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Pairing energy crops and root biostimulants for phytomanaging a former landfill

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ABSTRACT

Phytomanagement of contaminated soils is increasingly valued both to produce biomass for the bioenergy sector and as nature-based soil remediation option. However, degraded soil conditions may hinder energy crop growth. Biostimulants and micro-organisms could boost crop yield and phytomanagement performance but field-based evidences remain scarce. This study aimed at determining the shoot dry weight (SDW) yield, metal uptake and decrease in soil contaminants for *Cannabis sativa*, *Sorghum bicolor*, and *Miscanthus × giganteus* at a former landfill mainly contaminated by Cu, Zn and PCBs. The 3-year field trial, carried out in a randomized block design, consisted in control plots (C) and plots amended by humic substances paired with mycorrhizal fungi (Hs+M). The technosol exhibited high topsoil stoniness due to abundant debris and an unfavorable sandy-loam texture. SDW yields and metal concentrations (ICP - OES) were measured annually. Sorghum produced the highest SDW yields (3-year average: 20 Mg DW ha^{-1}) and responded best to the Hs+M treatment (+16 %-35 %). Treatment did not influence shoot metal concentrations, which tended to decrease over the years. However, higher SDW yields of sorghum increased its shoot Cu and Zn removals (+ 60 % and 46 %-65 % vs. control plants, respectively). Over the three years, shoot Cu uptake was similar for Hs+M sorghum and C hemp. DTPA-extractable soil metals did not change, final soil PCB concentrations as well. Consequently, crops studied would not promote environmental pollutant dispersion. Sorghum paired with the Hs+M treatment was the most promising option, maximizing biomass yield and gradual stripping of bioavailable soil Cu and Zn.

1. Introduction

Climate change and the necessity to secure stable energy supplies are key drivers of the global expansion of renewable energy, particularly in Europe, where the sector aims to cover 42.5 % of energy consumption in 2030. Bioenergy is pivotal in the European Union's ecological transition. Increasing lignocellulosic biomass production must support not only renewable energy generation but also the development of other bio-based value chains through biorefineries (Asgher et al., 2020; Baldinelli et al., 2024; Brodin et al., 2017; Clifton-Brown et al., 2023). The primary constraint lies in available land suitable for biomass cultivation without competing with food crops, impacting High Nature Value Farmland, or triggering the Indirect Land Use Change (ILUC) effect (Kulovesi and Oberthür, 2020). A strategy to address this challenge is the sustainable use of marginal and degraded lands for implementing low-input cropping systems that can enhance biomass production while providing essential ecosystem services (Von Cossel et al., 2019). Hence, today the interest increases for the phytomanagement of metal-contaminated soils through cropping lignocellulosic species. It is a dual solution for biomass production avoiding ILUC effect and remediating pollutant linkages, generally through Cd/Zn phytoextraction, Cu/Pb phytostabilization and organic contaminant rhizodegradation

Abbreviations: ILUC, Indirect Land Use Change; SDW, Shoot dry weight; Hs+M, Plots amended by humic substances paired with mycorrhizal fungi, C, control plots; PCB, Polychlorinated biphenyls; WHC, Soil water holding capacity; DTPA, Diethylenetriaminepentaacetic acid; ND, Not determined; SFW, Shoot fresh weight; DW, Dry weight; SMU, Shoot metal uptake; SMC, Shoot metal concentration; BCF_{tot}, Bioconcentration Factor based on total soil metal; BCFext, Bioconcentration Factor based on DTPA-extractable soil metal; MC $soil_{tot}$, Pseudo-total soil metal concentration; MC $soil_{ext}$, DTPA-extractable soil metal concentration; RDA, Redundancy analysis; HDPE, High-density polyethylene.

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(Robinson et al., 2009; Moreira et al., 2021). Such phytomanagement option is a potential cost-effective alternative to physico-chemical soil remediation methods, when there is no urgent land planning and pollutant linkages, as it significantly reduces costs, generally minimizes side effects (e.g., alteration of soil fertility and structure), and contributes to rehabilitate soil functions (Perlein et al., 2023). The long-term nature of phytomanagement strategies provides an opportunity to establish local value chains based on biomass utilization, thereby making extended-duration projects more desirable (Edgar et al., 2021).

Several successful case studies of phytomanagement are reported (Kidd et al., 2015; Mench et al., 2010, 2018; Vamerali et al., 2012, 2014; Vangronsveld et al., 2009). However, crop growth can be limited by various abiotic and biotic adversities at contaminated marginal sites, e.g., brownfields and landfills (Hostyn et al., 2022). These aspects are not irrelevant for the large-scale dissemination of phytomanagement systems, especially as many contaminated soils are located near these point sources of pollution and not in well-equipped agricultural contexts (e.g., machinery, hydraulic infrastructures) (Panagos et al., 2013; Römkens et al., 2023). In Europe about 100 000 ha are considered as landfills, largely used as dump sites, and about 70 000 ha as brownfields (Römkens et al., 2023). However, these estimates could further increase as many sites out of more than 2 million contaminated sites in Europe are waiting to be reclaimed (Panagos et al., 2013; Payá Pérez and Rodríguez Eugenio, 2018). Although lignocellulosic crops display marked resilience, factors beyond the presence of pollutants can influence their growth, e.g., unfavorable soil structure and pH, nutrient deficiencies, low organic matter content and water holding capacity, and high salinity (Kidd et al., 2015). Consequently, when both biomass production and soil remediation are of concern, phytomanagement options must be adapted to promote vigorous and sustainable crop growth.

Biostimulants and microorganisms are gaining increasing attention as effective strategy to support crop development under abiotic stress conditions (Leogrande et al., 2022; Rouphael and Colla, 2020). Their use appears promising to improve plant growth even in contaminated soils (Ciadamidaro et al., 2017; Visconti et al., 2023). However, their use still needs to be thoroughly investigated in-situ at more sites (Bartucca et al., 2022). Available results remain few (Nsanganwimana et al., 2015; Ofori-Agyemang et al., 2024a, b Rostami and Azhdarpoor, 2019). Most studies with biostimulants focus on high-profit food crops, e.g., grain cereals and horticultural crops (Li et al., 2022). Moreover, many studies were carried out in controlled-environment conditions and are often not followed by real field applications, where evidences of their beneficial effect are more lacking (Muratova et al., 2023; Nurzhanova et al., 2023). Under controlled conditions, treatment efficacy can be preliminarily evaluated on key parameters such as shoot dry weight (SDW) yield and shoot metal concentrations, but such conclusions are largely influenced by experimental factors and, therefore, not exhaustive for subsequent large-scale applications. Indeed, pot size can limit crop rooting (especially taproot and rhizomatous crops); climatic conditions provided in greenhouses do not replicate properly crop life cycle; weeds and pests do not occur and the metal(loid) bioavailability is influenced by soil sieving, air-drying and homogenization (Adamo et al., 2014; Nsanganwimana et al., 2021; Ofori-Agyemang et al., 2024b; Peroni et al., 2024). Therefore, conducting extensive open-field trials across multiple growing seasons is crucial to evaluate the efficacy of biostimulants and microorganisms in promoting energy crop growth under complex contamination scenarios and significant pedoclimatic constraints. This enables the development of innovative phytomanagement systems that can efficiently implement such nature-based products (Kidd et al., 2015; Ye-Tao et al., 2012).

This study investigated the agronomic and phytomanagement performance of three lignocellulosic crops (*Cannabis sativa* L., *Sorghum bicolor* (L.) Moench, and *Miscanthus* × *giganteus* (Greef et Deuter)) grown in a contaminated soil with (Hs+M) and without (C) the application of humic-based biostimulants paired with a mycorrhizal fungi inoculum over a three-year period (2022–2024). The study area was part of a former illegal landfill contaminated with multiple metals (Zn, Cu, Ni and Pb) and polychlorinated biphenyls (PCBs). The plant species were selected based on their promising characteristics for biomass production and phytomanagement of contaminated soils (Ofori-Agyemang et al., 2024a; Peroni et al., 2024). These species present a high versatility, resilience to adverse soil conditions, and ability to tolerate moderate metal concentrations in the soil (Priya et al., 2023; Scordia et al., 2022). Sorghum is an annual C4 plant species and ranks as the fifth most cultivated cereal crop globally (Umakanth et al., 2018). It is a major annual biomass crops in the Mediterranean region due to its high shoot yield potential (up to 29 Mg ha^{-1}) and exceptional tolerance to drought and high temperatures (Al Chami et al., 2015; Nazli et al., 2020; Zegada-Lizarazu and Monti, 2012). Additionally, sorghum displays a moderate capacity to accumulate certain metals, particularly Zn and Cd in stems (Al Chami et al., 2015; Ofori-Agyemang et al., 2024a; Zhuang et al., 2009). Hemp is a C3 annual herbaceous. It is a well-known industrial crop worldwide due to its ease of cultivation and multiple end products (Amaducci et al., 2015; Svennerstedt and Svensson, 2006). It also demonstrates good shoot biomass yields (12–15 Mg ha^{-1}) under diverse soil and climatic conditions (Prade et al., 2011). Hemp is one of the most relevant lignocellulosic species for phytomanagement, as it can tolerate high soil concentrations of various metals, including Pb, Ni, Cd, and Zn (Citterio et al., 2003; Rheay et al., 2021), effectively translocating Zn/Cd into the shoots and leaves, particularly in acidic soils (Citterio et al., 2003; Galić et al. 2019). Miscanthus is a rhizomatous, C4 perennial crop characterized by exceptional resilience to various abiotic and biotic stresses, thanks to its efficient antioxidant system (Wang et al., 2021). If the establishment phase is completed correctly, especially in the first year, it can subsequently achieve high biomass yields with minimal agronomic inputs (exceeding 15 Mg ha^{-1} in the Mediterranean region) and exhibit remarkable water-use efficiency (Heaton et al., 2010a; Monti et al., 2015). Miscanthus is of particular interest for phytomanagement due to its potential for coupling high SDW production with metal phytostabilization, as well as for revegetating degraded areas (Mamirova and Pidlisnyuk, 2024; Nsanganwimana et al., 2014). Although the potential of these crops for phytomanaging metal-contaminated soils is known, data from field trials over multiple agronomic seasons are less available when compared to studies on hyperaccumulators and tree species such as poplars and willows (Ciadamidaro et al., 2017, 2022; Guidi Nissim et al., 2018; Nkrumah et al., 2016; Vamerali et al., 2010). Application of these crops on soils contaminated with organic pollutants is further reduced, although some preliminary studies exist (Burdová et al. 2023; Muratova et al., 2009; Zhang et al., 2009), and almost absent in PCB-contaminated soils where the use of Cucurbits and maize are more promising as they can translocate into shoots these extremely recalcitrant organic compounds (Luo et al., 2015). Studies on their use pairing biostimulant applications under field conditions are even fewer (Nsanganwimana et al., 2015; Ofori-Agyemang et al., 2024a). Anyway, under greenhouse conditions, pairing humic substances and mycorrhizal fungi was effective in increasing SDW yield and shoot metal uptakes of all the three selected crops, as well as in reducing Cd and Zn bioavailability when applied to miscanthus and hemp (Ofori-Agyemang et al., 2024b; Peroni et al., 2024). However, under field conditions, this treatment did not improve SDW yield of sorghum, phytoextraction yield or phytostabilization efficiency when tested over two years in a contaminated farmland in Northern France (Ofori-Agyemang et al., 2024a). Differently it positively affected *Miscanthus lutarioriparius* SDW yield and Cd uptake in the second year of cultivation on former agricultural land in Hunan Province, China (Iqbal et al., 2025). Thus, further evidence on its potential application in open field and marginal contexts is required (Falconi et al., 2025). This study pursues this scope by comparing different plant species (two annuals and one perennial) both treated and untreated in a non-agricultural multi-contaminated soil. The aim is to identify the most effective crop-by-treatment strategy to maximize shoot SDW yield, metal uptake, and the reduction of soil contaminants. The impact of

Hs+M treatment on plant species was further assessed through the analysis of biometric parameters, shoot metal concentrations, and Bioaccumulation Indices. Additionally, a multivariate analysis was performed to evaluate how initial exposure to soil contaminants influenced crop yield and overall phytomanagement performances.

2. Materials and methods

2.1. Experimental set-up

Sorghum, hemp and miscanthus grown on C and Hs+M field plots were evaluated over three years in a randomized block design, using 10 m^2 plots (2.5 m x 4 m) in triplicates. Hs+M resulted from the combination of biostimulants: humic substances extracted from Leonardite including humic acids (75 % m/m), fulvic acids (5 % m/m) and organic carbon (20 % m/m) (Lonite 80SP®, Alba Milagro, IT) and an inoculum mixture of 7 species of arbuscular endo-mycorrhizal fungi naturally occurring in European soils: *Claroideoglomus claroideum*, *Rhizophagus irregularis*, *Claroideoglomus etunicatum*, *Funneliformis mosseae*, *Claroideoglomus lamellosum*, *Septoglomus deserticola* and *Rhizophagus diaphanus* (Symbivit®, Symbiom, CZ).

2.2. Site description and characterization

2.2.1. Soil analysis

The trial was carried out at the Chiarini site located on the outskirts of Bologna, Italy (44° 50' N, 11° 28' E), part of a former illegal landfill used for many years to dump illegally construction, craft and industrial wastes. Soil was characterized in spring 2022 before starting the trial. One composite topsoil sample per plot was collected as conventionally done for phytoremediation field trials (5 replicates, 0–0.3 m depth, nearly 500 g each) (Robinson et al., 2015; Vangronsveld et al., 2009). Soil samples were air-dried, homogenized, and then divided into sub-samples. The first one was used to determine soil stoniness (coarse fragments > 2 cm) as volumetric ratio based on Stavi et al. (2018). Then, a second sub-sample from each plot was taken and all were mixed together to obtain a composite soil sample to analyze its physico-chemical properties (Table 1). The soil pH was analyzed separately for each plot by potentiometric method in a 0.01 M $CaCl_2$ solution using a pH meter (D.M. 13 settembre 1999; UNI EN ISO 10390:2022). The original soil would have been an Inceptisol, but the high presence of residues and waste have profoundly changed its structure, making it a Technosol (WRB, 2022). Overall, it is sandy-loamy with more than 52 % of sandy component and a 20.4 ± 5.6 % of stoniness in the topsoil hence defined marginal according to JRC guidelines. This soil is slightly alkaline (pH = 8.3 ± 0.1) and calcareous, but with good supply of humified organic matter (no previous agricultural activities).

Two soil aliquots (20 g, sieved at 2 mm) of each plot were used for subsequent metal analysis. Pseudo-total Zn, Cu, Ni and Pb concentrations were determined via soil acid digestion in *aqua regia* (HCl: HNO_3 3:1 v/v) followed by inductively coupled plasma optical emission (ICP-OES) (UNIEN13657:2004 +UNIENISO11885:2009). Acid-soluble Zn, Cu, Ni, Pb, Cd, Co, Cr, Fe and Mn fractions were analyzed in the second aliquot using diethylenetriaminepentaacetic acid (DTPA) extractant as a proxy of the potential bioavailable fractions according to Lindsay and Norvell (1978) and following local technical indications. Topsoil Cu and Zn concentrations (Table 2) exceeded the legal thresholds established by the Italian Legislative Decree 152/06. In addition, excess of Ni and Pb were recorded in some plots. DTPA-extractable metal concentrations were low as compared to pseudo-total ones (Table 2). Finally, a soil subsample from each plot taken at the trial beginning was analysed for PCBs by congener separation in high-resolution gas chromatography (HRGC) and detection by high-resolution mass spectrometry (HRMS), according to EPA 1668B. Eleven PCB congeners were detected (Table 3); their sum exceeded the legal threshold value and most abundant compounds were the PCB-dioxin like PCB-18 and PCB-105.

Table 1
Soil physicochemical properties at the Chiarini site.

Parameter	Unit	Value
Clay	g kg^{-1}	149
Silt	g kg^{-1}	329
Sand	g kg^{-1}	522
WHC*	%	20
Total nitrogen	g kg^{-1}	1
Assimilable phosphorus	mg kg^{-1}	20
Exchangeable potassium	mg kg^{-1}	318
Total limestone	g kg^{-1}	160
Active limestone	g kg^{-1}	52
Total organic carbon	g kg^{-1}	10
Organic Matter	g kg^{-1}	17
C/N		11

*WHC soil water holding capacity.

2.2.2. Climatic characterization

During the three agronomic seasons (2022, 2023 and 2024), average daily temperatures from "San Luca" station and cumulative monthly rainfall from "Casalecchio Canonica" station were recorded. The climate is typical of a humid temperate region, with cold winters and hot, dry summers, 700 mm $year^{-1}$ of average rainfall. The year 2022 was the driest with a consistent reduction in cumulative monthly precipitation during the highest growth period: May-July (-36 %, Fig. 1a) and average monthly temperatures 2–3 °C above the historical trend (Fig. 1b). On the other hand, cumulative precipitation increased during the year in 2023, mainly due to floods in May (more than 300 mm, Fig. 1a). The rainiest year was 2024 (1031 mm) with particularly intense events at the end of the growing season (Fig. 1).

2.3. Agronomic management

Preliminary operations during year 1 were: removal of (i) the high-density thermoplastic polymer sheet that covered the entire site and (ii) the associated weed flora by excavator, stripping and harrowing (Bianchi, Casteggio, Italy) at 0.10 m. Subsequent crop agronomic management was carried out similarly each year manually. Chemical weeding was used to control the perennial invasive flora (e.g., *Robinia pseudoacacia*, *Carduus* perennial species) by systemic herbicide (Kyleo, Nufarm Italy, Bologna, Italy) with 6 L ha^{-1} dose one month before hemp seeding. Seedbed preparation was performed before each crop planting (sowing annuals and transplanting miscanthus rhizomes, dates are shown in Table 4) by hoeing and soil refinement.

Sorghum was sown at a rate of 6.7 kg ha^{-1} , 50 cm of inter-row

Table 2
Average pseudo-total metal concentrations of soil samples from the Chiarini site, their corresponding threshold values set by Italian law (D. Lgs 152/06), DPTA-extractable metal fractions and their percentage over the pseudo-total concentration.

Parameter	Total content (mg kg^{-1})	Legal threshold (mg kg^{-1})	DTPA-extractable fraction (mg kg^{-1})	DTPA vs. Total (%)
Copper (Cu)	155 ± 217	120	7.35 ± 4.81	4.74
Zinc (Zn)	276 ± 226	150	8.02 ± 5.96	2.91
Nickel (Ni)	48 ± 29	120	0.35 ± 0.21	0.7
Lead (Pb)	79 ± 56	100	4.77 ± 1.38	6.04
Cadmium (Cd)	*N.D.*	2	0.06 ± 0.04	*N.D.*
Cobalt (Co)	*N.D.*	20	0.04 ± 0.04	*N.D.*
Chromium (Cr)	*N.D.*	150	0.004 ± 0.004	*N.D.*
Iron (Fe)	*N.D.*	*N.D.*	4.32 ± 1.23	*N.D.*
Manganese (Mn)	*N.D.*	*N.D.*	4.93 ± 2.60	*N.D*

ND: not determined

Table 3
Average concentrations of soil PCB congeners (± standard deviation), their sum and the corresponding legal threshold values set by Italian law (D. Lgs 152/06).

PCB	Concentration	Unit
77	1440 ± 4639	ng kg^{-1} D.M.
81	65 ± 207	
105*	44505 ± 136248	
114	1814 ± 5689	
118*	89289 ± 260771	
123	8165 ± 25201	
126	155 ± 508	
156	12050 ± 36378	
157	3031 ± 9160	
167	5228 ± 16382	
189	437 ± 1285	
Sum	0.17	mg kg^{-1} D.M.
Legal Treshold	0.06	

*PCB-dioxin like

spacing. Hemp was sown at a rate of 50 kg ha^{-1} , 12.5 cm of inter-row spacing. Crop emergence was monitored by counting the plant number within a 1 m^2 area over three consecutive weeks, until reaching 18 plants m^{-2} for sorghum and 120 plants m^{-2} for hemp. Miscanthus was transplanted at a density of 2.5 rhizomes m^{-2}, 10 cm soil depth, 50 cm of inter-row spacing. Mycorrhizal inoculum was applied to all Hs+M plots at a rate of 20 g m^{-1} along the sowing furrows for annual crops each year, and 10 g per rhizome hole at the time of miscanthus transplanting. Humic substances were applied twice for all the species: the first application occurred at the 5th true leaf stage, while the second was carried out one month later. The application rate was 50 kg ha^{-1} , with a dilution ratio of 1.4 g L^{-1} of irrigation water. Prior to application, the product was dissolved in 1 L of tap water and vigorously stirred. Nitrogen fertilization (YaraVera, Granular Urea, Yara Italia, Milano, Italy), was applied concurrently with the first humic substance treatment, dosages followed local agronomic conventions. In sorghum plots, nitrogen application rates were progressively increased to mitigate soil fatigue due to continuous monoculture (Zegada-Lizarazu and Monti, 2012), with 120, 150 and 160 kg ha^{-1} applied respectively in 2022, 2023, and 2024. Lower nitrogen rates were applied to hemp and miscanthus (Amaducci et al., 2002; 2015; Monti et al., 2019): 100 kg ha^{-1} and 60 kg ha^{-1} in year 1 and 2, increasing to 120 kg ha^{-1} for hemp and reduced to 40 kg ha^{-1} for miscanthus in year 3. Irrigation was provided at the time of humic substance application, ensuring that C plots received the same water amount but without the treatment. In 2022, irrigation was applied in early vegetative phase to mitigate the effects of extreme drought, based on the needs of individual crops to emerge successfully (no fixed in-ground sensors were used owing to regulatory constraints). In total 30 mm for sorghum, 20 mm for hemp, and 46 mm for miscanthus of irrigation water were applied. From early July onwards, hemp flowering stage development was assessed. Harvesting occurred at full flowering with a minimum crop cycle of 90 days, and the accumulation of 2.000 growing degree days (GDD), to maximize cellulose yield while minimizing lignification (Amaducci et al., 2015; Rahemi et al., 2021). Sorghum harvesting was conducted at initial grain formation, before the onset of the milky ripening stage, to maximize lignocellulosic biomass accumulation (Zegada-Lizarazu and Monti, 2012). In year 1, miscanthus was harvested in winter to allow mineral re-translocation from shoots to rhizomes (Table 4), ensuring successful crop establishment (Heaton et al., 2010). In subsequent years, autumn harvesting was performed after complete shoot senescence.

2.4. Plant and soil sampling, laboratory analyses and calculations

Harvesting was performed within 2 m^2 sampling areas in the middle of each plot. Ten plants for sorghum and hemp and 5 plants for miscanthus were selected for biometric measurements: shoot height and basal diameter, number of ramifications / tillers per hemp and miscanthus plant, number of leaves per sorghum plant, number of leaves on the main miscanthus tiller. Then all plants in the sampling areas were manually cut to determine their fresh weight (FW). Subsequently, a shoot sub-sample of each plot (0.78 ± 0.53 kg) was dried in an oven at 60 °C until a constant weight was reached to determine the SDW. Each sub-sample of SDW was ground and then a 0.5 g aliquote for each plot and crop was wet-digested with nitric acid (HNO_3, 65 %, 5 mL) under pressure (UNIEN 13805:201). Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used to analyze the total metal concentrations in plant digestates. Shoot metal uptake (SMU) was then calculated as the product of SDW yield and shoot metal concentration (SMC).

$$SMU\ (\text{g ha}^{-1}) = SDW\ (\text{Mg ha}^{-1}) \times SMC\ (\text{mg kg}^{-1}\ \text{DM}) \quad (1)$$

The degree of metal translocation from soil to shoots was calculated in two ways, through the total bioconcentration factor (BCF_{tot}) relating the SMC and the pseudo-total metal concentration in the soil ($MC\ soil_{tot}$)

$$BCF_{tot} = SMC\ (\text{mg kg}^{-1}\ \text{DM})\ /\ MC\ soil_{tot}\ (\text{mg kg}^{-1}\ \text{DM}) \quad (2)$$

and the extractable bioconcentration factor (BCF_{ext}) by relating the SMC and the initial DTPA-extractable metal concentration in the soil ($MC\ soil_{ext}$)

$$BCF_{ext} = SMC\ (\text{mg kg}^{-1}\ \text{DM})\ /\ MC\ soil_{ext}\ (\text{mg kg}^{-1}\ \text{DM}) \quad (3)$$

as reported in Antoniadis et al. (2017); Ofori-Agyemang et al.

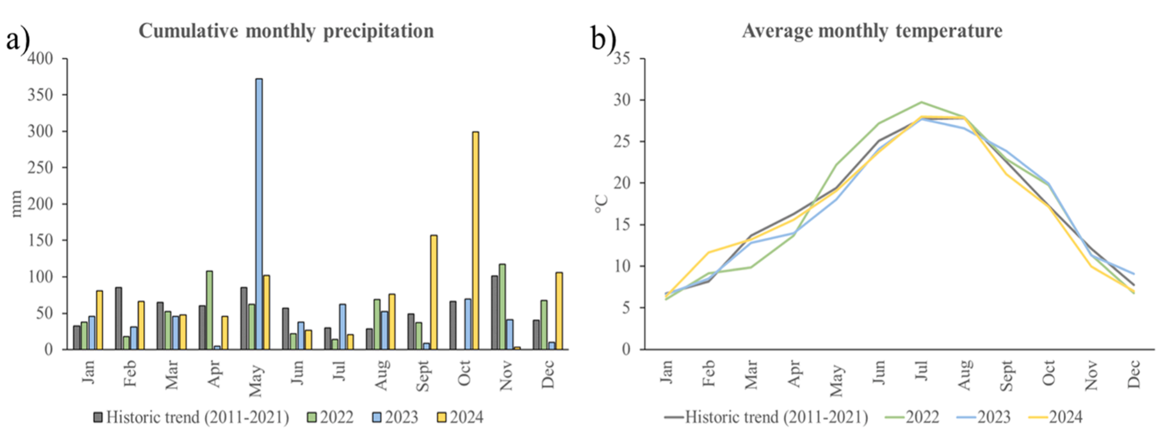

Fig. 1. Cumulative monthly precipitation (a) and average monthly temperature (b) recorded for the Chiarini area during 2022 (green bars and line, respectively), 2023 (blue bars and line, respectively) and 2024 (yellow bars and line, respectively) compared to the historical trends recorded between 2011 and 2021 (grey bars and line, respectively).

Table 4
Sowing/ transplanting and harvesting dates of each crop during the 3-year trial.

Crop	Variety	2022		2023		2024	
		Planting	Harvesting	Planting	Harvesting	Planting	Harvesting
Hemp	Futura 75	May 10	Aug 10	Apr 12	Jul 26	Apr 16	Aug 6
Sorghum	Bulldozer	May 19	Oct 7	May 5	Oct 12	May 10	Oct 16
Miscanthus		May 18	Jan 31*		Nov 29		Nov 21

* Miscanthus harvest for the 2022 agronomic season was carried out on January 31, 2023 to allow mineral re-translocation into the rhizome.

(2024a); Perlein et al. (2021). After the final harvest of each crop in 2024, composite soil samples (5 replicates) were collected from each plot (0–0.3 m soil depth). Final DTPA-extractable metal fraction and PCB concentrations in 2024 were analysed with the same methods adopted in 2022 at the trial beginning. PCBs were not analysed in shoots due to scarce evidence of their uptake in the studied species.

2.5. Data analyses

All the analysis were conducted using R software, version 4.3.0. SDW yield and SMU were evaluated by a twofold method. First, by comparing hemp and sorghum data (C and Hs+M) during all three years. Second, by including miscanthus as well, but analysing only data from 2023 and 2024 (C and Hs+M), because miscanthus is a perennial and its yield in the establishment year is low (Heaton et al., 2010; Nsanganwimana et al., 2015; Tejera and Heaton, 2017). Time repeated-measures ANOVA was conducted with both datasets, as the same crop * treatment combinations were set on the same plots each year. Individual plots were considered as subjects of the analysis, crop and treatment as between-subject factors, and year as within-subject factors. The year factor was also considered as random, as the block. The analysis was conducted after fitting the appropriate model for each parameter and appropriately modelling the corresponding variance-covariance matrix, through the evaluation of the Akaike's Information Criterion (AIC) index (Pagliari et al., 2022). Were statistically significant differences ($p \leq 0.05$) were found, Fischer's LSD post-hoc test was conducted to compare pairs of mean values. For the biometric variables, the analysis was conducted similarly, but in this case a hierarchic analysis was made within each crop separately (Laurent et al., 2019), as from an agronomic point of view it would not have made sense to compare botanically different crops, but rather the effect of treatments on them. Considering that the repeated crop cycles may affect metal uptake from the soil, specific analysis on shoot metal concentrations as well as BCF_{tot} and BCF_{ext} were carried out following the same time repeated-measures ANOVA procedure, but considering year as a fixed factor (Guidi Nissim et al., 2018; Ofori-Agyemang et al., 2024a; Nsanganwimana et al., 2015). Given the considerable hierarchical structure of the study factors and the increased possibility of incurring false positives, type I error, given by the growth of the experiment-wise error rate, for these parameters significant differences were considered for $p \leq 0.01$ (Huzsvai et al., 2022; Webster, 2007). DTPA-extractable metal and PCB concentrations in the soil at the trial end were assessed by ANCOVA, considering the initial concentrations at each plot as covariates and crop and treatment as factors (Yang and Juskiw, 2011). No data standardization was performed because the initial and final amounts were expressed in the same measurement unit. A Redundancy Analysis (RDA) was performed for each crop. The pseudo-total concentrations of Zn, Cu, Ni and Pb and the total sum of PCBs in the soil of each plot were considered as explanatory variables. SDW yield, average shoot Cu and Zn uptake, DTPA-extractable Zn, Cu, Ni and Pb concentrations and the total sum of PCB in each plot at the trial end were considered as dependent variables. For SDW yield and SMU, average data calculated over the three years were considered for sorghum and hemp while only data from 2023 and 2024 were considered for miscanthus. Initial DTPA-extractable metal concentrations and individual PCB congener concentrations were not included for not creating collinearity and to keep the number of explanatory variables lower than the replicate number. All variables were standardized (Capblancq and Forester, 2021; Huang et al., 2024).

3. Results

3.1. SDW yield and biometric traits

Comparing Hs+M and C hemp and sorghum plants over the three years (Fig. 2a), Hs+M sorghum yielded the highest SDW yield. Its average output over the three years was 20.1 Mg ha^{-1}, significantly higher than C sorghum (+35 %) as well as C and Hs+M hemp SDW yields. Surprisingly, Hs+M treatment reduced hemp SDW yield by 3.65 Mg ha^{-1} compared to C (Fig. 2a). Based on years 2 and 3 (Fig. 2b), Hs+M sorghum remained the best productive treatment. Hs+M treatment did not significantly increase miscanthus SDW yield compared to C plants despite an arithmetic difference between both mean values: 15.4 and 14.0 Mg ha^{-1}, respectively (Fig. 2b). In contrast to C miscanthus, Hs+M miscanthus allowed higher SDW yield than Hs+M and C hemp, not statistically lower than C sorghum, although lower than Hs+M sorghum (18.4 Mg ha^{-1}). Considering the biometric traits (Table 5), C hemp plants resulted on average 19 cm taller than Hs+M ones, having a larger diameter (+10 %). Conversely Hs+M sorghum plants were taller than C ones by about 50 cm, with a larger basal diameter (+ 3 mm on average). For miscanthus, plant height did not differ, but Hs+M treatment determined larger basal diameters than C, as well as substantially more tillers per plant (+53 %).

3.2. Shoot metal concentrations, BCF values and metal phytoextraction

Shoot metal concentrations (Fig. 3), both Bioconcentration Factors (Table 6) and shoot metal uptakes (Fig. 4) refer only to Cu and Zn as they were detected as main contaminants in the Chiarini topsoil and because Pb and Ni concentrations were found only in traces in most shoot samples. SMC were generally low, as relative BCF_{tot}, typical of lignocellulosic, non-hyperaccumulator plant species. All BCF_{tot} for both metals were largely < 1, while higher values were found for BCF_{ext} especially for Zn (Table 6). Over the three years, SMC averaged 10 mg Cu kg^{-1} and 44.7 mg Zn $kg^{-1}$for hemp, 5.3 mg Cu kg^{-1} and 60.7 mg Zn kg^{-1} for sorghum, and 5.1 mg Cu kg^{-1} and 46.2 mg Zn kg^{-1} for miscanthus. The statistical analysis carried out showed a crop * year interaction for both Cu and Zn ($p \leq 0.01$), with no effect of the biostimulant treatment (Fig. 3).

Overall, a declining trend from 2022 to 2024 emerged, except for shoot Zn concentration of sorghum, which increased again in 2024 being higher than in 2023 but still lower than in 2022 (Fig. 4b). The greatest decrease in shoot Cu concentrations between years 1 and 3 occurred for hemp (-61 % compared to −36 % for sorghum and −37 % miscanthus). In fact, hemp in 2022 had the highest shoot Cu concentration detected during the experiment (14.0 mg kg^{-1} DM on average). In contrast, the greatest decline in shoot Zn concentration between years 1 and 3 was recorded for miscanthus: −64 %, just higher than hemp (-60 %) and much greater than the decrease for sorghum (-35 %). Shoot Zn concentration peaked for sorghum in 2022 (98.9 mg kg^{-1} DM), not statistically different, however, from the values recorded for hemp and miscanthus in the same year (64.3 and 78.3 mg kg^{-1}, respectively).

An analogous crop * year interaction ($p \leq 0.01$) was found for both

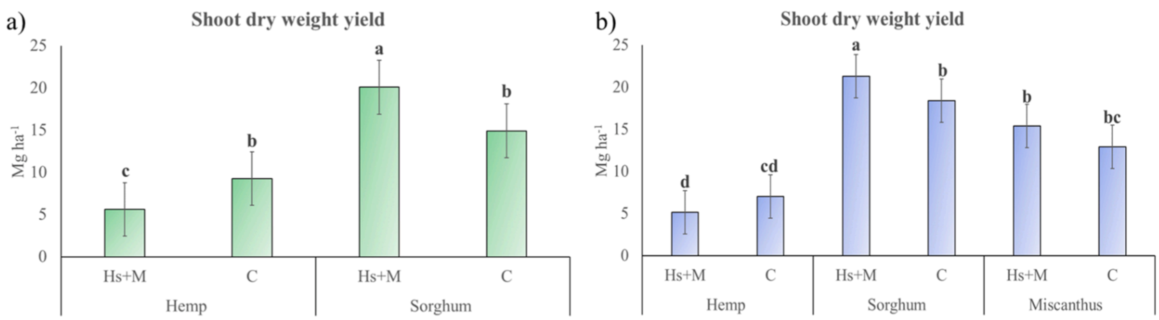

Fig. 2. SDW yield comparing (a) hemp and sorghum data for the three years and (b) hemp, sorghum and miscanthus data except year 1. All crops were evaluated with and without treatment application (Hs+M and C) and compared by time repeated-measures ANOVA ($p \leq 0.05$). Standard error is represented by the vertical black bars. The means were separated by Fisher's LSD test indicated by the letters above the histograms.

Table 5
Biometric traits measured at harvest for hemp, sorghum and miscanthus. All crops were evaluated separately with and without treatment application (Hs+M and C) and compared by time repeated-measures ANOVA ($p \leq 0.05$). The table shows the mean data (± the standard deviation). The means were separated by Fisher's LSD test indicated by the letters.

Crop	Treatment	Height (cm)	Basal diameter (mm)	Ramification/ Tiller per plant	Leaves on the main tiller
Hemp	Hs+M	175.5 ± 13.0 b	9.0 ± 0.9 b	12.9 ± 6.2 a	N.A.
	C	204.6 ± 12.0 a	9.9 ± 1.2 a	14.9 ± 5.4 a	N.A.
Sorghum	Hs+M	240.9 ± 53.5 a	20.2 ± 2.9 a	N.A.	14.4 ± 2.5 a
	C	189.1 ± 94.3 b	17.2 ± 2.1 b	N.A.	11.3 ± 3.2 b
Miscanthus	Hs+M	216.2 ± 71.5 a	10.3 ± 0.7 a	36.7 ± 7.1 a	15.6 ± 1.7 a
	C	201.4 ± 66.8 a	9.6 ± 0.6 b	24.0 ± 6.5 b	14.9 ± 1.4 a

metal BCF_{tot} and BCF_{ext} values (Table 6). Following shoot metal concentrations (Fig. 3), the same declining trend over the years, albeit with different magnitudes, was evidenced for the Cu and Zn BCF_{tot} in hemp and miscanthus, whose values in year 1 were significantly greater than in year 3. Considering the BCF_{ext} values of hemp, the value for Cu significantly decreased in year 3 (-62 %) as compared to year 1; conversely for miscanthus, a similar decrease (-74 %) was detected for Zn BCF_{ext}. Differently in sorghum, the Cu BCF_{tot} and BCF_{ext} showed no significant difference between the three years, while the Zn BCF_{tot} and BCF_{ext} in year 1 and 3 were not statistically different and greater than those in year 2. Overall, the highest Cu BCF_{tot} and Cu BCF_{ext} values were evidenced in hemp in 2022 and 2023 (BCF_{tot} = 0.13–0.17, BCF_{ext} = 2.67–3.01). The Zn BCF_{tot} of all crops in 2022, as well as hemp in 2023 and sorghum in 2024, did not statistically differ (BCF_{tot} = 0.22–0.49), and only miscanthus in 2024 and sorghum in 2022 showed statistically lower Zn BCF_{ext} values than the other treatments (BCF_{ext} = 5.76–16.13).

Shoot Cu uptake was similar for both Hs+M sorghum and C hemp when compared over the three years: 0.11 and 0.10 kg Cu ha^{-1}, respectively (Fig. 4a). Shoot Zn uptake (Fig. 4c) displayed a similar trend to that of SDW yield (Fig. 2a), as Hs+M sorghum allowed the greatest Zn recovery (1.27 kg ha^{-1}) compared to C sorghum, and both Hs+M and C hemp (about 1 kg Zn ha^{-1} less). Hs+M sorghum confirmed highest phytoextraction yields for both Cu (0.11 kg ha^{-1}) and Zn (1.05 kg ha^{-1}) when the three crops were considered on years 2 and 3 (Figs. 3b and 3d). No significant differences emerged for shoot Cu uptake among C sorghum and both Hs+M and C hemp and miscanthus, being all within the 0.05–0.07 kg Cu ha^{-1} range. In contrast, shoot Zn uptake of C sorghum (0.72 kg ha^{-1}, Fig. 4d) exceeded that of other treatments. Moreover, Hs+M application in miscanthus resulted in a higher Zn recovery (0.50 kg ha^{-1}) than in C miscanthus and both Hs+M and C hemp, which shoot Zn uptake varied in the 0.18–0.28 kg ha^{-1} range.

3.3. Changes in DTPA-extractable metal and PCB concentrations in the soil after three years

No significant difference was evidenced at the trial end for the DTPA-extractable concentrations of Cu, Zn, Ni and Pb (Table 7), as well as for the individual PCB congeners (Fig. 5). This lack of significant differences was likely due to the high statistical variance recorded for both parameters.

While the DTPA-extractable soil concentrations of metals did not

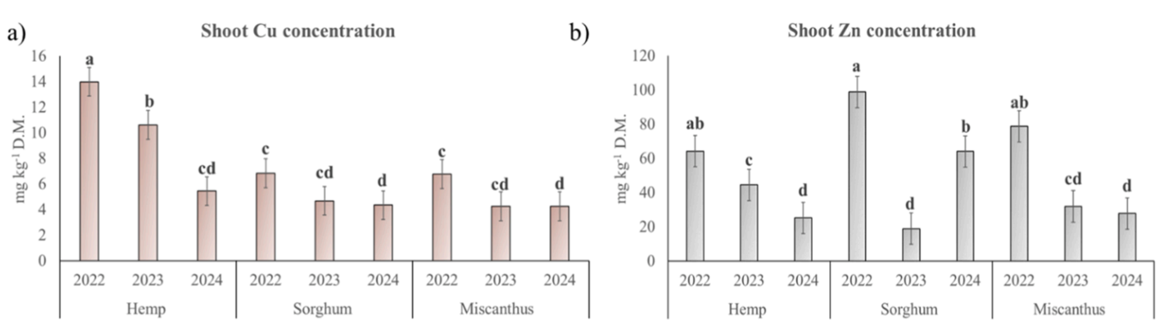

Fig. 3. (a) Shoot Cu concentrations and (b) shoot Zn concentrations of hemp, sorghum and miscanthus with the three-year data. All crops were evaluated with and without treatment application (HM and C) and compared by time repeated-measures ANOVA ($p \leq 0.05$). Standard error is represented by the vertical black bars. The means were separated by Fisher's LSD test indicated by the letters above the histograms.

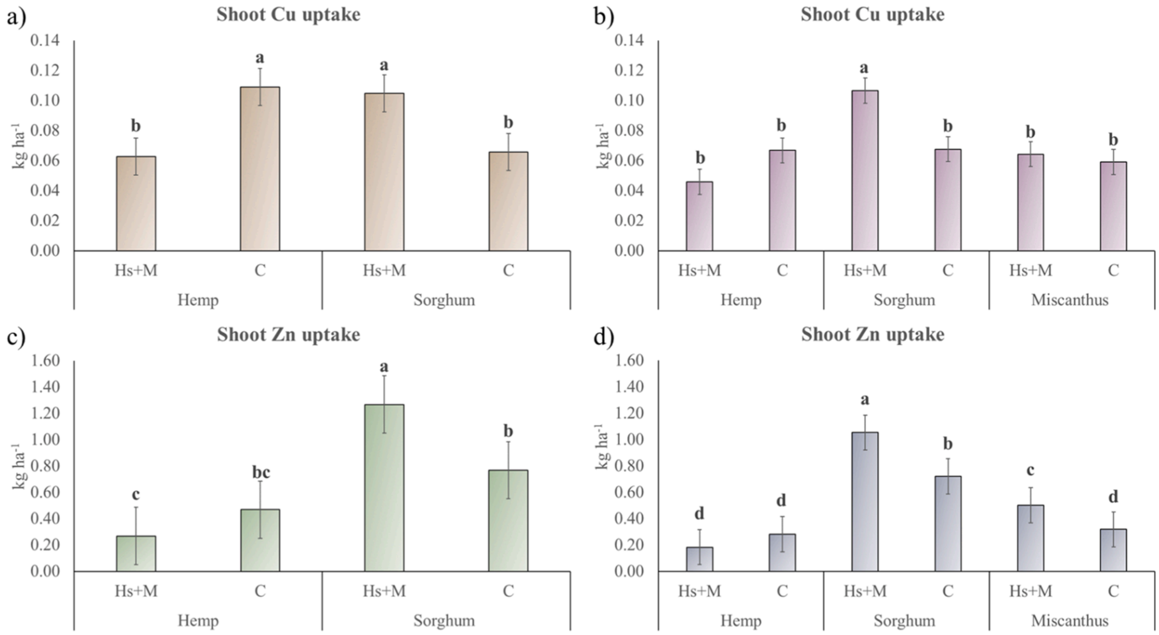

Fig. 4. Shoot Cu uptake (a) and shoot Zn uptake (c) comparing hemp and sorghum data over the three years; shoot Cu uptake (b) and shoot Zn uptake (d) based on hemp, sorghum and miscanthus data from years 2 and 3. All crops were evaluated with and without treatment application (Hs+M and C) and compared by time repeated-measures ANOVA ($p \leq 0.05$). Standard error is represented by the vertical black bars. The means were separated by Fisher's LSD test indicated by the letters above the histograms.

Table 6
Bioconcentration Factors of Cu and Zn calculated either as the ratio of shoot metal concentrations to the pseudo-total soil metal (BCF_{tot}) or the DTPA-extractable soil metal (BCF_{ext}). All crops were evaluated with and without treatment application (Hs+M and C) over the three years and compared by time repeated-measures ANOVA ($p \leq 0.01$). The table shows the mean data (± the standard deviation). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. If ANOVA found significant difference for at least $p \leq 0.01$, means were separated by Fisher's LSD and indicated by different letters a column.

Crop	Year	Cu BCF_{tot}	Zn BCF_{tot}	Cu BCF_{ext}	Zn BCF_{ext}
Hemp	2022	0.17 ± 0.08 a	0.32 ± 0.20 abc	2.93 ± 0.92 a	15.35 ± 8.17 a
	2023	0.13 ± 0.07 ab	0.23 ± 0.13 abc	2.19 ± 0.95 ab	10.27 ± 4.63 ab
	2024	0.06 ± 0.02 bc	0.13 ± 0.10c	1.10 ± 0.33 bc	5.77 ± 2.91 abc
Sorghum	2022	0.07 ± 0.04 bc	0.46 ± 0.31 ab	0.91 ± 0.35c	14.40 ± 11.17 ab
	2023	0.06 ± 0.04c	0.10 ± 0.08c	0.65 ± 0.33c	2.86 ± 2.78c
	2024	0.05 ± 0.05c	0.30 ± 0.19 ab	0.61 ± 0.42c	8.41 ± 5.23 abc
Miscanthus	2022	0.05 ± 0.04 bc	0.44 ± 0.37 a	1.10 ± 0.52c	16.22 ± 16.28 a
	2023	0.03 ± 0.02c	0.16 ± 0.04 bc	0.71 ± 0.26c	5.91 ± 3.20 abc
	2024	0.03 ± 0.02c	0.13 ± 0.07c	0.76 ± 0.39c	4.27 ± 2.25 bc

show clear trends, this was not true for the extracted soil PCBs. The plots cultivated with hemp C plants showed expected negative trends in particular for PCB-105, −118 and −156 (indicating either a potential dissipation, changes in chemical speciation, or in sorption properties) in contrast to the hemp Hs+M ones. Conversely, the Hs+M sorghum plots displayed such trend for the same congeners and not the C sorghum ones. For the miscanthus C and Hs+M plots, both displayed the same negative trend for the three PCB congeners mentioned above and three others to a lower extent. Overall, the greatest changes were recorded for dioxin-like congeners PCB-105 and PCB-118, which were the most abundant.

Table 7
Final DTPA-extractable soil concentrations of Cu, Zn, Ni and Pb. Hemp, sorghum and miscanthus were evaluated with and without treatment application (Hs+M and C) after the three years and compared by ANCOVA ($p \leq 0.01$) considering initial metal concentrations as covariates.

Crop	Treatment	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Pb (mg kg^{-1})	Ni (mg kg^{-1})
Hemp	Hs+M	7.92 ± 4.32	6.83 ± 1.23	5.47 ± 1.13	0.317 ± 0.067
	C	9.30 ± 2.90	13.45 ± 6.30	7.15 ± 1.25	0.375 ± 0.168
Sorghum	Hs+M	11.88 ± 11.71	16.35 ± 10.65	7.83 ± 1.72	0.518 ± 0.364
	C	10.37 ± 2.07	15.29 ± 9.98	12.67 ± 4.42	1.086 ± 0.590
Miscanthus	Hs+M	9.66 ± 0.13	13.38 ± 3.07	13.69 ± 5.38	0.479 ± 0.151
	C	7.66 ± 2.43	18.75 ± 13.19	18.32 ± 18.37	0.299 ± 0.101

3.4. Redundancy analysis

In the hemp RDA plot (Fig. 6a), the contribution rates of both axes were 39.7 % (RDA1) and 35.8 % (RDA2) respectively. The pseudo-total soil Cu concentration (Cu-Tot) greatly influenced the SDW yield and shoot Cu and Zn uptakes (Cu-SMU and Zn-SMU). The initial soil PCB content (PCB-S) directly affected their concentration at the trial end (PCB-F). For the sorghum RDA (Fig. 6b), RDA1 and RDA2 contributed to 42.7 % and 28 %, respectively. The initial pseudo-total soil metal concentrations directly impacted the DTPA-extractable soil metal concentrations in year 3, especially for Cu and Zn, but also on the final soil PCB content. However, PCB-S made the greatest contribution to RDA1 and, to a lesser extent, to SDW yields and phytoextracted metals. Fig. 6c showed the miscanthus RDA, whose axis contribution was 40.3 % for RDA1 and 34.8 % for RDA2. The PCB-S significantly shaped the shoot Zn uptake (Zn-SMU) and, vice versa, the initial pseudo-total soil Zn

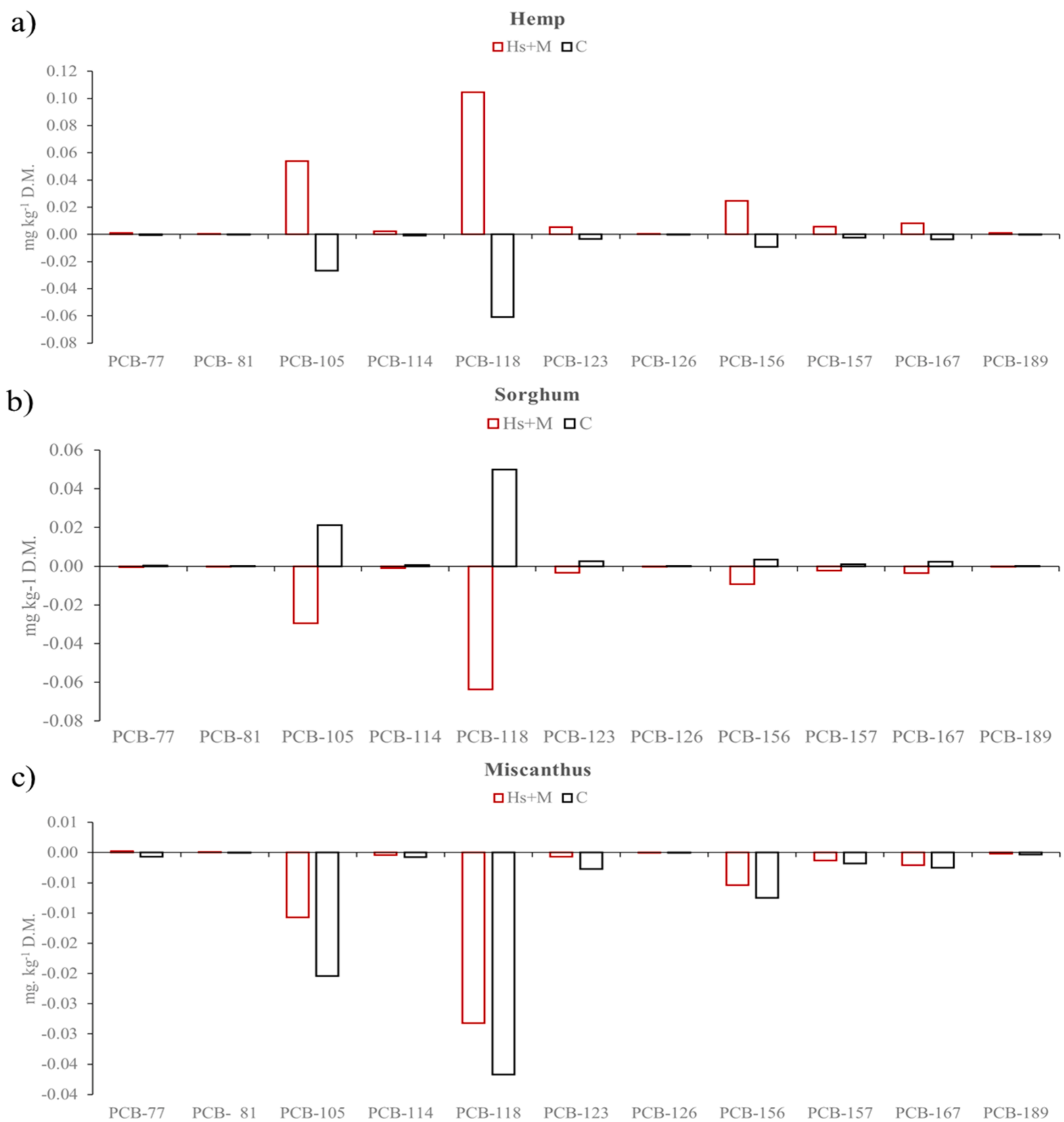

Fig. 5. Changes in the concentration of individual PCB congeners in the soil compared to the initial average value in (a) hemp, (b) sorghum and (c) miscanthus plots. Hemp, sorghum and miscanthus were evaluated with and without treatment (Hs+M and C) after the three years and compared using ANCOVA ($p \leq 0.01$) considering the initial concentrations of PCBs as covariates. The horizontal black line is the baseline on which changes in the individual congener were recorded, the red histograms refer to the treated plots (HM), the black ones to the untreated plots (C).

concentration (Zn-Tot) had the greatest impact on the PCB-F.

4. Discussion

4.1. Plant growth and biomass yield

Combined soil pollution and adverse marginal conditions characterize many contaminated soils and impact crop performances (Römkens et al., 2023). At the Chiarini site, the presence of multiple contaminants (Tables 2 and 3), the stoniness and sandy-loam texture (Table 1) of the soil were unsuitable for optimal crop cultivation. Normal crop rooting is largely affected by these adversities (Von Cossel et al., 2019). Sorghum requires a well-cultivated seedbed (Zegada-Lizarazu and Monti, 2012). For hemp taproot, soil penetration can be greatly reduced (Amaducci et al., 2015). In miscanthus such soil conditions can lead to water deficiency (Heaton et al., 2010). In this context, SDW yields, even without Hs+M application, are satisfactory for the three crops studied and within the literature ranges (Fig. 2, Amaducci et al., 2015; Blanco-Canqui, 2016; Zegada-Lizarazu et al., 2010). Specifically, on average, C sorghum and miscanthus produced more than 10 Mg ha^{-1} respectively (Figs. 1a and 1b), while C hemp slightly less (9.3 Mg ha^{-1}, Fig. 1a). Hs+M application increased sorghum SDW yield in both the statistical analysis performed (+16 %-35 %, Fig. 1a, b), mainly thanks to greater shoot height (Table 5). Although other biometric parameters, i.e. basal diameter and number of leaves, significantly promoted final SDW yield, their impact was less pronounced under the high seeding densities and maturity stage at harvest, as shoot biomass partitioning was markedly skewed towards stalks rather than leaves. Li et al. (2022) underlined how biostimulant application on field-grown grasses usually results in 10 %-16 % of grain yield increases, thus an estimated increase of approximately 20 %-30 % in aboveground biomass yield assuming an average Harvest Index value of

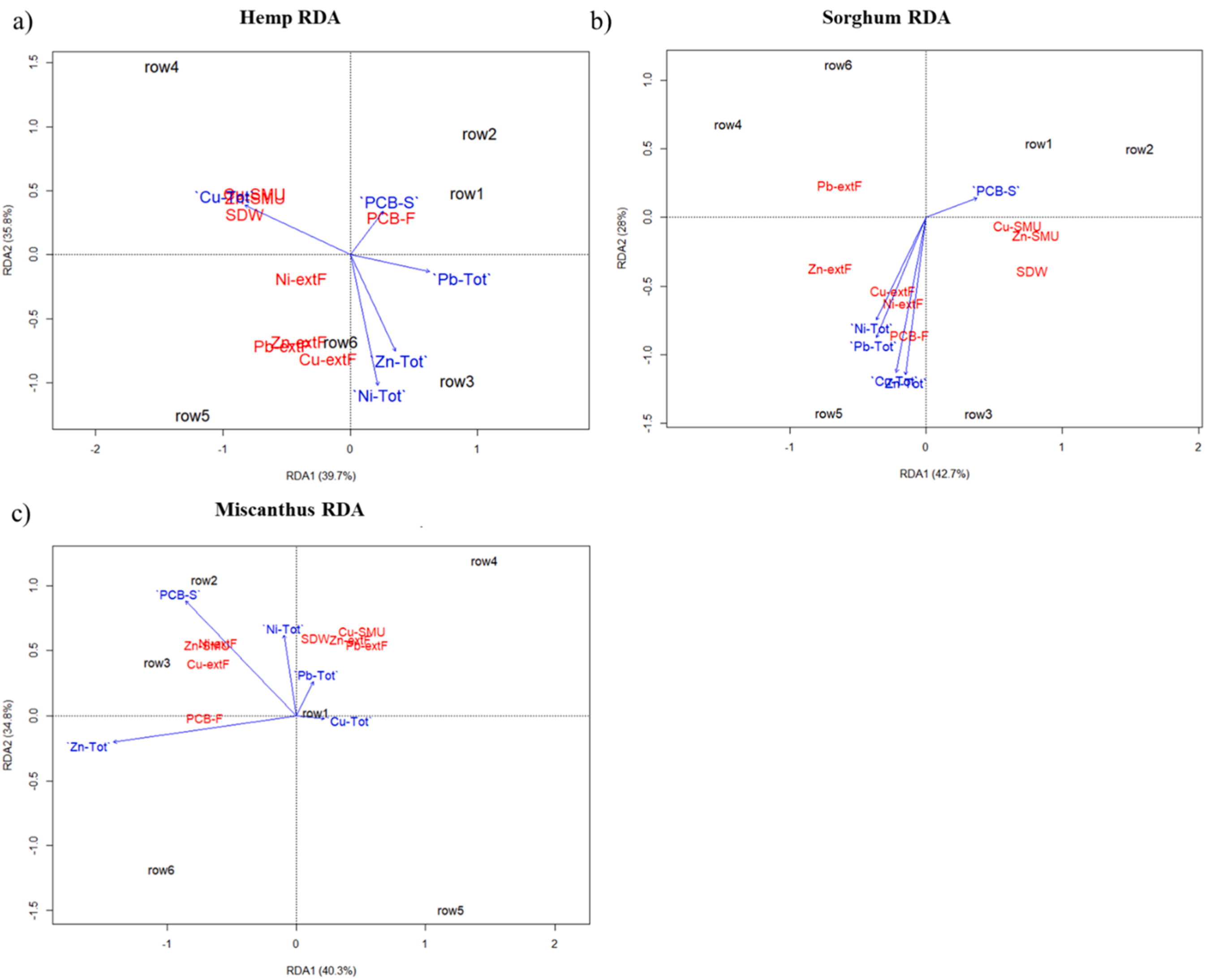

Fig. 6. Redundancy analysis (RDA) of (a) hemp, (b) sorghum and (c) miscanthus. The blue vectors represent the explanatory variables measured at the trial beginning: the pseudo-total concentrations of Cu, Zn, Ni and Pb (Zn-Tot, Cu-Tot, Ni-Tot, and Pb-Tot) and the total PCB content (PCB-S) in the topsoil of each plot. The terms in red represent the dependent variables: shoot DW yield (SDW), shoot Cu and Zn uptakes (Cu-SMU and Zn-SMU, respectively), the DTPA-extractable soil concentrations of Cu, Zn, Ni and Pb (Zn-extF, Cu-extF, Ni-extF and Pb-extF, respectively) and the total soil PCB content (PCB-F) at the trial end. The terms in black represent the individual plots on which the data were measured. RDA1 axes for each plot are indicated by the horizontal black line, RDA2 for each plot are indicated by the vertical black line.

0.5 (Yang and Zhang, 2006). Our results for Hs+M sorghum were in line with such findings. Similarly, miscanthus, exhibited an average increase of 13 tillers per plant (Table 5) resulting in + 19 % of SDW yield on Hs+M plots (Fig. 1b) although not significantly different from C miscanthus, but still greater than both C and Hs+M hemp plants, for which the Hs+M treatment had a detrimental effect. This appears linked to reduced morphological development, namely lower plant height and basal diameter (Table 5), while branching was unaffected, being primarily determined by sowing density. In hemp, the Hs+M treatment may have promoted metal accumulation in the roots (Peroni et al., 2024), without detectable differences in the shoots, likely due to limited translocation before full flowering, when biomass was harvested. This may have slowed development compared to the control, though this remains speculative without further evidence.

These findings underscored a more pronounced positive impact of the treatment on the growth of grass crops, in accordance with the observations of Iqbal et al. (2025) on *Miscanthus lutarioriparius* and *Panicum virgatum* L. Differently, Ofori-Agyemang et al. (2024a) did not reflect such positive improvements on SDW yield of the same sorghum cultivar following humic substances application, either single or paired with mycorrhizal fungi, probably because our study area (Po Valley), is much more suitable for sorghum cultivation than Northern France (Appiah-Nkansah et al., 2019; Zegada-Lizarazu and Monti, 2012). Furthermore, our study was conducted in a degraded and marginal non-agricultural area, which can accentuate the treatment's effects compared to untreated plants, particularly in enhancing root development and nutrient uptake. Indeed, humic substances promote soil fertility by influencing various physicochemical properties (Baltazar et al., 2021). Direct evidence in sorghum is lacking, but their primary effect on maize growth is root auxin activity stimulation, enhancing membrane ATPase activity, transporters, and vacuole pumps, leading to altered electrochemical gradients, which increase nitrogen uptake (Nardi et al., 2018; Jindo et al., 2020). According to Baltazar et al. (2021), humic substances increased plant vigour in the early stages of the crop cycle of barley and wheat. This would enabled sorghum and miscanthus to cope with the extreme weather conditions (drought in 2022 and flooding in 2023; Fig. 4.1), experienced in the most delicate emergence/establishment phase (Heaton et al., 2010; Monti et al., 2015;

Zegada-Lizarazu et al., 2012). Moreover, the applied arbuscular mycorrhizal fungi can infiltrate root cells by forming branched arbuscules (Chen et al., 2016) enhancing nutrient and water uptake efficiency while improving resilience to both biotic and abiotic stresses (Ciadamidaro et al., 2022; Jansa et al., 2008; Smith et al., 2009; Tedersoo et al., 2020).

Within the framework of the European GOLD project, the use of hemp, sorghum, and miscanthus biomass harvested at metal-contaminated sites was assessed for producing clean bioethanol followed by slag treatment and/or metal vitrification. Talluri and Miliotti (2024) evaluated three pre-treatment methods: TorWash(R) (developed by TNO, Dutch Organisation for Applied Scientific Research, The Netherlands), slow pyrolysis and torrefaction, followed by gasification, gas cleaning, and syngas conversion steps. Their findings indicate that the torrefaction-based pathway yields the highest bioethanol outputs. At the Chiarini site, the use of Hs+M sorghum biomass would maximize these yields: 2.8–3.0 Mg bioethanol ha^{-1} $year^{-1}$. In contrast, lower bioethanol yields, proportional to the SDW, could be expected from hemp (0.7 – 1.3 Mg bioethanol ha^{-1} $year^{-1}$), while miscanthus would provide intermediate yields, particularly when subjected to Hs+M treatment (1.8 – 2.1 Mg bioethanol ha^{-1} $year^{-1}$). According to previous data from phytomanagement field studies on sorghum, hemp, and miscanthus (Table 8), our shoot metal concentrations (Fig. 3a, b) fall within commonly reported ranges and remained well below phytotoxicity thresholds, i.e. 25–30 mg Cu kg^{-1} and 100–300 mg Zn kg^{-1} (Mench et al., 2010, 2018; Vangronsveld et al., 2009). Therefore, data recorded at the Chiarini site would not hinder the local processing of the harvested biomass. This is pivotal for enabling the use of biomass from the three energy crops cultivated on degraded and contaminated lands, such as former landfills, alongside biomass sourced from other marginal areas and from innovative cropping systems, such as double-cropping, without the need for specific pre-treatment steps. This, in turn, facilitates both harvest planning and logistics for processing facilities and local biorefineries (Edgar et al., 2021; Scordia et al., 2022). Parenti et al. (2024) empathized this multi-site / multi-source approach to promote a single advanced bioethanol production plant with a capacity of 250 000 Mg ha^{-1} as the most desirable option to replace approximately 5–7 % of the ethanol demand in the Emilia-Romagna Region, where this study was conducted. Based on their study, biomass sorghum as a feedstock would offer a more cost-effective alternative compared to conventional wheat straw.

Table 8
Cu and Zn concentrations in aboveground tissues of sorghum, hemp and miscanthus in open field trial reported in literature.

Plant species	Metal	Aboveground Biomass Concentration (mg kg^{-1})	Ref.
Sorghum	Cu	4.36 – 6.85 (shoots)	*This study*
		1.3 – 1.9 (stem), 3.1 – 4.6 (leaves)	(Angelova et al. 2011)
		28.6–48.9 (shoots)	(Marchiol et al. 2007)
	Zn	64.2–99 (shoots)	*This study*
		32.5 – 114.6	(Yuan et al. 2019)
		67.1 – 80.9 (leaves)	(Zhuang et al. 2009)
		11.1 – 15.7 (stem) 31.7 – 45.1 (leaves)	(Angelova et al. 2011)
		55.5 – 115 (shoots)	(Marchiol et al. 2007)
		125 – 133 (shoots)	Eissa & Almaroai (2019)
		127.7 (shoots)	(Mayerová et al. 2017)
		140 – 200 (shoots)	(Perlein et al. 2021)
		88.4 – 124.3 (shoots)	(Ofori-Agyemang et al. 2024a)
Hemp	Cu	5.5 – 14 (shoots)	*This study*
		4 – 6.2 (shoots)	(Angelova et al. 2004)
		2–6 (stem), 12–14 (leaves)	(Guidi Nissim et al. 2018b)
	Zn	25.3 – 64.3 (shoots)	*This study*
		12.7 – 54.5 (stem), 9.3 – 40.0 (leaves)	(Angelova et al. 2004)
		278.7 (leaves), 45.5 (stems)	(Canu et al. 2022)
		20–30 (stem), 100–150 (leaves)	(Guidi Nissim et al. 2018b)
			(De Vos et al. 2023)
Miscanthus	Cu	4.26 – 6.78 (shoots)	*This study*
		3.8 – 10 (shoots)	(Galende et al. 2014)
		1.7 (shoots)	(Mench et al. 2022)
	Zn	27.8 – 78.8 (shoots)	*This study*
		12.5 – 90.7 (stem) 21.1 – 60.6 (leaves)	(Nsanganwimana et al. 2015)
		50.3 – 131.4 (shoots)	(Grzegórska et al. 2023)
		103.0 (shoots)	(Rusinowski et al. 2019)
		26.9 – 57.1 (leaves)	(Pidlisnyuk et al. 2022)
		31.4 – 64.6 (leaves)	(Bastia et al. 2023)
		14.1 – 39.7 (shots)	(Antonkiewicz et al. 2016)
		93.7	(Deans et al. 2023)

4.2. *Plant metal phytoextraction, phytostabilization and PCB removal*

Despite no direct treatment effect, shoot Cu and Zn concentrations significantly decreased over the years for the three crops, except for Zn in sorghum (Fig. 4a, b). Extreme rainfall in early spring of year 1 (Fig. 1) may have limited root development and uptake, and promoted either metal leaching downward or partial Zn precipitation in the alkaline soil, reducing its availability during peak crop uptake phases (Bidar et al., 2009; Pan et al., 2016). The general metal concentration drop in shoots may be attributed to decreased metal phytoavailability and/or an increased shoot yield, leading to metal dilution effect. This latter hypothesis is likely for miscanthus, classically showing less shoot production in year 1 (1.4–1.6 Mg ha^{-1}), followed by a significant increase in year 2 (11.3–14 Mg ha^{-1}), and typically peaking in year 3 (14.6–16.8 Mg ha^{-1}) (Monti et al., 2015), while shoot metal concentrations dropped from 6.8 to 4.3 mg Cu kg^{-1} and from 78.8 to 27.8 mg Zn kg^{-1} DM (Fig. 3a, b). However, this interpretation cannot be exhaustive for hemp and sorghum. Rather, the cultivation lack and, especially, the previous soil coverage with an HDPE sheet, adopted to reduce metal leaching, may have enhanced a substantial share and absorption of the bioavailable metal fraction in 2022. Although the DTPA-extractable metal fraction did not significantly change (Table 7), a decrease in the more phytoavailable metal pools may occur. In Ofori-Agyemang et al. (2024a), at a farmland near the former Metaleurop smelter, after two sorghum crops, 0.01 M $Ca(NO_3)_2$–extractable soil Cd, Pb, and Zn concentrations dropped drastically, particularly for Zn (-95 %). Conversely, Guidi Nissim et al. (2018) found no significant differences in DTPA-extractable Zn and Cu fractions after two years of hemp cropping at a military base, both metals having a low initial bioavailability. Generally, DTPA extraction tends to overestimate the soil fraction of metals readily exchangeable and assimilable by roots, as DTPA can react with metals present in organic phases, carbonates, Al/Fe/Mn (oxy)hydroxides and clays (Soriano-Disla et al., 2010). DTPA-extraction was adopted in our study as a method to assess the *potential* bioavailable soil metal fraction in a contaminated area, as required by local technical guidance, serving to closely monitor the possible physicochemical mobility of metal(loid)s into other environmental compartments (Peijnenburg et al., 2007). The results often correlate with pseudo-total soil metal concentration in aqua regia and the root concentration of metals strongly sorbed with organic compounds (e.g., Cu and Pb), but poorly correlate with shoot metal concentration, for which it is preferable to determine the exchangeable fraction using weak, (un)buffered saline solutions (Bakircioglu et al., 2011; Gryschko et al., 2005; Meers et al., 2007; Mench et al., 1994; Soriano-Disla et al., 2010). Hence, our results indicated that energy crops can be cultivated in former landfills without causing increased environmental dispersion of the detected metals, especially at the Chiarini site with low initial metal bioavailability and sub-alkaline pH.

Higher SDW yields mainly contributed to highest shoot Cu and Zn uptakes as reflected in all crop RDA (Fig. 6a, b, c). This was significant as phytomanagement practices with high-yielding crops can promote the phytoextraction of some potentially phytoavailable metal(loid)s (e.g. Zn) from contaminated soils (Mayerová et al. 2017; Moreira et al., 2021). Direct relationship between higher SDW yield and metal(loid) uptake is common in several experiments with industrial crops, particularly sorghum (Guidi Nissim et al., 2018; Marchiol et al., 2007; Zhuang et al., 2009). Here, Hs+M sorghum displayed the highest shoot Cu and Zn uptakes (Fig. 3a, b, c, d), resulting particularly efficient in bioavailable Zn stripping (Marchiol et al., 2007; Perlein et al., 2021). The higher Cu and Zn uptakes could be traced back to root uptake, transfer and storage into the greater number of leaves (Table 5) (Angelova et al., 2011; Zhuang et al., 2009). The Cu amount (kg ha^{-1}) phytoextracted by the harvested shoots of hemp (C: 0.11 – Hs+M: 0.06) and sorghum (C: 0.07 – Hs+M: 0.10) (Fig. 3a) are of the same magnitude order as the highest for sunflower (0.02 – 0.09, Mench et al., 2018). The decreasing trend for shoot Cu concentration of hemp was also noted by Guidi Nissim et al. (2018) suggesting it is due to reduced Cu phytoavailability over time not detectable by the DTPA-extraction. This progressively induced declining BCF_{tot} and BCF_{ext} values (Table 6), as observed for Zn in miscanthus (Fig. 4b, Table 6).

Sorghum RDA (Fig. 6b), highlighted that the initial soil PCB concentration (PCB-S) mainly contributed to RDA1 and SDW (Fig. 6b). This result, along with the trend for changes in PCB congeners (Fig. 5b), strictly aligned with sorghum SDW yield. A bit of PCB phytoextraction and/or dissipation might occur. There is no direct evidence of root-to-shoot translocation of PCB in sorghum, but accumulations of polychlorinated dibenzo-p-dioxins and dibenzofurans in roots have been reported (Zhang et al., 2009), and PCB phytoextraction has been documented in maize (Luo et al., 2015; Wang et al., 2011). Differently, in hemp RDA (Fig. 6a), the initial pseudo-total soil Cu concentration (Cu-Tot) influenced both hemp SDW and the SMU. Shoot Cu concentrations were at the bottom range of frequent values for plant species (Table 8), likely mirroring the low Cu bioavailability in the Chiarini soil (Table 2). This may have constrained both hemp growth and phytoextraction efficiency. Nevertheless, its shoot Cu concentrations (5–16 mg kg^{-1} DM) were similar or exceeded those determined in field studies conducted under similar soil Cu levels (Angelova et al., 2004; Guidi Nissim et al., 2018), which was particularly relevant given the alkaline pH and favourable organic matter content of Chiarini soil (Table1) (Galić et al. 2019). A dual relationship emerged in the miscanthus RDA (Fig. 6c). The initial soil PCB concentration (PCB-S) directly influenced shoot Zn uptake, while the initial pseudo-total soil Zn concentration (Zn-Tot) affected the final soil PCB concentration (Fig. 6c). Unlike sorghum and hemp, changes in soil PCB concentration of miscanthus-plots were unrelated to differences in SDW yield between C and Hs+M plants (Fig. 5c). Among the three tested crops, miscanthus, due to its perennial nature, extensive root system, and high rhizodeposition (which may enhance microbial activity), could have the strongest potential to promote organic pollutant dissipation, although research has primarily focused on PAHs and petroleum-derived sludge rather than PCBs (Burdová et al. 2023; Figala et al., 2015; Muratova et al., 2022; Techer et al., 2012). Unexpectedly, initial pseudo-total soil Pb and Ni concentrations likely influenced SDW and consequently shoot Cu uptake of Miscanthus (Fig. 6c). However, shoot concentrations of Cu and Zn (both essential micronutrients) were similar to those in sorghum and only slightly lower than shoot Cu concentrations of hemp in the first two years (Fig. 3a, b). Under Hs+M treatment, miscanthus produced a higher SDW yield than hemp (Fig. 2b), resulting in shoot Cu uptake similar to hemp and a greater shoot Zn uptake. These results reinforced also miscanthus potential for landfill phytomanagement, such as the Chiarini site. Indeed, its perennial nature also confers agronomic advantages over annual crops, reducing management inputs in year 2 and promoting soil health through carbon sequestration, erosion control, and other ecosystem services (Nsanganwimana et al., 2014; Rusinowski et al., 2019).

5. Conclusion

The cultivation of sorghum, hemp and miscanthus at a former Italian landfill was successfully carried out over three years, producing satisfactory SDW yields for these crops despite the context characterized by soil contaminants (i.e. Cu, Zn and PCBs) and adverse agronomic constraints. Sorghum was the most productive crop, with a notable increase in SDW yield (+16 %-35 %) thanks to the incorporation into the soil of humic substances paired with mycorrhizal fungi (Hs+M treatment), which resulted in taller plants. This higher shoot biomass resulted in greatest Cu and Zn phytoextraction yields. Hemp also demonstrated potential as an effective species for bioavailable soil Cu stripping, exhibiting shoot Cu uptake similar to that of sorghum (0.11 and 0.10 kg Cu ha^{-1}, respectively). However, the Hs+M treatment adversely affected hemp's growth, its SDW yield, and overall phytoextraction performance. Consequently, the use of biostimulants in this study was more promising for grass crops. The Hs+M treatment did not induce differences in shoot Cu and Zn concentrations. However, these ones exhibited a decreasing trend over the three years and suggested that the biomass obtained can be directly processed, promoting higher bioethanol yields and enabling its integration with other biomass sources, supporting the supply of local biorefineries. Changes in DTPA-extractable soil metals did not confirm a fast, effective phytoextraction process. Biomass production with the three crops did not increase the potential danger of metals being dispersed into the environment. Similarly, soil PCB concentrations did not significantly change. Further years would be needed to monitor tangible changes in soil metal and PCB concentrations. The cultivation of sorghum paired with humic substances and mycorrhizal fungi has emerged as the most promising option out of those considered for phytomanaging the Chiarini contaminated soil. It should be extended and tested in other landfill types. As usual, further agronomic and risk assessment should be carried out to assess long-term feasibility, without disregarding the implications that an annual cereal would impose in terms of tillage, crop rotation and fertilization. In light of these considerations, also miscanthus stands out as a compelling choice, combining minimal agronomic demands with a perennial growth cycle that supports repeated harvesting without necessitating crop rotation.

CRediT authorship contribution statement

Michel Mench: Writing – review & editing, Validation, Methodology, Funding acquisition. **Andrea Monti:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Walter Zegada-Lizarazu:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Data curation, Conceptualization. **Erika Facciolla:** Writing – review & editing, Supervision, Methodology, Investigation. **Pietro Peroni:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Andrea Parenti:** Writing – review & editing, Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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