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RESEARCH ARTICLE

Phytoremediation properties of maize grown on heavy metal-contaminated soil and stimulated with biochar

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Abstract

Plant biomass is an attractive raw material for energy purposes because it is easy to grow, widely available, and relatively inexpensive. One of the crops with great and versatile potential is maize. Therefore, a study was conducted to determine the effects of biochar on maize biomass production, calorific value, and other energetic parameters as well as the chemical composition of the biomass. The study was conducted with soils contaminated with zinc and copper at concentrations of 0, 105, 210, and 420 mg kg^{-1}. Biochar was added to the soil as a remediation agent at levels of 0 and 15 g kg^{-1} soil. The heavy metal contamination of the soil had a relatively little effect on the heat of combustion and the calorific value of the biomass as well as on the C, H, S, N, O, and ash contents of the maize biomass. In contrast, the heavy metals tested both biomass and energy production. Translocation factors (TFs) were determined from the HM content of maize aerial parts and roots. The series without biochar had 15.29% higher TF values for plants from Zn^{2+} sites and 12.00% higher TF values for plants from Cu^{2+} sites compared to the series with biochar. Higher accumulation factor (AF) values for the tested heavy metals were also recorded in maize grown on Zn^{2+} and Cu^{2+}-contaminated soils where no biochar was applied. Nevertheless, the values of the TF and AF coefficients were > 1. The use of biochar had a positive effect on the heat of combustion, the calorific value of the biomass, and its chemical composition. The use of biochar can, thus, be an effective means for the phytostabilization of soils contaminated with heavy metals.

Keywords Phytostabilization plant · Zn^{2+}; Cu^{2+} · Energy parameters · Translocation factor · Accumulation factor

Introduction

Due to human economic activities and rapid urbanization, environmental pollution by heavy metals (HM) has increased significantly in recent times (Taheran et al. 2018; Bilal et al. 2019; Gavrilescu 2022; Wang et al. 2022). Significant damage to the soil environment and to terrestrial and aquatic ecosystems is caused by HMs (Gavrilescu 2022; Wang et al. 2022). These HMs include zinc (Zn) and copper (Cu), which are required in small amounts by living organisms to ensure their proper body functions, but whose excessive accumulation can exert harmful effects on the environment (Varjani et al. 2019; Arthur et al. 2020). The accumulation of HMs in the environment is a consequence of activities such as industrial emissions (Haider et al. 2021), mining of mineral resources (Xiao et al. 2017), electroplating (Liu et al. 2011), paint and varnish processing (Udosen et al. 2016), and electronics manufacturing (Haider et al. 2021; Kaya 2016). Soil contamination with HM has been identified as a major environmental challenge worldwide (Ahmad et al. 2019; El-Naggar et al. 2020; Yang et al. 2025). Technologies must therefore be sought that support soil remediation sustainably, efficiently, and on a large scale (Yuan et al. 2019; Alipour et al. 2021). One such cost-effective remediation method is phytoremediation, including with maize, which is a valuable source of raw materials for the food and chemical industries and makes excellent animal feed (Abd El-Mageed et al. 2020). The future direction of its use could be its cultivation for energy purposes, mainly for the production of biogas and bioethanol (Križan et al. 2017; Skoufogianni et al. 2019; Konieczna et al. 2021). Maize cultivation on soils contaminated with heavy metals affords the possibility

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of its simultaneous remediation, which can be supported by the use of various soil additives and sorbents (Ge et al. 2017), improving the enzymatic and microbiological properties of the soil. Soil enzymes in particular are sensitive markers of heavy metal-induced changes (Liu et al. 2017; Razavi et al. 2016). They reflect the interaction between plants and microorganisms and can be used to monitor the soil microbiome.

To effectively remediate soils contaminated with heavy metals, various techniques must be used to employ the growth and development of energy crops. One such technique is the use of biochar, which improves the soil's physicochemical properties and increases plants' tolerance to contamination. However, it should be noted that crops intended for human consumption or as animal feed should not be grown in contaminated areas (Premalatha et al. 2023; Bolan et al. 2023). Consequently, research into the cultivation of energy crops and their response to biochar is becoming an essential part of phytoremediation strategies in contaminated areas. Introducing such practices can improve soil and environmental quality, facilitating the sustainable use of areas contaminated with heavy metals (Saletnik and Saletnik 2025; Thirumalaivasan et al. 2024).

In the phytoremediation of heavy metal-contaminated soils, indices are used to investigate the plant-soil interaction, transport mechanism, and accumulation pattern of trace elements in plants (Tauqeer et al. 2024). The tolerance index (TI), also known as the growth rate (GR) (Buendía-González et al. 2010), is an important factor in assessing the growth performance of plants in heavy metal-contaminated sites compared to control soils (Zhang et al. 2021a). The translocation index is regularly used to assess the potential for heavy metal transfer from soil to plants (Chamba et al. 2017; Raj et al. 2019). Similarly, the bioaccumulation in aboveground parts (BF_{GP}) and bioaccumulation in roots (B_R) indices show the ability of a plant species to accumulate heavy metals in specific parts of the plant (shoots, leaves, roots) from soil (Peng et al. 2020; Zhang et al. 2021b).

Immobilizing HMs in the soil is one way to remediate contaminated soils and thereby improve plant growth and development, especially when plants are not sufficiently supplied with nutrients (Beesley et al. 2011; Mohan et al. 2014). To this end, biochar can be used due to its high potential to improve soil fertility, reduce the bioavailability of heavy metals, and improve the physicochemical properties of the soil (Rizwan et al. 2016; Shaaban et al. 2018), leading to climate change mitigation (Jiang et al. 2016; Kammann et al. 2017; Abbas et al. 2018; Haddad and Lemanowicz 2021). Studies by Yuan et al. (2019) and Younis et al. (2016) also show that biochar can mitigate the adverse effects of HM. According to El-Naggar et al. (2020) and Virk et al. (2021), the application of biochar on HM-contaminated soils elicits positive effects on plant growth and development as well as accumulation in plants. The above information inspired a study to determine the effects of biochar on the yield, heat of combustion, heating value, energy production, and chemical composition of maize biomass used as a phytostabilizer on Zn^{2+} and Cu^{2+}-contaminated soils. The enzymatic properties of the soils under HM pressure were also investigated. Despite extensive research on biochar, limited studies have assessed its role in enhancing maize's phytoremediation efficiency in metal-contaminated soils. These objectives were pursued based on the following research hypotheses: (1) Soil contamination with zinc and copper affects the size and chemical composition of maize and its heating value; and (2) Biochar improves soil quality, which may affect the yield of maize biomass and energy production.

In light of the complex interactions between heavy metals, soil, and biochar, conducting advanced research on the most effective methods of growing energy crops is crucial for the successful implementation of phytoremediation in contaminated areas. Analyzing their response to the addition of biochar is an important part of these strategies. Implementing such innovative practices has the potential to significantly improve soil quality and the environment as a whole, while enabling the sustainable use of areas degraded by heavy metal contamination. This makes phytoremediation a more effective tool in environmental remediation.

Material and methods

Methodological assumptions of the study

The study was based on a pot experiment conducted in the vegetation hall of the University of Warmia and Mazury in Olsztyn (north-eastern Poland). The soil used for the study was an Eutric Cambisol humic horizon made of sandy loam (IUSS Working Group WRB 2014). It had the following granulometric composition: sand 69.41%, silt 27.71%, and clay 2.88%. Its pH_{KCl} was 5.80; hydrolytic acidity (HAC)—13.50 mmol(+) kg^{-1}; total base exchange cations (EBC)—31.00 mM(+) kg^{-1}; sorption capacity (CEC)—44.50 mM(+) kg^{-1}; degree of saturation with base cations (BS)—69. 65%; C_{org} content—0.78 g kg^{-1}; and N_{Total} content—0.12 g kg^{-1}. This was a three-factor experiment:

1. The type of heavy metals: Zn^{2+} and Cu^{2+} administered in the form of chlorides.
2. Heavy metals dosage: 0, 105, 210, and 420 mg kg^{-1} d.m. soil.
3. The third-order factor was the application of biochar to the soil at 0 and 15 g kg^{-1} soil. The biochar (company NTP Sp. zoo. Poland) had a pH of 9.79. It contained 0.91% N_{Total} and 83.92% C_{org}.

The soil was also enriched with macronutrients adapted to the plant's nutrient requirements (in mg kg^{-1} soil): N—150, P—70; K—120; Mg—15. Heavy metals, biochar, and macronutrients were mixed in a batch of 3.4 kg of soil per 1 pot, and the soil was then placed in a polyethylene pot. The tested crop was maize (*Zea mays* L.), variety LG 32.58. Soil moisture was kept constant throughout the experiment (50% water capacity). The experiment was conducted in four replicates. The maize was harvested at BBCH 51, when the above-ground parts and roots of the plants and soil samples were taken for laboratory analyses. The leaf greenness index (SPAD) of maize was determined in relative units according to the Biologische Bundesanstalt, Bundessortenamt, and chemical scale at the BBCH 33 stage using a Spectrum Technologies, Inc. chlorophyll meter (KONICA MINOLTA, Inc., Chiyoda, Japan).

Methods of laboratory

Prior to the chemical and physicochemical analyses, the soil material was air-dried and sieved through a 2 mm mesh sieve. The granulometric composition of the soil was determined by laser diffraction using a Mastersizer 200 instrument. (Malvern Instruments Ltd., Malvern, Worcestershire, UK). The above-ground parts and roots of the maize were dried at 60 °C and ground. The parameters determined in the above-ground parts of the maize are listed in Table 1. The detailed procedure for determining the heat of combustion is described in Wyszkowska et al (2022b), while the other parameters are described in Wyszkowska et al. (2022a). Table 2 lists the biochemical, chemical, and physicochemical properties determined in the soil samples. The biochemical activity of the soil was determined using fresh soil material. Detailed procedures for determination of enzymes can be found in our previous research (Wyszkowska et al. 2023a, 2023b).

Calculations and statistical analysis

Taking into account the yield of the plants and the heavy metal content of the above-ground parts, roots, and soil were counted: uptake of heavy metals (D) from the soil by maize, indices of tolerance (TI), indices translocation (TF), indices bioaccumulation in the above-ground parts (BF_{GP}), and roots (BF_R), indices accumulation (AF) were calculated. The coefficients were calculated using the following formulas:

$$D = \left(Y_A x\ Hm_{AP}\right) + \left(Y_R x\ Hm_R\right) \tag{1}$$

$$TI = \frac{Y_{Hm}}{Y_C} x\ 100 \tag{2}$$

$$TF = \frac{Hm_{AP}}{Hm_R} \tag{3}$$

$$BF_{GP} = \frac{Hm_{AP}}{Hm_S} \tag{4}$$

$$BF_R = \frac{Hm_R}{Hm_{SO}} \tag{5}$$

$$AF = \frac{Hm_{AP} + Hm_R}{Hm_{SO}} \tag{6}$$

where:

Y_A—yield of above-ground parts (g kg^{-1}).

Y_R—yield roots (g kg^{-1}).

Y_{Hm}—yield of above-ground parts/roots from soil contaminated with heavy metals (g kg^{-1}).

Y_C—yield of above-ground parts/roots from uncontaminated soil (g kg^{-1}).

Hm_{AP}—content of heavy metals in the above-ground part (mg kg^{-1}).

Hm_R—content of heavy metals in the roots (mg kg^{-1}).

Hm_{SO}—content of heavy metals in the soil (mg kg^{-1}).

Results were statistically processed using Statistica software (TIBCO Software Inc Statistica Version 13 2017) with the Shapiro–Wilk test ($p \leq 0.05$) for non-parametric statistics. For homogeneous groups, Tukey's test was used at a significance level of $p \leq 0.05$.

Results

Effects of zinc and copper on maize

Contamination of soil with Zn^{2+} and Cu^{2+} and application of biochar (B) significantly affected maize yield (Figs. 1 and 2), tolerance index (TI) (Fig. 3), and leaf greenness index (Fig. 4) of maize, its energetic properties (Table 3), as well as C, H, S, N, O, and ash contents of plants (Table 4). Zn^{2+} applied at 210 and 420 mg kg^{-1} d.m. soil significantly increased the yield of above-ground parts of the biomass (Figs. 1 and 2). The opposite was true for Cu^{2+}, where increasing soil contamination with this metal caused a successive decrease in maize biomass yield. In the series without biochar, soil contamination with Cu^{2+} at the highest dose tested (420 mg kg^{-1} d.m. soil) reduced the yield of dry above-ground parts of maize biomass by 72.43% compared to uncontaminated soil. Increasing soil contamination with HM had a negative effect on maize root biomass. Both Cu^{2+} and Zn^{2+} applied in the highest dose tested (420 mg kg^{-1} d.m. soil) reduced maize root biomass by 61.82% and 19.74%, respectively, compared

Table 1 Chemical analyses performed on above-ground parts of maize

Plant	Analyses	References
	• heat of combustion (Q) of the above-ground parts was determined in a C-2000 calorimeter from IKA WERKE, USA	PN-EN ISO 18125:2017 (2010)
	• the calorific value (Hv), according to the formula • $Hv = \frac{Q\ (100-Mc)}{100} - Mc \cdot 0.0244$ where: Hv – calorific value of air-dried plant mass (MJ kg-1) Q – heat of combustion of dry plant matter MC – biomass moisture content (%) 0.0244 – correction coefficient for water vaporization enthalpy (MJ kg−1 per 1% moisture content)	PN-EN ISO 18125:2017 (2010)
	• carbon, hydrogen, and sulfur contents were determined using an automatic analyzer ELTRA CHS 500 (Carbon Hydrogen Sulphur Determinator, Neuss, Germany)	PN-G-04517 (1981) and PN-G- 04584 (2001)
	• nitrogen content of the biomass was determined. The determinations were carried out using a digestion kit – K-424 digestion unit and a distillation kit – B-324 nitrogen distiller (BUCHI Labotechnik AG, Flawil, Switzerland)	PN-EN ISO 20483 (2014)
	• background oxygen content of the biomass was determined using the total content of C, H, S, N, and ash	Protásio *et al.* (2011)
	• ash content was determined in an ELTRA THERMOSTEP muffle furnace (thermogravimetric analyzer, Neuss, Germany). Plant samples were wet-mineralized	

to the control series. The application of biochar to the soil had a positive effect on the dry matter yield of the above-ground parts and roots of maize.

Maize was more tolerant to soil contamination with Zn^{2+} than with Cu^{2+} (Fig. 3). In the series without biochar, tolerance indices (TI) for GP from the Zn^{2+}-contaminated soils ranged from 0.991 to 1.101 and for Cu^{2+}—from 0.276 to 0.847. Roots were also more tolerant to Zn^{2+} than to Cu^{2+}. Biochar reduced the sensitivity of the above-ground parts to Cu^{2+}, but not that of the roots.

Leaf greenness index values of maize were dependent on the type of metal contaminating the soil and its fertilization with biochar (Fig. 4). The highest SPAD values were determined for the maize leaves from the soil contaminated with Cu^{2+} and not fertilized with biochar. Biochar application contributed to a significant reduction of SPAD in maize leaves grown on the Cu^{2+}-contaminated soil. Soil contamination with Zn^{2+} had an ambiguous effect on the value of this index.

Table 2 Biochemical, chemical, and physicochemical analyses of soil

Soil	Analyses	References
	Biochemical	
	➢ dehydrogenases (Deh)	Öhlinger (1996)
	➢ catalase (Cat)	Johnson and Temple (1964)
	➢ urease (Ure) ➢ acid phosphatase (Pac) ➢ alkaline phosphatase (Pal) ➢ β-glucosidase (Glu) ➢ aryl sulfatase (Aryl) Soil enzyme activity, excluding catalase activity, was determined using a PerkinElmer Lambda 25 spectrophotometer (Waltham, MA, USA).	Alef, Nannpieri (1988)
	Chemical	
	➢ C_{org} ➢ N_{Total} Contents using a Vario MaxCube CN elemental Microanalyzer (Hanau, Germany)	PN-R-04032; Soil and Mineral Materials—Sampling and Determination of Particle Size Distribution. Polish Committee for Standardization: Warsaw, Poland, 1998
	➢ Total heavy metals by the flame atomic absorption spectrometry (FAAS) using an air-acetylene flame after wet-digestion in a mixture of concentrated hydrochloric acid and nitric acid in the MARS 6 microwave digestion system (CEM Corporation, Matthews, NC, USA) according to US-EPA3051	US Environmental Protection Agency 2007; CEM Corporation 2017)
	Physicochemical	
	➢ soil pH by the potentiometric method in a 1 M dm^{-3} aqueous KCl solution	ISO 10390 (2005)
	➢ hydrolytic acidity (HAC) a ➢ sum of exchangeable base cations (EBC)	Kappen method (Carter 1993)
	➢ based on the hydrolytic acidity (HAC) and total exchangeable bases (EBC), the total cation exchange capacity (CEC) and base cation saturation (BS)	Klute (1996)

The suitability of maize biomass for energy purposes was determined based on its heat of combustion, calorific value, and energy production (Table 3). The first two indices were not affected by Zn^{2+} and Cu^{2+}. The differences between the series were within the statistical error. In contrast, the third indicator (Y_{EP}) was significantly reduced by the effect of

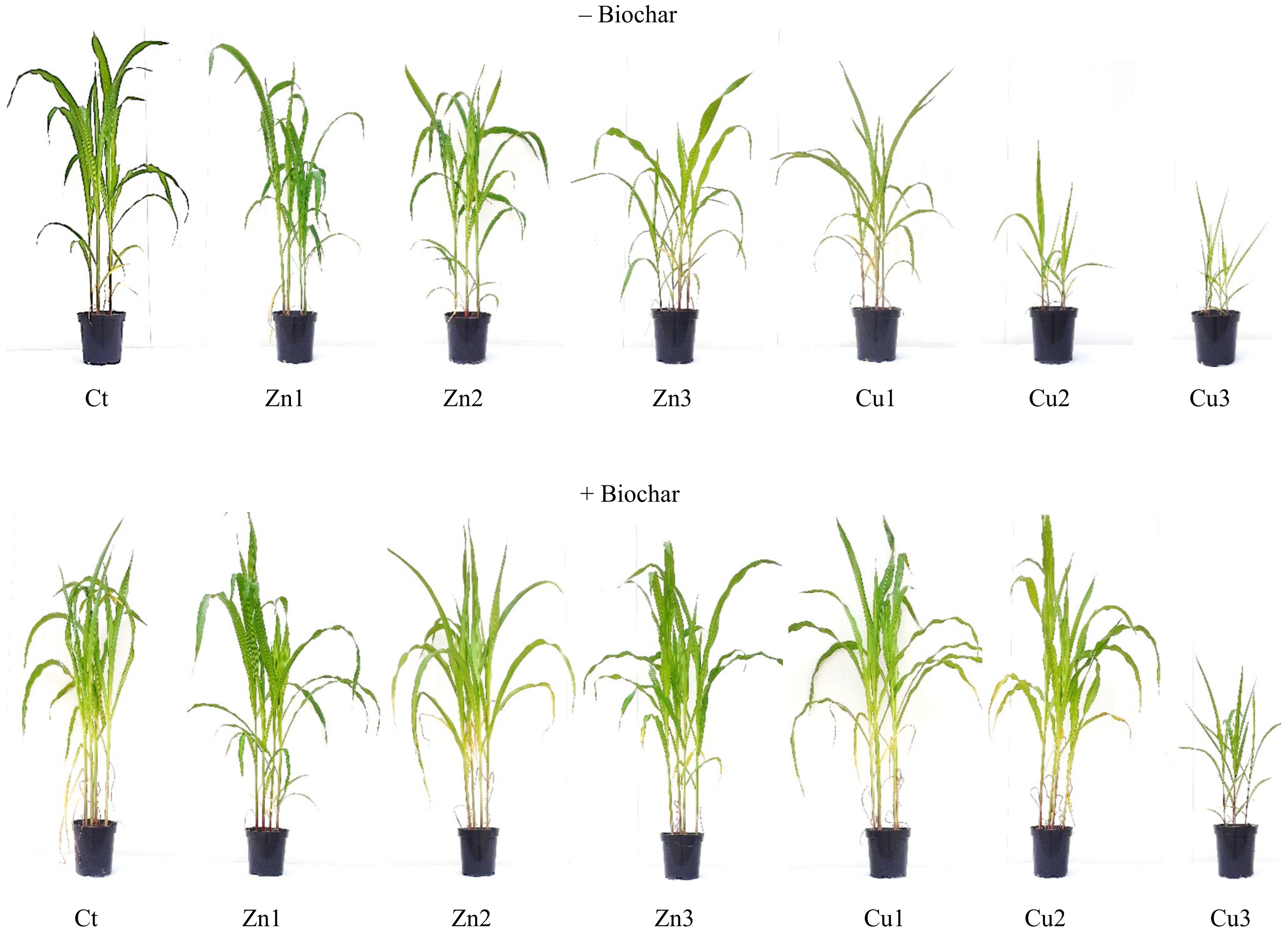

Fig. 1 Maize height on day of harvest. Explanations: Dose of Zn^{2+} or Cu^{2+} in mg kg^{-1} soil: 1—105; 2—210; 3—420; Ct—control; – Biochar—soil without biochar; + Biochar—soil with biochar

Cu^{2+}. This decrease is attributed to the toxic effect of this element applied at a dose of 420 mg.

kg^{-1} soil, on maize growth and development.

Soil contamination with Zn^{2+} and Cu^{2+} and biochar application affected the C, H, S, N, O, and ash contents of maize biomass (Table 4). In the above-ground biomass of maize grown on the soil not fertilized with biochar, Zn^{2+} and Cu^{2+} did not significantly affect the oxygen content. However, both elements reduced the carbon content. Both metals contributed to an increase in nitrogen content. Only Cu^{2+} significantly reduced sulfur accumulation, whereas Zn^{2+} increased ash content. The maize biomass produced in the biochar-supplemented series had increased ash, N, and S contents and a decreased oxygen content under the pressure of Zn^{2+} and Cu^{2+}. In contrast, H content increased only under the influence of Cu^{2+}. C accumulation was negatively influenced by Zn^{2+}, while remained unaffected by Cu^{2+}.

Maize grown on the HM-contaminated soils in the series without and with biochar had increased levels of Zn^{2+} and Cu^{2+}, both in the above-ground parts and roots (Table 5). Greater accumulation of Zn^{2+} in GP and R was found in the series without biochar. Cu^{2+} uptake was higher in GP in the soil without biochar and in R in the series with B. Significantly more heavy metals were found in R than in GP. This was true for both unfertilized and biochar-fertilized soils. Of course, higher levels of both HMs were determined in the experimental series with biochar.

The uptake of Cu^{2+} and Zn^{2+} by maize grown on the biochar-amended soil without HM pressure was higher than by the maize grown on the soil not fertilized with biochar (Table 5). Biochar application significantly enhanced the uptake of Zn^{2+} from the soils contaminated with this metal. In contrast, the uptake of Cu^{2+} from copper-contaminated soils was reduced. The mobility of HM in maize was determined using the translocation factor (TF), calculated from the HM content of its above-ground parts and roots. In the series without biochar, the TF values were 15.29% higher for the plants from the soil treated with Zn^{2+} and 12.00% higher for those from the treatments with Cu^{2+} compared to the series with biochar. The translocation values for Zn^{2+} were

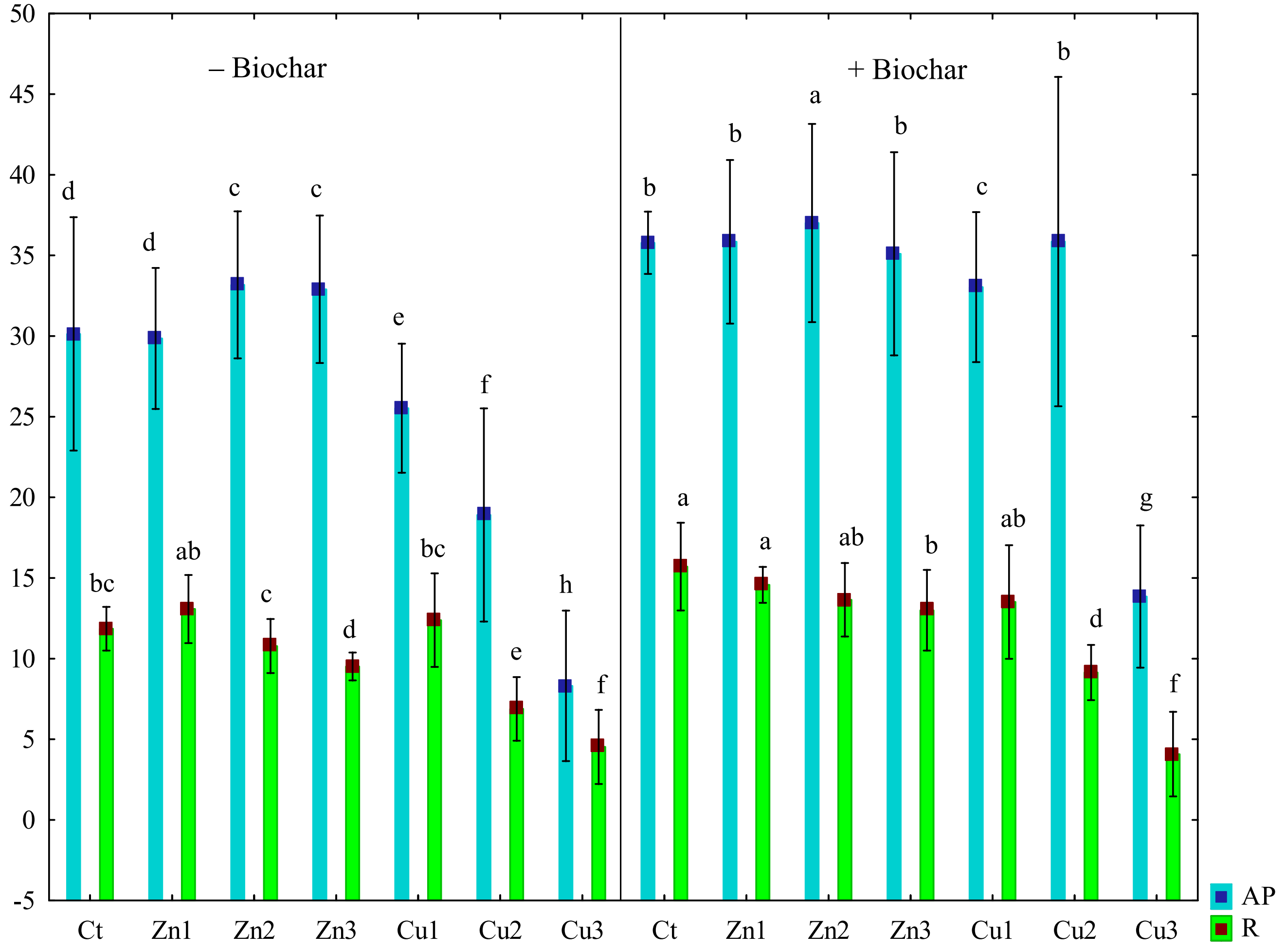

Fig. 2 Effect of zinc and copper on yield of above-ground parts (GP) and roots (R) of maize in g d.m. pot.$^{-1}$. Explanations: Dose of Zn^{2+} or Cu^{2+} in mg kg^{-1} soil: 1—105; 2—210; 3—420; Ct—control; – Biochar—soil without biochar; + Biochar—soil with biochar. The same letters (a–h) indicate homogeneous groups separately for GP and R

significantly lower than for Cu^{2+}, although in both cases they were below 1.0. The highest accumulation factors (AF) of the tested heavy metals were recorded in maize grown on the soil contaminated with Zn^{2+} and Cu^{2+}, where no biochar was applied (Table 5). AF > 1 was also recorded in plants grown on the soil exposed to Zn^{2+} and Cu^{2+} and fertilized with biochar. However, it was significantly lower than in the experimental series without this fertilizer. The accumulation indices of the tested heavy metals in the above-ground parts and roots of maize were also higher in the series without biochar. This was in comparison to the treatments with biochar-amended soil. Correlation coefficients (Table 6) indicate a significant positive correlation of heavy metals (Zn^{2+} and Cu^{2+}) with heat of combustion (Q), calorific value (Hv), hydrogen content (H), ash, Zn^{2+}, and Cu^{2+} content in above-ground parts (GP), roots (R), and soil (S), uptake (D) of heavy metals (HM) by maize, HM translocation rates (TF), accumulation (AF), bioaccumulation in above-ground parts (BF_{GP}), and bioaccumulation in roots (B_R). In contrast, heavy metals (Zn^{2+} and Cu^{2+}) were significantly negatively correlated with energy production (Y_{EP}) of maize. Biochar was only significantly positively correlated with carbon (C), sulphur (S), and ash content and negatively correlated with oxygen (O) content.

The effect of zinc and copper on selected soil properties

The response of enzymes to the effects of the heavy metals tested was inconclusive (Table 7). Copper was a strong inhibitor of dehydrogenase, urease, acid, and alkaline phosphatase activity. Application of biochar did not always improve enzyme activity. A positive effect of this substance in alleviating the inhibitory effect of copper was observed only for dehydrogenase, urease, and acid phosphatase. However, this effect was considerably weaker than in the control

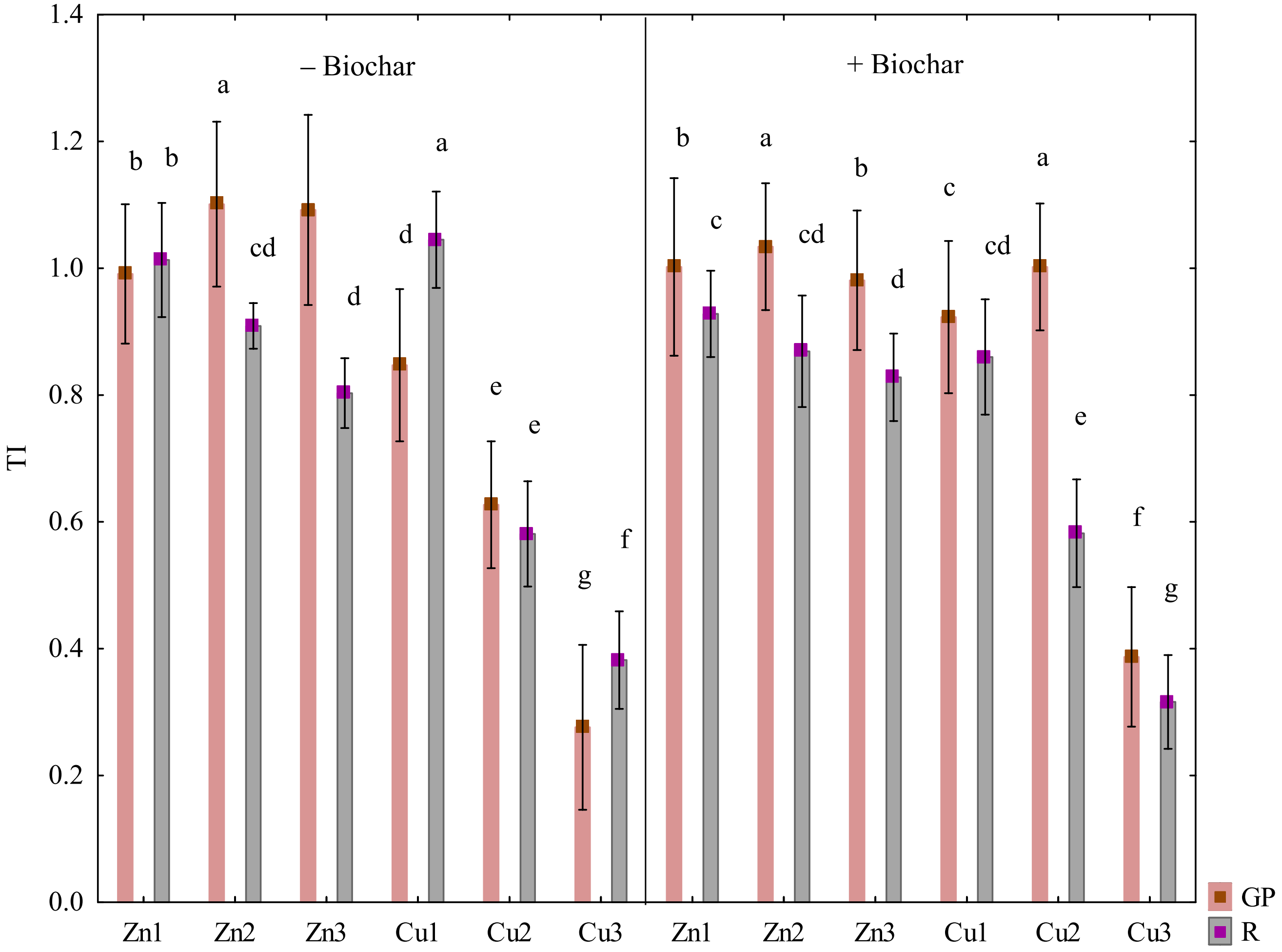

Fig. 3 Tolerance index (TI) of maize to soil zinc and copper content. Explanations: Dose of Zn^{2+} or Cu^{2+} in mg kg^{-1} soil: 1—105; 2—210; 3—420; – Biochar—soil without biochar; + Biochar—soil with biochar. The same letters (a–g) indicate homogeneous groups separately for GP and R

series without fertilizer application. Higher *β*-glucosidase and arylsulfatase activities were found in zinc- and copper-contaminated soils. Comparing the effects of both heavy metals on soil enzymes, it is evident that zinc is a weaker suppressor. It has a less pronounced impact on soil biochemical activity than copper. When present in the soil at 105 mg kg^{-1} soil, it generally stimulated enzyme activity. Its inhibitory effect on some enzymes could only be observed at higher contamination levels.

The effects of the heavy metals tested on the soil physicochemical properties (Table 8) were much weaker than on its biochemical properties. However, there were some regularities. Namely, both metals degraded the soil by reducing its sorption capacity and the degree of saturation with base cations. However, their effects on nitrogen and carbon contents were inconclusive. In the zinc-contaminated soil fertilized with biochar, higher levels of C_{org} were recorded than in the control soil. They were also higher than in the soil not fertilized with biochar. No such a clear effect was found for copper. N_{Total} was highest in the biochar-fertilized control plot. On the other hand, in copper-contaminated soils, the content of this element was highest in the objects with biochar, and at the same time lowest in the objects without this fertilizer. On the other hand, in zinc-contaminated sites without and with biochar, the N_{Total} content remained at a similar level.

Discussion

Environmental conditions and the physical properties of soil significantly affect plant growth and yield. This was confirmed by the results of our study. In moist soils, soil temperature is an important factor that influences germination and the initial growth of plants. If the temperature is too low, several processes are negatively

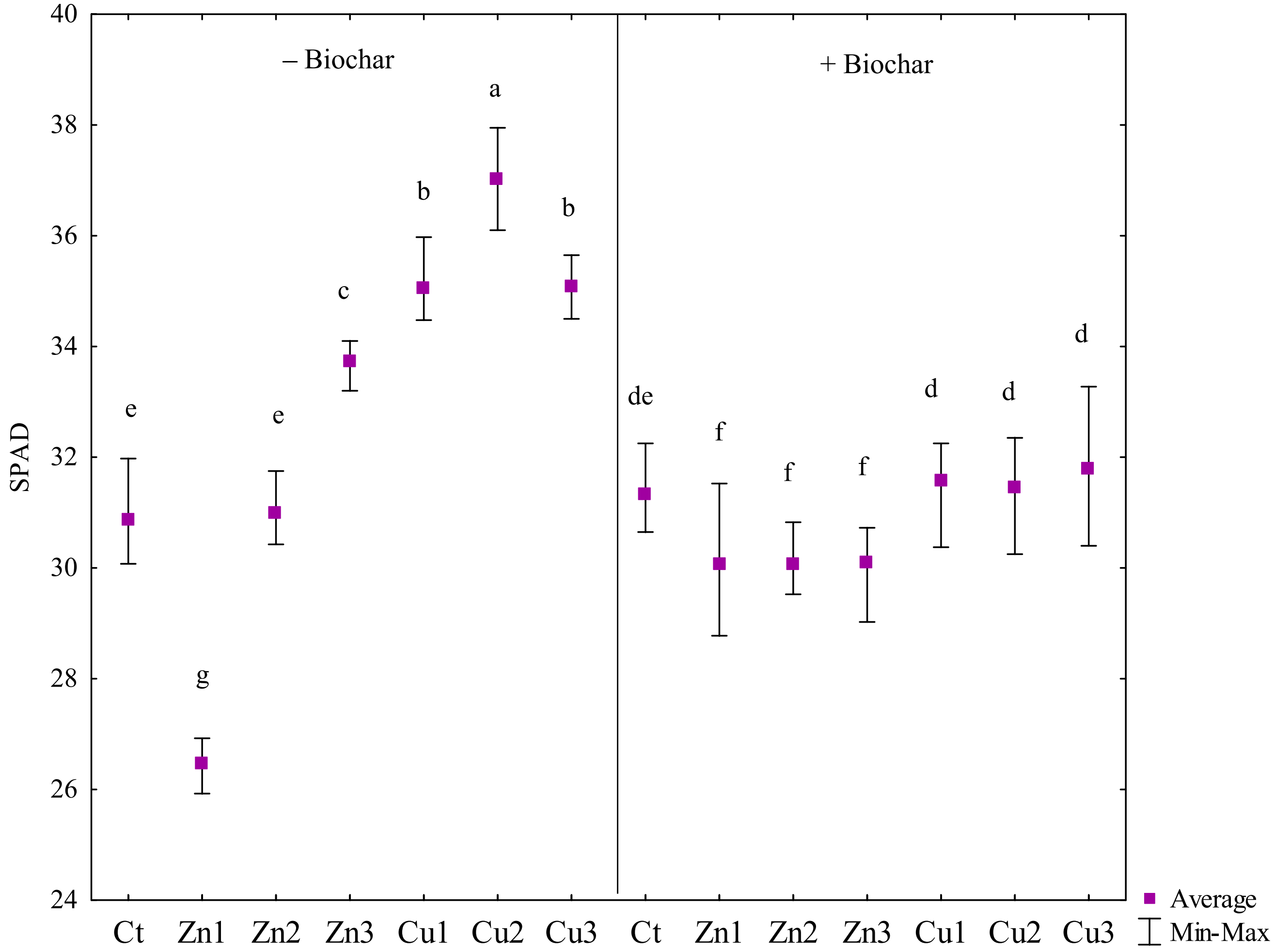

Fig. 4 Effect of zinc and copper on the greenness index (SPAD) of maize leaves. Explanations: Dose of Zn^{2+} or Cu^{2+} in mg kg^{-1} soil: 1—105; 2—210; 3—420; Ct—control; – Biochar—soil without biochar; + Biochar—soil with biochar. The same letters (a–g) denote homogeneous groups

Table 3 Heat of combustion (Q) and calorific value (Hv) in MJ kg^{-1} d.m. of maize and energy production (Y_{EP}) in MJ of maize grown in 1 kg of soil

Object	Soil without biochar	Soil with biochar
Q		
Ct	17.23^{ab}	17.29^{ab}
Zn^{2+}	17.15^{bc}	17.29^{ab}
Cu^{2+}	17.59^{a}	17.50^{a}
Hv		
Ct	15.42^{a}	15.53^{a}
Zn^{2+}	15.35^{a}	15.53^{a}
Cu^{2+}	15.74^{a}	15.72^{a}
Y_{EP}		
Ct	0.13^{b}	0.16^{a}
Zn^{2+}	0.14^{ab}	0.16^{a}
Cu^{2+}	0.04^{c}	0.06^{c}

The same letters (a–c) denote homogeneous groups for each parameter separately

affected. The solubility of substances decreases. The diffusion and absorption of ions are also reduced. Additionally, the activity of soil enzymes decreases, leading to poorer plant growth (Lipiec et al. 2011). As a thermophilic crop, maize responds to lower temperatures with delayed seed germination, reduced growth rate, and plant vigor (Walne 2022). Although soil structure has no direct effect on plant vegetation, it does have a significant influence on several related factors. These include the availability of water and oxygen for the plants, the temperature, and the resistance of the roots to mechanical penetration of the soil substrate (Ahmed et al. 2022; Giuliani et al. 2024; Zheng et al. 2024). In their study, Aladesanmi et al. (2019) observed a slight decrease in the biomass yield of maize grown on sandy soils. By contrast, the same plant grown on clay soils showed a significant increase in plant yield. Additionally, there was a decrease in Cu content in shoot biomass. Maize prefers clay or silty loam soils with a permeable subsoil

Table 4 Effect of zinc and copper on C, H, S, N, O, and ash contents of maize above-ground parts in %

Object	Soil without biochar	Soil with biochar
C		
Ct	48.46^{a}	48.51^{a}
Zn^{2+}	46.53^{d}	48.10^{b}
Cu^{2+}	47.45^{c}	48.25^{a}
H		
Ct	5.12^{c}	5.32^{b}
Zn^{2+}	5.15^{c}	5.38^{b}
Cu^{2+}	5.59^{a}	5.62^{a}
S		
Ct	0.07^{c}	0.08^{b}
Zn^{2+}	0.06^{cd}	0.09^{a}
Cu^{2+}	0.06^{d}	0.08^{ab}
N		
Ct	0.58^{c}	0.62^{b}
Zn^{2+}	0.68^{ab}	0.72^{a}
Cu^{2+}	0.67^{ab}	0.74^{a}
O		
Ct	41.02^{ab}	40.61^{b}
Zn^{2+}	41.94^{a}	39.48^{cd}
Cu^{2+}	41.32^{ab}	38.98^{d}
Ash		
Ct	4.77^{c}	4.81^{c}
Zn^{2+}	5.64^{b}	6.34^{a}
Cu^{2+}	4.92^{c}	6.34^{a}

The same letters (a–d) denote homogeneous groups separately for each element and ash

Ct control

(Wuana and Okieimen 2011). The growing season can have a significant effect on the biomass yield of maize as well. The light soil used in our experiment is characterized by the granulometric composition of sandy loam, which is permeable and well aerated, warms up quickly, and is easy to cultivate. It is also more easily penetrated by the roots than other soils. This is due to the good soil-seed contact and the lower mechanical resistance of the soil in the rhizosphere of the plants. Unfortunately, it is also characterized by lower water retention (Abdelfattah et al. 2021; Atkinson et al. 2009). This means that maintaining optimum soil moisture was particularly important during the period of intensive maize growth. It is important to supply nutrients to the plant in the form of a fertilizer, usually the mineral one. Biochar can be used as a type of organic fertilizer. It adds organic matter, N, P, K, Ca, Mg, and other nutrients to the soil. Additionally, biochar increases microbial diversity and the activity of soil enzymes. On the other hand, water retention in the rhizosphere (Ni et al. 2020) can be improved by the presence of biochar, which additionally promotes

Table 5 Effect of soil contamination by zinc and copper on the content of these elements in maize biomass and soil and indicators determining the suitability of maize for remediation

Object	Above-ground parts	Roots	Soil	D (µg kg^{-1})	TF	AF	BF_{GP}	BF_{R}
	Cu and Zn content, mg kg^{-1} d.m							
Soil without biochar								
Ct	Zn—0.07^{g} Cu—0.09^{g}	Zn—6.82^{g} Cu—6.00^{g}	Zn—8.60^{e} Cu—8.75^{e}	Zn—23.70^{h} Cu—26.08^{g}	Zn—0.01^{g} Cu—0.02^{d}	Zn—0.80^{g} Cu—0.70^{h}	Zn—0.01^{f} Cu—0.01^{f}	Zn—0.79^{f} Cu—0.69^{g}
Zn^{2+}	44.80^{a}	285.41^{a}	105.00^{d}	1196.86^{b}	0.16^{b}	3.15^{a}	0.43^{a}	2.72^{a}
Cu^{2+}	43.80^{b}	251.00^{d}	109.00^{c}	925.50^{c}	0.18^{a}	2.71^{b}	0.40^{b}	2.30^{b}
Soil with biochar								
Ct	Zn—0.57^{f} Cu—0.90^{e}	Zn—6.41^{f} Cu—8.81^{e}	Zn—7.86^{e} Cu—8.50^{e}	Zn—34.59^{f} Cu—48.73^{e}	Zn—0.09^{f} Cu—0.10^{e}	Zn—0.89^{f} Cu—1.14^{e}	Zn—0.073^{e} Cu—0.11^{d}	Zn—0.82^{f} Cu—1.04^{e}
Zn^{2+}	37.20^{d}	278.71^{b}	172.00^{b}	1408.11^{a}	0.13^{c}	1.84^{c}	0.22^{c}	1.62^{c}
Cu^{2+}	40.90^{c}	265.00^{c}	186.00^{a}	538.18^{d}	0.15^{b}	1.65^{d}	0.22^{c}	1.43^{d}

The same letters (a–h) denote homogeneous groups separately for each element, ash, and indicator

Ct control, *D* uptake of heavy metals by maize, *TF* translocation, *AF* accumulation, BF_{GP} bioaccumulation in above-ground parts, B_{R} bioaccumulation in roots

Table 6 Correlation coefficients between variables in soil contaminated with Zn^{2+} and Cu^{2+} and treated with biochar

Variable factors	Q	Hv	Y_{EP}	C	H	S	N	O
HM	0.762*	0.727*	−0.824*	−0.363	0.722*	−0.022	0.319	−0.258
B	0.119	0.319	0.219	0.564	0.347	0.894*	0.186	−0.827*
Q	1.000	0.979*	−0.906*	0.151	0.924*	−0.068	0.154	−0.339
Hv		1.000	−0.820*	0.260	0.953*	0.119	0.186	−0.494*
Y_{EP}			1.000	0.106	−0.788*	0.349	−0.132	0.105
C				1.000	0.169	0.482*	−0.108	−0.635*
H					1.000	0.081	0.299	−0.450
S						1.000	0.268	−0.856*
N							1.000	−0.346
O								1.000
Ash								
Cu and Zn content GP								
Cu and Zn content R								
Cu and Zn content S								
D								
TF								
AF								
BF_{GP}								

Variable factors	Ash	Cu and Zn content GP	Cu and Zn content R	Cu and Zn content S	D	TF	AF	BF_{GP}	BF_R
HM	0.506*	0.873*	0.823*	0.811*	0.534	0.826	0.619	0.707	0.599
B	0.529*	−0.083	0.011	0.341	−0.050	0.116	−0.411	−0.344	−0.424
Q	0.066	0.381	0.303	0.410	0.021	0.490*	0.128	0.261	0.099
Hv	0.172	0.347	0.291	0.462	0.010	0.492*	0.038	0.178	0.007
Y_{EP}	−0.023	−0.488*	−0.381	−0.390	−0.042	−0.477*	−0.305	−0.410	−0.282
C	−0.107	−0.666*	−0.616*	−0.245	−0.643*	−0.583*	−0.919*	−0.869*	−0.927*
H	0.164	0.366	0.297	0.431	−0.025	0.579*	0.111	0.255	0.080
S	0.720*	0.018	0.157	0.483*	0.182	0.029	−0.359	−0.337	−0.363
N	0.354	0.344	0.363	0.399	0.288	0.336	0.205	0.231	0.199
O	−0.661*	−0.074	−0.164	−0.557*	0.004	−0.074	0.416	0.342	0.431
Ash	1.000	0.637*	0.737*	0.903*	0.616*	0.488*	0.254	0.269	0.250
Cu and Zn content GP		1.000	0.987*	0.856*	0.845*	0.891*	0.858*	0.887*	0.849*
Cu and Zn content R			1.000	0.904*	0.890*	0.855*	0.807*	0.827*	0.800*
Cu and Zn content S				1.000	0.728*	0.724*	0.482*	0.530*	0.470*
D					1.000	0.704*	0.788*	0.763*	0.791*
TF						1.000	0.812*	0.877*	0.795*
AF							1.000	0.989	0.999
BF_{GP}								1.000	0.984

HM heavy metals, *B* biochar, *Q* heat of combustion, *Hv* calorific value, Y_{EP} energy production, *D* uptake of heavy metals by maize, *TF* translocation, *AF* accumulation, BF_{GP} bioaccumulation in above-ground parts, B_R bioaccumulation in roots

*r—coefficient of correlation significant at: $p=0.05$, $n=18$

plant growth and nutrient uptake. Finally, it increases plant biomass and dilutes the HM content in plant tissues to reduce phytotoxicity. Meier et al. (2017) indicated that the addition of 5% biochar derived from chicken manure can reduce Cu uptake from 66.9 to 36.6 mg kg^{-1} in the aboveground parts of *Oenothera picensis*. The present study results also demonstrated a lower HM content in the above-ground parts and roots of maize. In Zn^{2+}-contaminated

Table 7 Enzyme activity in 1 kg d.m. soils contaminated with zinc and copper

Object	Dose mg kg^{-1} d.m. soil	Deh		Cat		Ure	
		Bw	B	Bw	B	Bw	B
Ct	0	3.013^{e}	3.644^{c}	0.375^{d}	0.400^{cd}	0.336^{fg}	0.987^{a}
	105	3.906^{a}	3.731^{b}	0.424^{b}	0.440^{ab}	0.510^{e}	0.929^{b}
Zn^{2+}	210	3.353^{d}	3.018^{e}	0.417^{bc}	0.403^{c}	0.397^{f}	0.732^{d}
	420	1.641^{i}	2.019^{h}	0.367^{e}	0.403^{c}	0.224^{h}	0.705^{d}
	105	1.027^{l}	1.129^{k}	0.406^{c}	0.424^{b}	0.225^{h}	0.765^{d}
Cu^{2+}	210	0.337^{n}	0.534^{m}	0.383^{d}	0.404^{c}	0.281^{g}	0.399^{f}
	420	0.150^{g}	0.372^{n}	0.371^{d}	0.391^{cd}	0.226^{h}	0.143^{i}

Object	Pac		Pal		Glu		Aryl	
	Bw	B	Bw	B	Bw	B	Bw	B
Ct	1.490^{de}	1.850^{b}	0.853^{f}	1.030^{d}	0.412^{i}	0.688^{f}	0.400^{j}	0.645^{h}
	2.067^{a}	2.098^{a}	1.276^{ab}	1.295^{a}	0.674^{fg}	0.980^{e}	0.864^{g}	1.421^{c}
Zn^{2+}	2.030^{ab}	1.617^{d}	1.205^{b}	1.166^{c}	0.696^{f}	1.079^{d}	1.243^{d}	1.637^{a}
	1.259^{g}	1.535^{d}	0.942^{e}	1.122^{d}	0.423^{i}	0.426^{i}	0.907^{g}	1.666^{a}
	1.687^{c}	1.524^{d}	0.813^{f}	0.766^{g}	0.642^{g}	1.175^{b}	1.019^{f}	1.079^{ef}
Cu^{2+}	1.143^{h}	1.354^{f}	0.753^{g}	0.692^{h}	0.645^{g}	1.472^{a}	1.123^{e}	0.516^{i}
	0.937^{i}	1.421^{e}	0.744^{g}	0.744^{g}	0.423^{i}	0.471^{i}	1.132^{e}	0.901^{g}

The same letters (a–h) indicate homogeneous groups separately for each enzyme

Ct control, *Deh* dehydrogenases in μmol TFF kg^{-1} d.m. h^{-1}, *Cat* catalase in mol O_2 kg^{-1} d.m. h^{-1}, *Ure* urease in mmol N-NH_4 kg^{-1} d.m. h^{-1}, *Pac* acid phosphatase in mmol PNP kg^{-1} d.m. h^{-1}, *Pal* alkaline phosphatase in mmol PNP kg^{-1} d.m. h^{-1}, *Glu* β-glucosidase in mmol PNP kg^{-1} d.m. h^{-1}, *Aryl* arylsulfatase in mmol PNP kg^{-1} d.m. h^{-1}, *Bw* soil without biochar, *B* soil with biochar

samples without biochar addition, the content of this element in the above-ground parts of corn was 16.96% higher, and in the roots 6.62% higher, than in samples with the addition of biochar. However, in soil contaminated with Cu^{2+}, only the above-ground parts showed a 2.35% higher content of this metal in the absence of biochar. Soil contamination with Zn^{2+}, Cu^{2+}, and its amendment with biochar (B), applied to reduce the adverse effects of HM, had a positive effect. This amendment improved the heat of combustion, calorific value, and energy production of maize biomass. In uncontaminated samples, energy production from corn biomass increased from 0.13 MJ kg^{-1} in samples without biochar to 0.16 MJ kg^{-1} in samples where biochar was used. The results obtained are comparable to the findings reported by other authors (Xiong et al. 2010; Lizotte et al. 2015; Wojcieszak et al. 2022). In the experiment of Xiong et al. (2010), the average calorific value of the above-ground parts of maize was 18.92 MJ kg^{-1}. Similar results were obtained by Lizotte et al. (2015). They recorded the highest calorific value of 17.72 MJ kg^{-1} for maize stalks. In contrast, the highest calorific value (18.2 MJ kg^{-1}) was reported for leaves by Wojcieszak et al. (2022). Another advantage of maize is its ability to bioaccumulate HMs from contaminated soils. These metals are then transferred from the roots to the stalks. Due to its ability to trap HMs and its sensitivity to high levels of metal contamination, maize is considered an accumulator and element-tolerant plant, especially for zinc and cadmium (Aladesanmi et al. 2019). The transfer and accumulation of HM from soil to plants is a complex process. It depends on many factors, e.g., soil pH, soil organic matter content, plant species, and climatic conditions (Aladesanmi et al. 2019; Bali et al. 2010). A study by Zojaji et al. (2014) showed that the accumulation of HM (Pb, Cd, Cr, Cu, and Zn) was mainly higher in the roots of cucurbits than in other parts of the soil. In our study, the counted TF, AF, BFGP, and BFR indices provide information on the uptake, translocation, and distribution of heavy metals in maize growing on Zn^{2+} and Cu^{2+} contaminated soil. The TF and BF_{GP} indices, whose values were less than 1, indicate a higher accumulation of Zn^{2+} and Cu^{2+} in maize roots than in the above-ground parts of maize. This finding suggests that maize could be used in the phytostabilization process. This confirms our previous studies which also showed that maize is a useful plant for phytostabilization of soils contaminated with heavy metals (Ni^{2+}, Co^{2+}, Cd^{2+}) (Boros-Lajszner et al. 2021). The translocation factor (TF), defined as the ratio of the amount of metal in the aerial parts to its content in the roots, is a key indicator of phytoremediation efficiency. A higher TF indicates a more efficient transfer of metals from the roots to the aerial parts, allowing suitable plant species to be selected for phytoremediation (Aziz et al. 2023; Tauqeer et al. 2024).

Table 8 Physicochemical properties of the soils contaminated with zinc and copper

Object	Dose mg kg^{-1} d.m. soil	C_{org}		N_{Total}		pH_{KCl}	
		g kg^{-1} d.m. soil					
		Bw	B	Bw	B	Bw	B
Ct	0	7.56^{d}	11.77^{bc}	1.20^{b}	1.36^{a}	5.70^{bc}	5.90^{a}
Zn^{2+}	105	7.75^{d}	12.83^{a}	1.15^{b}	1.23^{b}	5.70^{bc}	5.80^{ab}
	210	8.07^{d}	12.45^{a}	1.31^{a}	1.17^{b}	5.70^{bc}	5.95^{a}
	420	7.94^{d}	13.05^{a}	1.31^{a}	1.31^{a}	5.73^{b}	6.00^{a}
Cu^{2+}	105	7.91^{d}	11.61^{c}	1.20^{b}	1.17^{b}	5.75^{b}	5.80^{ab}
	210	7.54^{d}	12.85^{a}	1.09^{c}	1.31^{a}	5.70^{bc}	5.90^{a}
	420	7.32^{d}	12.19^{b}	1.29^{a}	1.40^{a}	5.90^{a}	6.10^{a}

Object	HAC		EBC		CEC		BS %	
	mmol(+)kg^{-1} d.m. soil							
	Bw	B	Bw	B	Bw	B	Bw	B
Ct	15.38^{b}	13.13^{de}	34.00^{c}	38.00a	50.38^{a}	51.13^{a}	69.47^{d}	74.33^{b}
Zn^{2+}	15.94^{a}	13.69^{d}	29.00^{fg}	33.00^{de}	44.94^{e}	46.69^{c}	64.42^{f}	70.61^{d}
	15.56^{ab}	13.69^{d}	31.60^{e}	36.00^{b}	47.16^{b}	49.69^{ab}	67.00^{e}	72.42^{c}
	15.19^{bc}	12.56^{e}	31.00^{ef}	30.00^{f}	46.19^{bc}	42.56^{e}	67.11^{e}	70.49^{d}
Cu^{2+}	15.00^{c}	14.44^{cd}	30.00^{f}	32.00^{e}	45.00^{c}	46.44^{bc}	66.62^{e}	68.87^{de}
	15.00^{c}	14.44^{cd}	30.93^{ef}	33.00^{de}	45.93^{c}	47.44^{b}	67.34^{e}	69.48^{d}
	8.63^{f}	12.56^{e}	30.94^{ef}	33.00^{de}	45.56^{c}	45.56^{c}	81.07^{a}	72.48^{c}

The same letters (a–h) indicate homogeneous groups separately for each parameter

The same letters (a–h) indicate homogeneous groups separately for each parameter

Ct control, C_{org} total organic carbon. N_{total} total nitrogen. *HAC* hydrolytic acidity. *EBC* total exchangeable cations. *BS* base cations saturation ratio in soil. *Bw* soil without biochar. *B* soil with biochar

The use of biochar in soils contaminated with heavy metals can lead to different translocation effects depending on the specific properties of the soil, the metals, and the biochar used (Majewska and Hanaka 2025). Biochar increases the uptake and translocation of metals. However, due to its high sorption capacity, it can also limit their availability, leading to a decrease in the translocation factor (Ibrahim et al. 2022). In this study, the TF of maize in Zn^{2+}- and Cu^{2+}-contaminated plots containing biochar was found to be 18.75% and 16.67% lower, respectively, than in plots without biochar.

The present study also indicates an opportunity for farmers to generate additional income by growing energy crops on HM-contaminated or partially degraded soils. Growing annual crops such as maize also allows for a quick change in the production. This flexibility is important in the event of unprofitability or the emergence of new energy trends. Maize is a crop with high potential for thermal energy production. This potential is due to its yield, fuel properties, and ecological aspects. Research in this area is therefore necessary and remains relevant.

Enzyme activity is one of the most important indicators for monitoring the impact of soil management, agricultural practices, or contaminants on soil health (Oleszczuk et al. 2014). It also indirectly reflects the self-cleaning capacity of a soil contaminant (Cui et al. 2013). In our study, HMs suppressed activities of the soil enzymes tested, and the application of biochar mitigated this negative effect, with the exception of arylsulfatase. The results obtained indicate that, of the enzymes studied, dehydrogenases were the most sensitive to Zn^{2+} and Cu^{2+} soil contamination. This was particularly evident in sites with the highest contamination (420 mg kg^{-1}). Through the sorption of organic and inorganic molecules, biochar can block reactive sites and inhibit the activity of some enzymes (Haddad and Lemanowicz 2021). A study by Niemi et al. (2015) showed that biochar had a weak effect on the activity of enzymes such as arylsulfatase, β-glucosidase, and cellobiosidase. Enzyme activity is highly dependent on organic matter, pH, and organic and mineral nutrient content. These factors are related to soil type (Wyszkowska et al. 2023b). Wang et al. (2025) showed that a reduction in HM availability promoted the growth of soil microorganisms, thereby resulting in their enhanced activity. The addition of biochar can alter the level of HM stress in the soil, which is another factor affecting enzyme activity.

In our own studies of uncontaminated sites, the addition of biochar increased the content of C_{org}, N_{Total}, pH, and the sum of exchangeable base cations, as well as the saturation of the soil with base cations, while reducing hydrolytic acidity. This effect of biochar can be attributed to its physical and chemical properties, as reported by other researchers (Singh et al. 2022; Zhu et al. 2025). In soil contaminated with the highest dose of Zn^{2+} and Cu^{2+} (420 mg kg^{-1} d.m. soil), biochar had a similar effect to that observed in uncontaminated soil on the organic carbon content, pH, and total exchangeable base cations. The different effects of these two heavy metals on other physical and chemical properties can be explained by their distinct chemical properties (Jara-Chávez et al. 2024). Both heavy metals tested reduced the activity of most soil enzymes. Copper had a greater impact, demonstrating that excess copper in the soil is more toxic to soil microorganisms than excess zinc and that microorganisms are the primary producers of soil enzymes (Rashid et al. 2023; Campillo-Cora et al. 2025).

By adding biochar, HM cations can be immobilized in the contaminated soil. This process occurs through adsorption and complexation. Consequently, the bioavailability of heavy metals in the contaminated soil is reduced. Biochar proves to be an effective component for improving soil properties (Wang et al. 2025). Ippolito et al. (2017) observed that the addition of biochar contributed to reduced bioavailability of Cu, Zn, and Cd in soil. However, the responses of different enzymes to heavy metals have been shown to vary (Haddad and Lemanowicz 2021).

The above studies were carried out under controlled conditions. This included maintaining optimum light, humidity, and temperature. The studies aimed to investigate plant behavior, specifically in maize. The researchers assessed the plant's suitability and survival following soil contamination with zinc and copper, as well as its response to biochar. Thus, the research carried out provides a basis for carrying out field studies. These studies will verify the effectiveness of maize tested in the laboratory on naturally heavy metal-contaminated soils. Such environments are characterized by high competition and variability in biotic and abiotic conditions (temperature, humidity, water availability, etc.).

Conclusion

In the series without biochar, soil contamination with Zn^{2+} and Cu^{2+} affected the heat of combustion and the calorific value of biomass, while very strongly reducing the dry matter yield and the energy production from maize biomass. The lowest tested dose of Zn^{2+} and Cu^{2+} (105 mg kg^{-1} d.m. soil) had a positive effect on plant biomass. Soil contamination with Zn^{2+} and Cu^{2+} decreased C content and increased H, S, N, O, and ash contents in the above-ground parts of maize. Copper had a more negative effect on soil enzyme activity than zinc. Soil physicochemical properties were slightly modified by HM. The application of biochar to the soil had a positive effect on maize biomass height, heat of combustion, biomass calorific value, and C, H, S, N, O, and ash contents. The same was true for the enzymatic and physicochemical properties of the soil. The use of biochar appears to be a successful complement to the phytostabilization of HM-contaminated soils by growing energy crops.

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Data availability The datasets analyzed in this study are available on request.

Declarations

Ethical approval This is not applicable.

Consent to participate This is not applicable.

Consent for publication This is not applicable.

Competing interests The authors declare no competing interests.

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