bottom of the planting furrow, below the seeds.

Plant growth and physiological parameters

Shoot fresh weight and total plant height were determined at the end of September 2022 on at least 30 plants randomly collected from each experimental plot. Another growth parameter, leaf thickness, was determined using MultispeQ (PhotosynQ, East Lansing, MI, USA) at the time of determination of the plant physiological parameters in situ, not at the end of the growing season, as described below.

Plant physiological parameters – performance of the photosynthetic apparatus, photosynthesis rate, transpiration rate, water use efficiency, and plant pigments content – were determined in situ at the end of August 2022. At that time, samples from the same plants and leaves were also collected for future analyses of selected metabolites (total sugars, proline, and lipid peroxidation products) and the mineral profile. Samples of 0.2 g fresh weight (FW) were frozen in liquid nitrogen and stored at −80 °C for the biochemical analyses of the metabolites concentrations. Samples of the same leaves were washed thoroughly with tap water, then rinsed in distilled water and dried at 105 °C until constant dry weight (DW) for determining element concentrations.

Photosynthetic rate, transpiration rate, and water use efficiency

Gas exchange-based parameters (photosynthetic rate, A, expressed in μmol CO_2 m^{-2} s^{-1}; transpiration rate, T, expressed in mmol H_2O m^{-2} s^{-1}; stomatal conductance, g_s, expressed in μmol H_2O m^{-2} s^{-1}) were determined using a portable infrared gas analyser (Targas 1.0, PP-Systems, Amesbury, MA, USA). The examined leaves were placed in the measuring chamber, and five measurements were taken on each leaf after allowing the readings to stabilize (about 1.5 min). The water use efficiency index (WUE) was calculated as the ratio of the CO_2 assimilation rate to the simultaneously measured stomatal conductance ($WUE = A/g_s$) and expressed as mol CO_2 photosynthetically fixed per mol of H_2O transpired (Hoover et al. 2023).

Performance of the photosynthetic apparatus and pigments content

We followed the approach outlined in published articles (Bury et al. 2021; Sitko et al. 2019; Szopiński et al. 2020). Analyses of chlorophyll *a* fluorescence and pigments contents were performed on the same leaves as the measurements of gas exchange parameters. Chlorophyll fluorescence parameters were determined using the Plant Efficiency Analyser (PocketPEA fluorimeter; Hansatech Ltd., Pentney, UK). Before measurement, each selected leaf was adapted in the dark for 30 min using leaf clips. After adaptation, a saturating light pulse of 3500 μmol photons m^{-2} s^{-1} was applied for 1 s, which closed all of the reaction centres, and the fluorescence parameters were analysed and displayed. Additionally, Pulse-Amplitude-Modulated fluorescence was measured using a PAM fluorimeter (MultispeQ, PhotosynQ, East Lansing, MI, USA) with the Photosynthesis RIDES protocol (www.photosynq.com/software). The contents of chlorophylls, flavonols, and anthocyanins in leaves were estimated using a pigment content meter (Dualex Scientific+, Force-A, Orsay, France). Measurements were taken after the device was automatically calibrated by placing a leaf blade between the measuring heads. Care was taken to measure the leaf surface without major veins. Both fluorescence and pigment content measurements were performed in situ in a non-invasive manner, thus without damaging the plant material. Ten plants per plot were examined; fifteen measurements of pigment contents, five measurements of chlorophyll fluorescence, and five measurements of PAM fluorescence per plant and were performed.

Lipid peroxidation levels

Thiobarbituric acid reactive substances (TBARS) were used as a marker of lipid peroxidation. For this purpose, the method described by Krzemińska et al. (2024) was adopted. Briefly, 0.5 g of frozen leaf tissues was homogenised in 5 mL of 0.1% w/v trichloroacetic acid (TCA), and the homogenate was centrifuged (10,000 rpm, 4 °C, 10 min, Beckman Avanti™ 30 centrifuge). 2 mL of the supernatant was mixed with 2 mL of 0.5% 2-thiobarbituric acid (TBA) dissolved in 20% TCA. The mixture was incubated for 30 min at 95 °C, cooled on ice, and centrifuged again under the same conditions as before. The absorbance of the supernatant was measured on a CPS-240A Shimadzu spectrophotometer at $\lambda = 532$ nm and corrected for unspecific absorbance at $\lambda = 600$ nm. The amount of TBARS was calculated on the basis of the extinction coefficient (155 mM^{-1} cm^{-1}).

Proline concentrations

Free proline was determined according to the method described by Ábrahám et al. (2010) and Rienth et al. (2014) with some modifications. 0.2 g of the frozen leaf samples was homogenized on ice with 2 ml of 3% (w/v) sulfosalicylic acid. The homogenate was centrifuged at 4500 rpm for 15 min in a Beckman Avanti™ 30 centrifuge (Beckman Coulter, Inc., IN, USA). 1 mL of the supernatant was transferred to a glass tube and mixed with 1 mL of glacial acetic acid and 1 mL of acid ninhydrin. The acid ninhydrin was prepared just before used for analysis by adding 1.25 g of ninhydrin to 30 mL of glacial acetic acid and 20 mL of 6 M orthophosphoric acid. This mixture was incubated for 1 h at 100 °C in a water bath and cooled on ice to stop the reaction. After adding 2 mL of toluene, the reaction mixture was vortexed vigorously for 15–20 s and left for 5 min until the aqueous and organic layers separated. The proline content was determined in the organic layer on a CPS-240A Shimadzu spectrophotometer at 520 nm against toluene as a blank. The proline concentration was calculated based on the calibration curve and expressed in µmol g^{-1} FW.

Total carbohydrate concentrations

Determination of total carbohydrate concentrations in leaf tissues was performed based on the modified method described by Nowotny (1979) and Chow and Landhäusser (2004). 0.2 g of frozen leaf samples was homogenised with 4 mL of concentrated ethyl alcohol and centrifuged for 5 min at 2500 rpm in a Beckman Avanti™ 30 centrifuge (Beckman Coulter, Inc., IN, USA). 0.5 mL of supernatant was collected and mixed with 0.5 mL of 5% phenol and 2.5 mL of concentrated sulfuric acid. The mixture was stirred and after cooling on ice, diluted four times. The absorbance was measured on a CPS-240A Shimadzu spectrophotometer at 490 nm. The total carbohydrate concentration was calculated based on the calibration curve and expressed in µg g^{-1} FW.

Element concentrations in the leaves

The dry plant samples were ground into powder and digested in concentrated HNO_3 in Teflon PFA vessels in a microwave-accelerated reaction system (MarsXpress; CEM Corp., Matthews, NC, USA). The concentration of elements in extracts was determined using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500CE, Santa Clara, CA, USA). To assess analytical precision and quality control, the NIST certified reference material – 1573a tomato leaves and oriental tobacco leaves (CTTA-OTL-1), reagent blanks, and duplicates of every tenth sample were used.

Statistical analysis

Statistical analysis of the data was performed using Origin Statistica 14.0.0.15 (StatSoft, Cracow, Poland). For the evaluation of statistically significant differences, obtained data for each species separately were analysed using one-way ANOVA. The differences were considered significant at $p < 0.05$. Before running the analyses, the assumptions for normality of data distribution and the homogeneity of variance were checked using Shapiro–Wilk's and Levene's tests, respectively. A post-hoc Fisher's test was carried out to estimate statistically significant differences among the means. The principal component analysis (PCA) was performed to obtain a holistic view of the results and to evaluate some independent trends in the variations among the investigated data. The analyses were performed using the statistical package MVSP program version 3.1.

Results

Plant growth parameters

Application of HS increased the shoot fresh weight of both hemp and sorghum (Fig. 1A). At the end of the growing season, the average fresh weight of hemp was approximately 22.3% higher, and that of sorghum approximately 56% higher, compared to the plants grown without biostimulants. The combined application of HS + M resulted in lower fresh weights of both crops compared to HS alone; although the differences were not statistically significant relative to the control. The average plant height in both hemp and sorghum was unaffected by any treatment (Fig. 1B). HS application increased hemp leaf thickness by

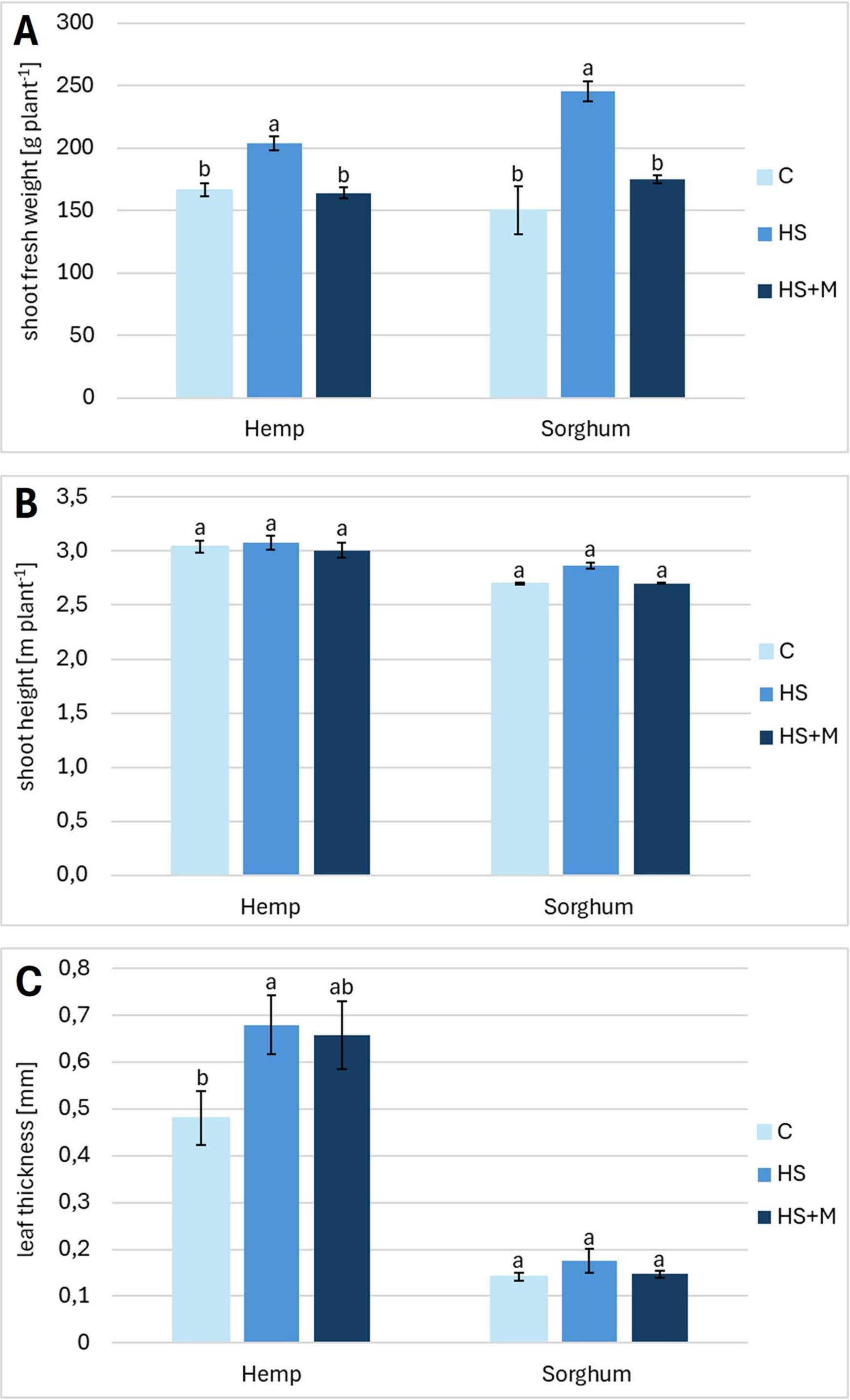


Fig. 1 Growth parameters: shoot fresh weight (**A**), shoot height (**B**), and leaf thickness (**C**) of hemp and sorghum grown on metal(loid)-polluted soil without (C – control) or with application of humic substances (HS) or humic substances and mycorrhiza (HS + M). Data are means ± SE. Means followed by the same letters for each crop separately are not significantly different (Fisher LSD test, $p < 0.05$)

approximately 41.5% compared to untreated plants (Fig. 1C). This effect was not found after treatment with HS + M. The leaves of sorghum were about three times thinner than those of hemp, but none of the treatments had a significant effect on their thickness.

Photosynthetic rate and other gas exchange-related parameters

In hemp leaves, the photosynthetic rate, measured as the amount of CO_2 assimilated per m^2 of leaf per second, was significantly increased only by the application of HS + M (Fig. 2A), reaching approximately twice the rate found in plants grown without biostimulants or with HS alone. In case of sorghum, however, both biostimulants positively affected the CO_2 assimilation rate, with the HS + M treatment giving the strongest effect (Fig. 2A). After treatment with HS and HS + M, photosynthetic rates were approximately 35% and 58% higher, respectively, compared to the control.

Biostimulants did not affect the transpiration rate in hemp (Fig. 2B). In sorghum, only HS + M treatment raised transpiration by approximately 30% compared to plants grown without biostimulants. The water use efficiency index nearly doubled in hemp grown with HS + M in comparison to other groups (Fig. 2C). In sorghum, both HS and HS + M application caused increases in WUE by 21.6% and 33.5%, respectively, compared to control plants.

Performance of the photosynthetic apparatus

The performance of the photosynthetic apparatus was evaluated using chlorophyll *a* fluorescence parameters (Fig. 3), including: F_0 – minimal fluorescence; F_m – maximal fluorescence; F_v – variable fluorescence; φD_0 – quantum yield (at t = 0) of energy dissipation; φP_0 – maximum quantum yield of primary photochemical reactions; ΨE_0 – probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-; φE_0 – quantum efficiency of electron transfer from Q_A^- to plastoquinone; δR_0 – probability with which an electron from the intersystem electron carriers will move to reduce the end acceptors at the PSI acceptor side; φR_0 – quantum yield for the reduction of terminal electron acceptors on the acceptor side of PSI; and φNPQ – non-photochemical quenching. Application of biostimulants did not alter any of these parameters in hemp leaves, except for ΨE_0, which was significantly lower in HS + M treated plants compared to the control plants (Fig. 3A). In sorghum, application of HS + M decreased F_0 and ΨE_0 while increasing δR_0 values as compared to the control (Fig. 3B). However, no significant changes in fluorescence parameters were observed with HS alone.

As the electron flow is traced, only one effect was detected: in hemp, HS treatment reduced electron transport flux per the excited cross section (Fig. 3C). No changes were recorded in sorghum leaves in response to either treatment (Fig. 3D).

Pigment content

The total chlorophyll content in hemp leaves increased by approximately 14% with HS treatment compared to the control, and by additional 7% when HS was combined with M (Fig. 4A). In sorghum the chlorophyll content remained similar across treatments, although it was slightly, but significantly higher in plants treated with HS alone than in those treated with HS + M (Fig. 4A). No treatment-related differences were found for the contents of flavonols (Fig. 4B) and anthocyanins (Fig. 4C) in sorghum. In contrast, hemp plants treated with HS + M showed lower flavonol levels, while anthocyanins content was significantly lowered in both HS and HS + M treatments (Figs. 4B, C). Interestingly, the content of chlorophylls was higher in the leaves of sorghum than hemp, whereas the opposite was found for anthocyanins.

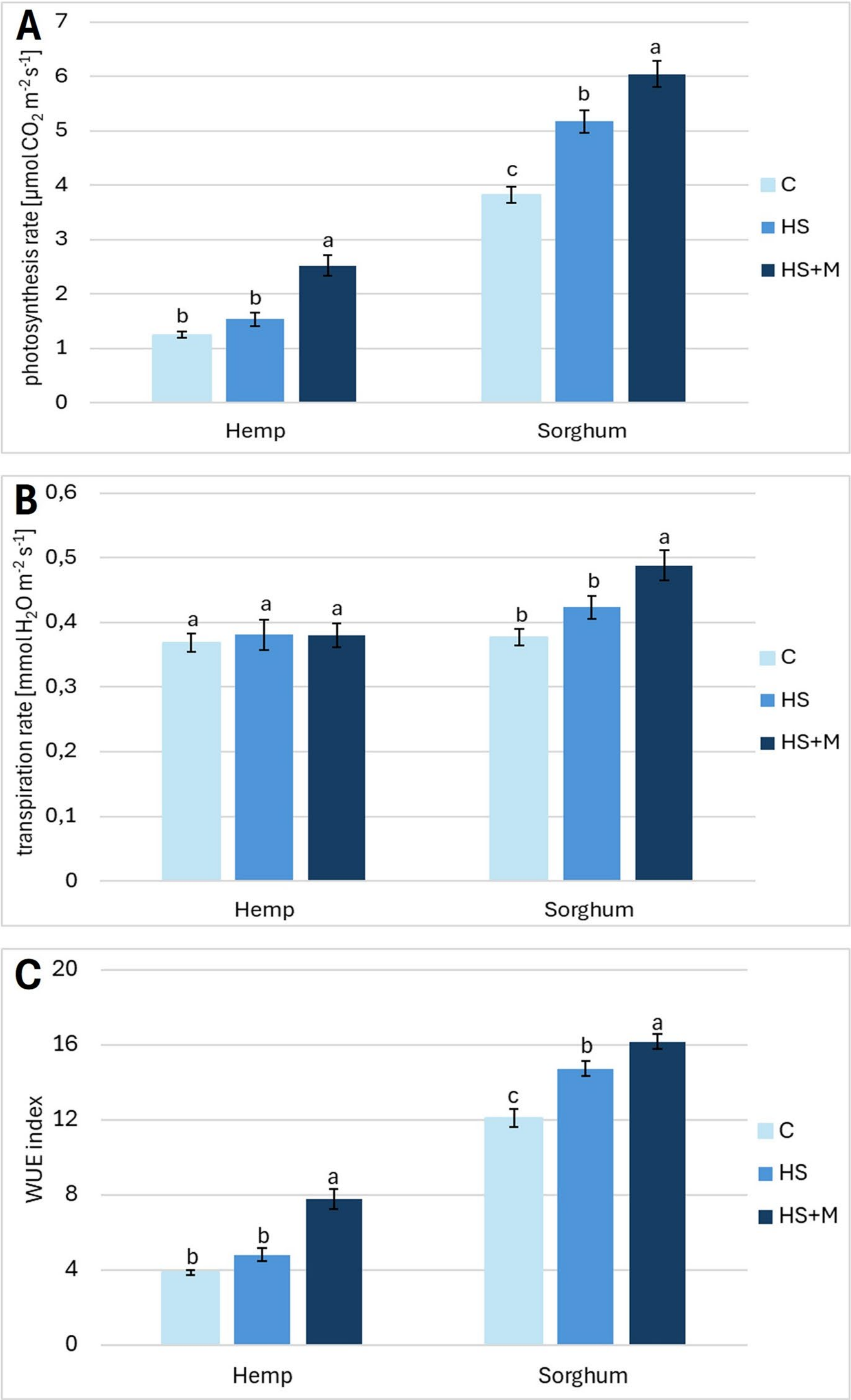
Level of lipid peroxidation and accumulation of proline and sugars

Application of biostimulants had no effect on lipid peroxidation levels, measured as TBARS concentrations, in either hemp or sorghum leaves (Fig. 5A). The proline concentrations in hemp leaves also remained unchanged across treatments (Fig. 5B). In contrast, application of biostimulants affected positively the proline concentration in sorghum (Fig. 5B). The concentration of proline was higher by 60.5% with HS and more than two-fold with HS + M compared to the control. Moreover, HS + M treated plants accumulated 35% more proline than those treated with HS alone. No differences were found in the concentrations of total sugars in hemp and sorghum grown with and without the addition of biostimulants (Fig. 5C).

Mineral profile

In general, the mineral profiles of hemp and sorghum leaves did not differ significantly between plants grown with or without the tested biostimulants (Table 1). Notable differences were observed only

Fig. 2 Gas exchange-based parameters: photosynthetic rate (**A**), transpiration rate (**B**), and water use efficiency index (**C**) in hemp and sorghum grown on metal(loid)-polluted soil without (C – control) or with application of humic substances (HS) or humic substances and mycorrhiza (HS + M). Data are means ± SE. Means followed by the same letters for each crop separately are not significantly different (Fisher LSD test, p < 0.05)



for Cr and Mn concentrations in hemp, where the Cr concentration was significantly lower in plants grown with HS + M compared to other experimental conditions. The Mn concentration was 36.8% higher in plants grown with HS + M than in untreated controls.

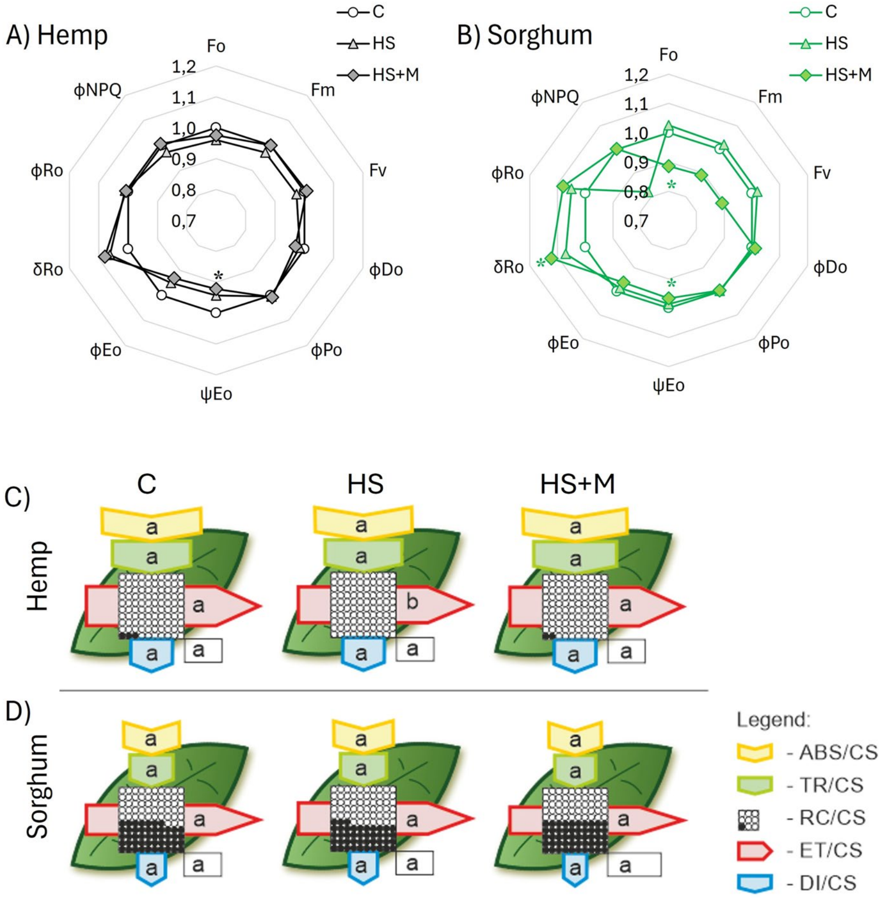
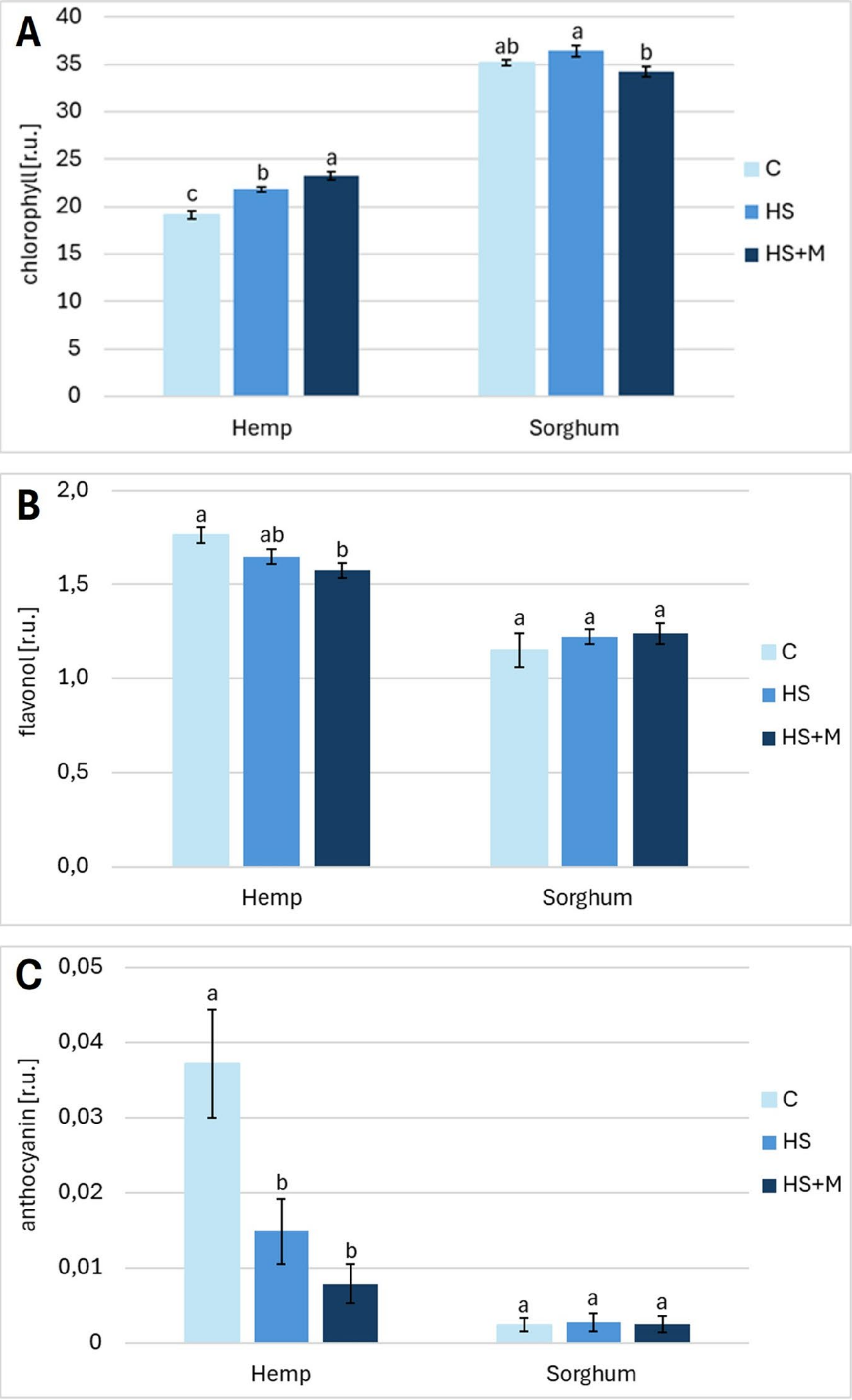


Fig. 3 Chlorophyll fluorescence parameters in hemp (**A**) and sorghum (**B**) leaves, and leaf models showing the phenomenological energy fluxes per the excited cross sections (CS) of the leaves of hemp (**C**) and sorghum (**D**) grown on metal(loid)-polluted soil without (C – control) or with application of humic substances (HS) or humic substances and mycorrhiza (HS + M). The width of each arrow in Fig. C and D corresponds to the intensity of the flux. Yellow arrow – ABS/CS, absorption flux per CS (approximated); green arrow – TR/CS, trapped energy flux per CS; red arrow – ET/CS, electron transport flux per CS; blue arrow – DI/CS, dissipated energy flux per CS; circles inscribed in squares – RC/CS, % of active/inactive reaction centres, white circles inscribed in squares represent reduced QA reaction centres (active), black circles represent non-reduced QA reaction centres (inactive); 100% of the active reaction centres responded with the highest mean value observed in the control conditions. Each relative value of the measured parameters is the mean ($n = 18$). Means followed by the same letter for each parameter in a row are not significantly different (Fisher LSD test, $p < 0.05$). Letters are inscribed into arrows, except for RC/CS, where they are placed in a box in the bottom right corner of the square with circles. Abbreviations: F_0 – minimal fluorescence; F_m – maximal fluorescence; F_v – variable fluorescence; φD_0 – quantum yield (at $t = 0$) of energy dissipation; φP_0 – maximum quantum yield of primary photochemical reactions, ΨE_0 – probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond QA^-; φE_0 – quantum efficiency of electron transfer from QA^- to plastoquinone; δR_0 – probability with which an electron from the intersystem electron carriers will move to reduce the end acceptors at the PSI acceptor side; φR_0 – quantum yield for the reduction of terminal electron acceptors on the acceptor side of PSI; φNPQ – non-photochemical quenching. Means followed by an asterisk are significantly different from the control

Principal component analysis

The biplot derived from the principal component analysis (PCA) of general growth and physiological parameters, along with shoot concentrations of the polluting elements in hemp and sorghum, explains 63.1% of the total variance in the dataset (Fig. 6, Table 2). The PCA revealed strong crop-specific responses to growth conditions. The first principal component (Axis 1), accounting for 51% of the

Fig. 4 Content of chlorophyll (**A**), flavanol (**B**), and anthocyanin (**C**) in hemp and sorghum grown on metal(loid)-polluted soil without (C – control) or with application of humic substances (HS) or humic substances and mycorrhiza (HS + M). Data are means ± SE. Means followed by the same letters for each crop separately are not significantly different (Fisher LSD test, $p < 0.05$)



variance, clearly separates hemp from sorghum. It is positively correlated with concentrations of TBARS, flavonols and anthocyanins as well as with plant height and Pb concentration – traits associated mainly with hemp. On the other hand, sorghum samples group together on the left part of the ordination space, characterised by higher CO_2 assimilation rate, chlorophyll content, WUE index, and elevated

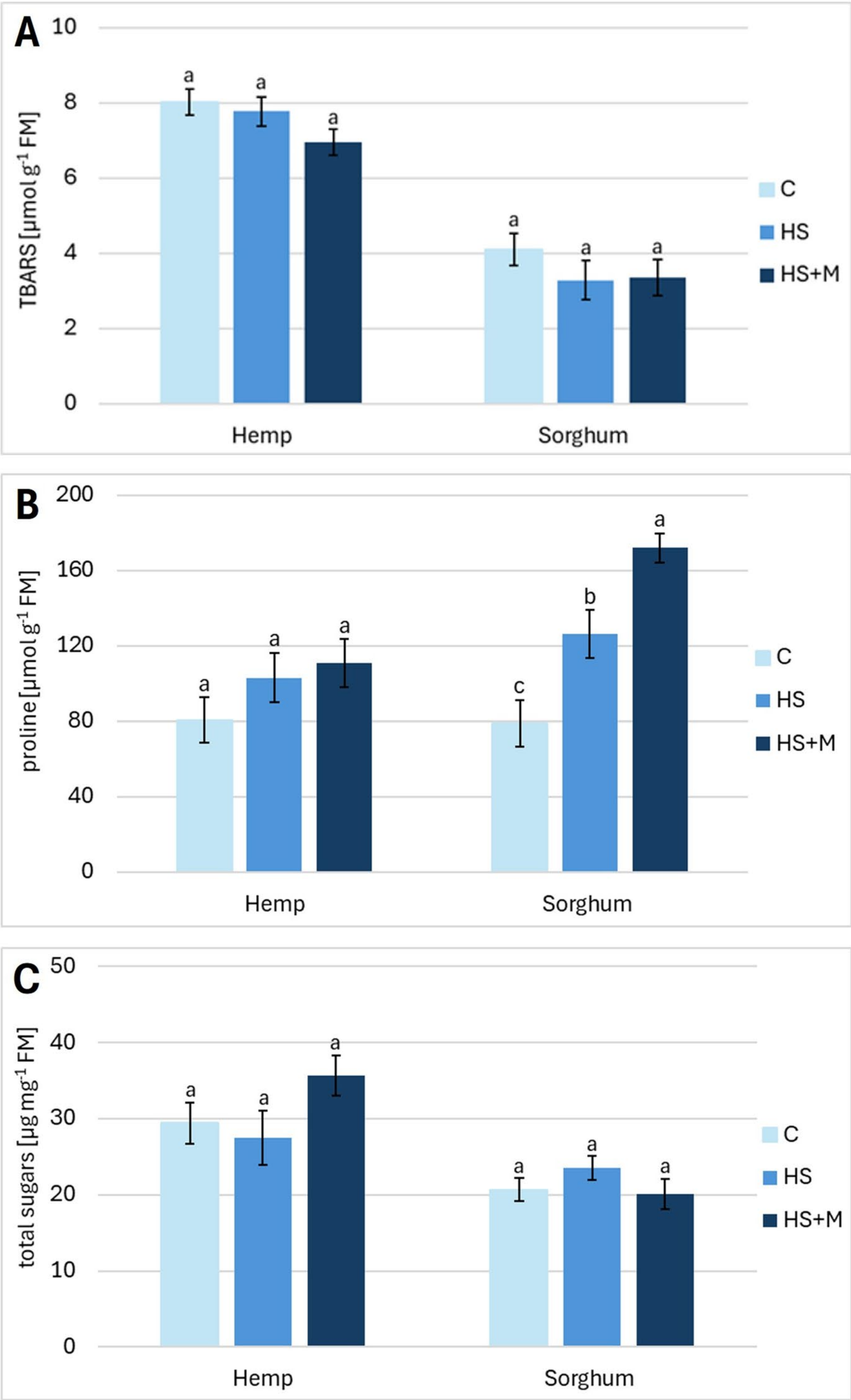


Fig. 5 Concentrations of TBARS (**A**), proline (**B**), and total sugars (**C**) in hemp and sorghum grown on metal(loid)-polluted soil without (C – control) or with application of humic substances (HS) or humic substances and mycorrhiza (HS + M). Data are means ± SE. Means followed by the same letters for each crop separately are not significantly different (Fisher LSD test, $p < 0.05$)

concentrations of Zn and Cd in the leaf tissues. The second principal component (Axis 2), which accounts for 12.12% of the total inertia, reveals the differences between treatments. It correlates strongly with plant fresh weight, transpiration rate, and proline concentration. Along this axis, a visible gradient is observed: control plants group in the lower part of the plot, HS + M-treated plants are located in the middle, and

Table 1 Element concentrations (mg kg^{-1} DM) in hemp and sorghum grown on metal(loid)-polluted soil without (C – control) or with application of humic substances (HS) or humic substances and mycorrhiza (HS + M)

Element [mg kg^{-1} DM]	Hemp			Sorghum		
	C	HS	HS + M	C	HS	HS + M
As	0.20 ± 0.00 a	0.21 ± 0.01 a	0.18 ± 0.01 a	0.11 ± 0.02 a	0.11 ± 0.01 a	0.1 ± 0.01 a
Cd	0.69 ± 0.11 a	0.48 ± 0.02 a	0.83 ± 0.28 a	11.93 ± 1.4 a	9.54 ± 0.38 a	8.47 ± 1.53 a
Pb	9.84 ± 0.10 a	9.40 ± 0.51 a	9.26 ± 0.10 a	5.90 ± 0.49 a	7.23 ± 0.37 a	7.32 ± 1.13 a
Zn	149.4 ± 19.9 a	160.9 ± 0.5 a	175.3 ± 6.7 a	189.8 ± 11.8 a	215.7 ± 22.0 a	185.2 ± 14.9 a
Cr	1.5 ± 0.11 a	1.3 ± 0.7 a	0.7 ± 0.02 b	3.1 ± 0.35 a	3.6 ± 0.37 a	3.21 ± 0.84 a
Mn	77.4 ± 2.67 b	80.1 ± 4.48 ab	105.8 ± 4.03 a	31.2 ± 0.91 a	36.9 ± 1.46 a	36.1 ± 1.84 a
Fe	217.2 ± 10.31 a	237.2 ± 6.62 a	194.6 ± 7.24 a	114.6 ± 7.79 a	127.3 ± 6.36 a	124.7 ± 6.64 a
Cu	6 ± 0.09 a	6.2 ± 0.36 a	5.1 ± 0.28 a	9.2 ± 0.83 a	9.5 ± 1.23 a	7.8 ± 0.84 a
Se	0.09 ± 0.01 a	0.08 ± 0.01 a	0.07 ± 0.00 a	0.03 ± 0.00 a	0.04 ± 0.00 a	0.04 ± 0.00 a
Na	66.2 ± 13.93 a	51.7 ± 2.76 a	18.1 ± 2.94 a	7.72 ± 1.2 a	16.9 ± 3.49 a	86.6 ± 28.14 a
Ca	51,324 ± 1193.1 a	56,678 ± 763.7 a	52,883 ± 2266 a	7229 ± 374.63 a	9174.2 ± 212.6 a	9025.6 ± 162.4 a
Mg	9472 ± 275.73 a	10,302 ± 271.2 a	9625.7 ± 101.1 a	3320 ± 178.33 a	4030.2 ± 262.8 a	3940.3 ± 282.1 a
K	35,599 ± 1958 a	38,084 ± 367.4 a	33,944 ± 1012 a	18,623 ± 66.88 a	18,831 ± 120.8 a	18,292 ± 591.5 a

Data are means ± SE. Means followed by the same letters for each crop separately are not significantly different (Fisher LSD test, $p < 0.05$)

these treated with HS cluster at the top. However, as Axis 2 accounts for a relatively small proportion of the total variance, any interpretation based on this component should be made cautiously.

Discussion

Effect of biostimulants on plant growth

The application of HS enhanced the fresh weight of sorghum and hemp grown on metal(loid)-polluted soil. In contrast, the combination of HS with mycorrhiza had no significant effect on growth for either crop. Similar experiments performed by Peroni et al. (2024) showed enhanced biomass production of the same species following application of HS + M but not HS alone. On the other hand, in an analogical experimental design, other researchers did not observe any stimulating effect of HS or HS + M on sorghum biomass production. Nevertheless, they reported increased plant height following the application of these biostimulants (Ofori-Agyemang et al. 2024b). Bayat et al. (2021) demonstrated that both humic acids and fulvic acids (components of the humic substances in Lonite) had a positive effect on the growth of yarrow (*Achillea millefolium* L.) when applied separately at different concentrations. Other studies have also mentioned positive effects of humic substances on growth parameters of plants grown under stress conditions (Hasanuzzaman et al. 2024; Nazli et al. 2020; Olivares et al. 2017; Schmidt et al. 2007).

Humic substances are the main components of organic fertilizers and inherently contain significant amounts of nutrients (Olivares et al. 2017). Positive effects of HS application on soil structure and the soil microbiome have also been reported. Additionally, the auxins-like compounds present in HS stimulate the development of lateral roots, root hairs and root elongation. These effects result in higher nutrient availability and improved cation exchange, which ultimately enhance plant growth (Alsudays et al. 2024; Nardi et al. 2016). The beneficial impact of AMF on crop yield has also been attributed to an increased absorption surface for nutrients and water – up to forty times greater – due to the fungal hyphae (Głuszek et al. 2020). Consistently, Peroni et al. (2024) observed positive effects of the HS + M treatment on Zn and Cu concentrations in sorghum shoots, but not in hemp. Since the concentrations of most macro- and microelements in leaves did not change following HS or HS + M application (Table 1), our results do not provide evidence for improved mineral nutrition. Only the Mn concentration in hemp grown with HS + M was higher in comparison to the control and HS treated plants. However,

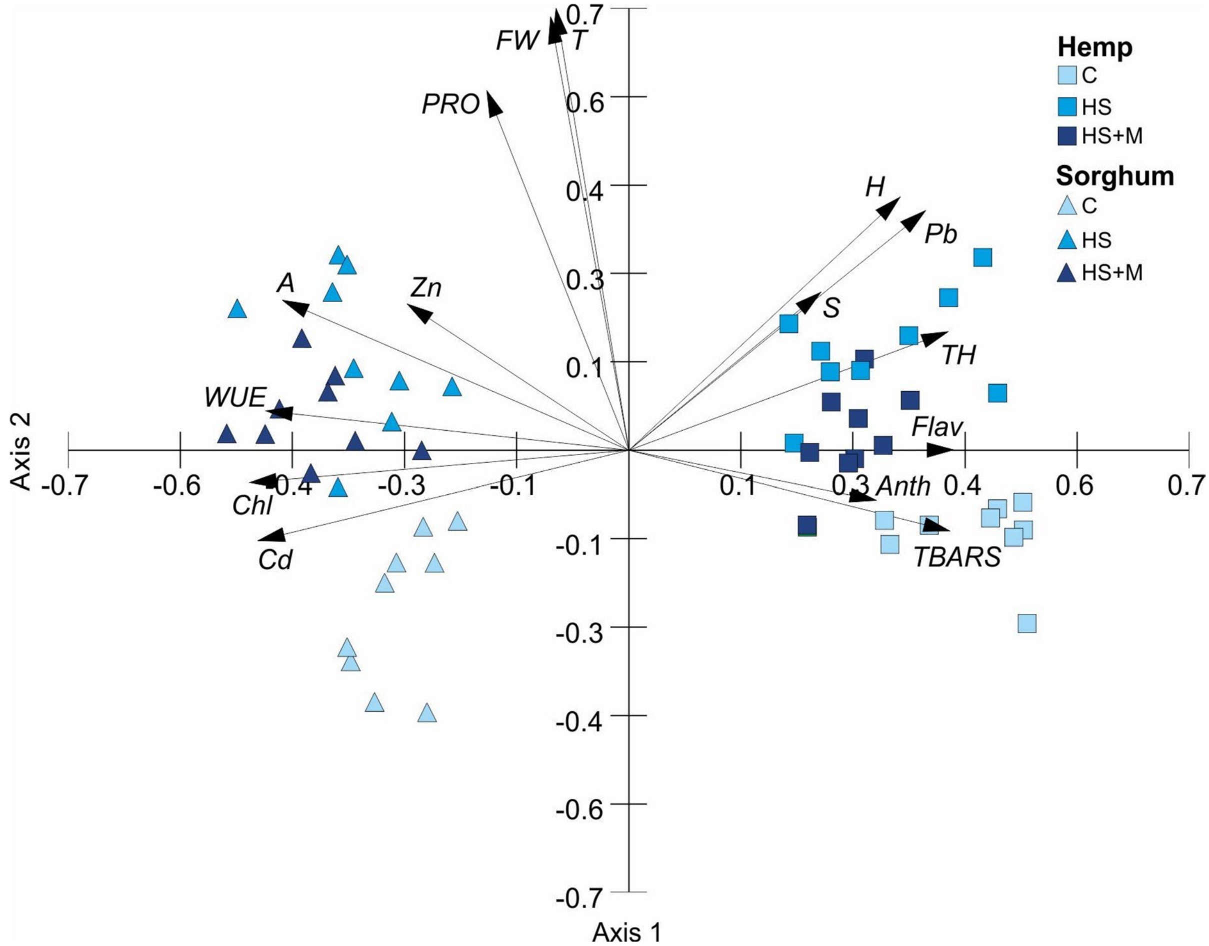


Fig. 6 Principal component analysis describing the significance of the studied variables in the response of hemp and sorghum grown on metal(loid)-polluted soil without (C – control) or with application of humic substances (HS) or humic substances and mycorrhiza (HS + M). Abbreviations: A – photosynthesis rate; Ant – anthocyanin content; Cd – Cd concentration; Chl – chlorophyll content; T – transpiration rate; Flav – flavonol content; FW – fresh weight; Pb – Pb concentration; Pro – proline concentration; TBARS – TBARS concentration; TH – leaf thickness; WUE – water use efficiency index; Zn – Zn concentration

no effects on shoot fresh weight or plant height were observed (Fig. 1A, B). Therefore, the mechanisms underlying the positive effect of HS on shoot biomass production in our experiment remain unclear and warrant further investigation.

It should be noted, that AFM root colonisation was not assessed in this study, and therefore all interpretations regarding fungal effectiveness remain speculative. No positive effects of AMF on the growth of hemp and sorghum were found (Fig. 1). Similarly, Qiao et al. (2015) did not observe significant effects of AMF, applied separately or in combination with biochar, on the growth of corn in soil with high Cd concentrations. AMF effectiveness is influenced by fungal species, native soil microbiota, and host plant genotype (Rouphael et al. 2015). Further, it was demonstrated that AMF activity is influenced by plant diversity, encompassing both crops and weeds (Jiao et al. 2011). Soil fertility also plays a crucial role. When the availability of nutrients and minerals is high, plants may not be colonized by AMF (Naher et al. 2013). Our experiment was conducted on fertile soil, and humic substances were additionally applied, potentially further enhancing nutrient bioavailability. This may have contributed to the failure to establish symbioses between fungi and plants. Soltangheisi et al. (2024) found that mycorrhizal fungi

Table 2 Results of PCA based on the most relevant analysed parameters (**A**) Eigenvalues and variance (%) explained by the first two PCA axes; (**B**) Loading components for each variable associated with the two axes

Chemical variables	Axis 1	Axis 2
(A)		
Eigenvalues	7.646	1.818
Percentage	50.976	12.117
Cum. percentage	50.976	63.093
(B)		
Chl	−0.348	−0.038
Flav	0.299	0
Anth	0.228	−0.058
T	−0.067	0.515
A	−0.318	0.175
WUE	−0.333	0.046
PRO	−0.131	0.419
TBARS	0.295	−0.094
S	0.177	0.184
TH	0.294	0.138
H	0.249	0.295
FW	−0.072	0.505
Cd	−0.34	−0.105
Zn	−0.204	0.17
Pb	0.273	0.279

For explanation of abbreviations see Fig. 6

did not alleviate Cu toxicity due to the fact that the Cu concentration was also toxic to the AMF. Similar results were obtained by Szada-Borzyszkowska et al. (2024), where high Hg concentrations in the soil led to reduced AMF colonisation on miscanthus roots. These findings suggest that the success of AMF-based biostimulation strategies may be limited in highly polluted or nutrient-rich soils. Accordingly, the high fertility and especially elevated metal(loid) concentrations in the soil of our experimental field may have negatively affected successful AMF colonisation. We plan to address this knowledge gap in future research to better understand the symbiotic relationship under such conditions.

Metal concentrations and stress metabolites

Metal accumulation and phytotoxicity

Considerable evidence shows that both hemp and sorghum are tolerant to elevated metal concentrations in the soil (Galić et al. 2019; Perlein et al. 2021). Therefore, both crops have been recommended for phytomanagement of metal-polluted soils (Ofori-Agyemang et al. 2024a,b; Testa et al. 2024; Vangronsveld et al. 2009).

Our experiment was conducted on soil significantly polluted with Zn, Pb, Cd, and As. As expected, substantial concentrations of these elements were also detected in the leaves of both plant species (Table 1). However, no toxicity symptoms such as chlorosis or stunted growth were observed. Zinc, as an essential micronutrient, was found at 150–175 mg kg^{-1} in hemp and 195–216 mg kg^{-1} in sorghum. Although these values slightly exceeded the optimal range for plant growth (25–150 mg kg^{-1}), they remained below toxicity thresholds, typically reported between 100–400 mg kg^{-1} (Kabata-Pendias and Mukherjee 2007; Kaur and Garg 2021). According to Marschner (1995), Zn toxicity symptoms, such as chlorosis, stunted growth, and oxidative stress, generally appear at concentrations above 100 to 700 mg Zn kg^{-1}. None of these symptoms were observed in our crops. Similarly, leaf concentrations of Pb (5.9–9.8 mg kg^{-1}), Cd (0.5–12 mg kg^{-1}), and As (5–20 mg kg^{-1}) were all below established toxicity thresholds for plants: 30–300 mg kg^{-1} for Pb, 5–30 mg kg^{-1} for Cd, and 5–20 mg kg^{-1} for As (Kabata-Pendias and Mukherjee 2007). These relatively low leaf concentrations likely reflect the low phytoavailability of these elements in the soil, which depends not only on their total concentrations, but also on their speciation, soil type, pH, organic matter content, and many other factors (Gul et al. 2016; Wójcik et al. 2014). It is therefore plausible that the potentially phytoavailable concentrations of these elements in the soil were too low to induce any stress symptoms, or that the cultivated crops possess an inherently high tolerance to them.

Biostimulant effects on metal uptake

The application of biostimulants, either HS or HS + M, did not affect the concentrations of Zn, Pb, Cd, or As in the leaves of the studied plants. These findings are consistent with those of Ofori-Agyemang et al. (2024b), who also reported no significant changes in metal accumulation following similar treatments. In contrast, other studies have shown increased metal accumulation in shoots upon application of these biostimulants (*e.g.* Peroni et al. 2024), highlighting the inconsistency in observed outcomes. The mechanisms by which HS or mycorrhizal fungi influence metal accumulation and tolerance in plants remain poorly understood (Guo et al. 2023). Several studies demonstrated that AMF can enhance the accumulation of metals such as Cr, Ni, Cd in shoots (Pan et al. 2024; Prasad et al. 2011). Conversely, other research reported a reduction in metal accumulation following AMF application (Kapoor et al. 2007; Liu et al. 2011). One possible explanation is that AMF may immobilize metals within the fungal biomass, thereby reducing their availability to host plants (Gonzalez-Chavez et al. 2002). Additionally, AMF can facilitate biochemical processes such as detoxification, translocation and transformation (Sanjana et al. 2024). Humic substances may also affect metal uptake through multiple pathways. They can reduce metal bioavailability via chelation and stabilization, or increase it by altering soil pH (Adhikari et al. 2023; Halim et al. 2003). The application of humic substances to soils polluted with various concentrations of Pb did not increase Pb concentrations in shoots of *Vetiveria zizanioides* (L.) Nash (Angin et al. 2008). The authors suggested that the lack of plant response may be due to insufficient metal(loid) availability in the soil.

Oxidative stress metabolites

The relatively low concentrations of metalloids in the leaves (Table 1) allow to assume that the studied plants did not experience significant metal-induced stress. Consequently, no alleviating effects of biostimulants could be expected in terms of stress mitigation. This is consistent with the general lack of changes in the concentrations of stress-related metabolites – the products of lipid peroxidation (TBARS), proline, and total sugars – between treatments in both crops (Fig. 5). The only exception was a significant increase in proline concentration in the leaves of sorghum after application of HS, and especially of HS + M. However, the reason for this remains unclear. Proline accumulation is typically associated with plant responses to abiotic stresses, including metal and oxidative stress (Hayat et al. 2012; Pandian et al. 2020). Under metal stress, proline acts as an excellent osmolyte, metal chelator, and an antioxidative defence molecule (Hayat et al. 2012; Rienth et al. 2014). However, in this case, the absence of TBARS elevation or growth impairment suggests that the increase in proline was not related to stress conditions, but possibly a shift in primary metabolism induced by the treatment.

Reactive oxygen species (ROS) are continuously produced in plant cells as by-products of normal metabolism, especially in chloroplasts, mitochondria, and plasma membranes (Mansoor et al. 2022). Their amount is kept under control by the antioxidative defence system comprising both enzymatic and non-enzymatic antioxidants (Hajam et al. 2024; Jorjani and Pehlivan Karakaş 2024). However, under stressful environmental conditions, production of ROS exceeds the quenching capacity of these antioxidants, leading to ROS accumulation and oxidative stress (Kamal and Ahmad 2022). Lipid peroxidation, typically quantified as TBARS, is a widely used marker of oxidative lipid injury and a general stress marker in plants (Morales and Munné-Bosch 2019). It remained unchanged in our experiment, indicating absence of oxidative stress.

Sugars and flavonoids (including flavonols and anthocyanins) also play roles in ROS detoxification and membrane protection (Jeandet et al. 2022; Keunen et al. 2013; Nawaz et al. 2024). Their stable levels across treatments further support the absence of stress-related metabolic responses. PCA analysis showed a positive correlation between TBARS and non-enzymatic antioxidants – flavonols, anthocyanins, and sugars (Fig. 6). This suggests that even under mild, sub-toxic exposure to metalloids, plants maintained homeostasis through basal non-enzymatic antioxidant defences, protecting the photosynthetic apparatus.

Photosynthesis and pigment content

Photosynthetic pigments participate in the absorption of light and its conversion to chemical energy through photosynthesis. Therefore, there is a strong correlation between chlorophyll concentration and photosynthetic efficiency (Atero-Calvo et al. 2024), which is also evident in our study (Fig. 6). In response to metal stress, the concentration of photosynthetic pigments, the rate of photosynthesis and the rate of transpiration usually decrease, which is, at least in part, the result of oxidative stress and oxidation of enzymes involved in the Calvin cycle and chlorophyll biosynthesis (Guidi et al. 2019; Irshad et al. 2024). Kalaji et al.

(2016) associate the decrease in chlorophyll content with reduced N and Mg absorption. In some studies, increases in these parameters could be attributed to the application of humic substances, both separately and in combination with AMF (Irshad et al. 2024; Zahra et al. 2024). In our study, Mg was not a factor influencing the chlorophyll content in leaves of any crop and treatment. Despite marked variations in chlorophyll content between treatments, particularly in hemp (Fig. 4, Table 1), no significant differences were observed in the concentrations of this element in leaf tissues. Additionally, the leaf concentrations of Fe, an element crucial for chlorophyll biosynthesis, did not differ between treatments. Interestingly, the concentrations of both elements were more than two times higher in hemp, while the chlorophyll content was almost two times higher in sorghum. This suggests that factors beyond nutrient availability – such as mesophyll structure, chlorophyll-binding protein expression, and biosynthesis and degradation rates – may influence chlorophyll content (Wang and Grimm 2021). The chlorophyll content was also negatively correlated with leaf thickness in these two crops. This was rather unexpected, as the opposite correlation is usually found, but may result from different leaf morpho-anatomical structure of the two crops (Pereyra et al. 2014).

In our experiment, hemp grown with HS and especially with HS + M exhibited higher chlorophyll contents compared to the control, yet this translated into enhanced photosynthesis rate and WUE index in the HS + M variant only (Figs. 2, 4). In sorghum, chlorophyll contents remained similar across treatments, but plants treated with HS + M also showed the highest CO_2 assimilation, transpiration, and WUE rates. These trends were further supported by PCA analysis, which confirmed a positive correlation between chlorophyll content, CO_2 assimilation rate, and the WUE index (Fig. 6). Moreover, higher values of these parameters clearly distinguished sorghum from hemp. More importantly, these parameters, along with the high transpiration rate, correlated with higher fresh weight in sorghum and were higher in plants grown with biostimulants.

The fact that the photosynthesis rate and WUE index were higher in sorghum than in hemp is not surprising, considering the different carbon fixation mechanisms related to morpho-anatomical and biochemical differences between the two species, which belong to C4 and C3 plants, respectively. In general, C4 plants have a significantly higher level of photosynthetic efficiency than most C3 species (Guidi et al. 2019). In C3 plants, only the Calvin cycle, which takes place inside the chloroplasts of mesophyll cells, is responsible for CO_2 fixation. In contrast, C4 plants have evolved a two-step CO_2 fixation process that is spatially separated between mesophyll cells and bundle sheath cells. In the first step, CO_2 entering the leaf interior is captured in the cytoplasm of mesophyll cells by phosphoenolpyruvate, forming oxaloacetate. Oxaloacetate is then reduced to malate, which is transported to bundle sheath cells, where it undergoes decarboxylation, releasing CO_2 for the Calvin cycle occurring inside their chloroplasts. This CO_2-concentrating mechanism in bundle sheath cells prevents the oxygenase activity of Rubisco and reduces energy losses due to photorespiration, which occurs in C3 plants, leading to a higher photosynthetic rate. Additionally, it maintains a high diffusion gradient for CO_2 at lower stomatal conductance (and reduced water loss by transpiration) while still providing sufficient CO_2 for photosynthesis; thus, the WUE index is also higher (Guidi et al. 2019; Hoover et al. 2023).

There exist rather little information concerning the comparison between C3 and C4 photosynthetic performance under the same stressful conditions and this was also not the purpose of our study. In general, C4 plants are expected to be less prone to PSII photoinhibition and photodamage than C3 plants under stressful conditions, as determined by chlorophyll fluorescence (Guidi et al. 2019). The F_v/F_m ratio is a key indicator of plant photosynthetic performance, with a significant decrease below 0.7 indicating PSII photoinhibition caused by a stress factor (Krzemińska et al. 2024; Lichtenthaler and Babani 2022). However, our data (Figs. 3A, B) show that this was not the case in hemp and sorghum grown either with or without biostimulants, further supporting our above-mentioned assumption that these plants were not under any significant stress.

In summary, the application of biostimulants, including humic substances combined with mycorrhiza, and especially when applied alone, significantly enhanced the fresh weight of sorghum and hemp cultivated on metal(loid)-polluted soil. This improvement can be attributed to more efficient photosynthesis, with enhanced CO_2 assimilation providing carbon skeletons directly used for plant growth and serving as a source of energy to drive all metabolic processes in the plant.

Conclusions and future research

Both hemp and sorghum are becoming increasingly important agricultural crops due to their versatile uses, resistance to changing climatic conditions, and tolerance to soil pollution. In this study, the effect of biostimulants – humic substances used alone or in combination with mycorrhiza – was tested as a contemporary approach to improving crop growth and stress tolerance. Our results demonstrated that the application of biostimulants, particularly humic substances, significantly increased the fresh weight of both hemp and sorghum grown on soil polluted with metal(loid)s (Zn, Pb, Cd, As). Enhanced biomass production, particularly in sorghum, was associated with more efficient CO_2 assimilation and a higher water use efficiency index, typical for C4 plants. The plants did not exhibit signs of metal(loid)-induced stress, most likely due to limited metal uptake and accumulation in the leaves, and/or the inherent high tolerance of both crops to these pollutants. Biostimulants did not affect mineral nutrition, the light phase of photosynthesis, or the concentrations of stress metabolites in plant leaves. An interdisciplinary approach is required to fully understand the effects of biostimulants on plant growth to ensure sustainable agriculture. For instance, it would be valuable to examine the mycorrhizal colonisation rates and the genetic and physiological profiles of the rhizosphere microbiomes following HS and/or HS + M application. This could help us to determine whether these amendments affect the occurrence of beneficial microbes and enhance their plant growth-promoting potential, thereby improving crop yields and tolerance to adverse environmental conditions.

Author contributions Conceptualization: Małgorzata Wójcik, Jaco Vangronsveld, Eleni G. Papazoglou, Efthymia Alexopoulou, Francois Rineau, Piotr Sugier, and Jolanta Jaroszuk-Ściseł; Methodology: Karolina Jaros-Tsoj, Krzysztof Sitko, and Małgorzata Wójcik; Formal analysis: Karolina Jaros-Tsoj, Krzysztof Sitko, Piotr Sugier, and Małgorzata Wójcik; Investigation: Karolina Jaros-Tsoj, Krzysztof Sitko, Małgorzata Rudnicka, Piotr Sugier, Jolanta Jaroszuk-Ściseł, Adam Rostański, and Małgorzata Wójcik; Writing—original draft preparation: Karolina Jaros-Tsoj and Krzysztof Sitko; Writing—review and editing: Jaco Vangronsveld and Małgorzata Wójcik; Funding acquisition: Eleni G. Papazoglou, Efthymia Alexopoulou, Jaco Vangronsveld, Małgorzata Wójcik; Supervision: Małgorzata Wójcik.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interest The authors have no relevant financial or non-financial interests to disclose.

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