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ORIGINAL PAPER

Effect of Dry Olive Residue–Based Biochar and Arbuscular Mycorrhizal Fungi Inoculation on the Nutrient Status and Trace Element Contents in Wheat Grown in the As-, Cd-, Pb-, and Zn-Contaminated Soils

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Abstract
The Mediterranean countries are the largest producers of olives with Spain taking the lead in olive oil production. A two-phase extraction system is used to produce oil and dry olive residue (DOR), a waste product. DOR biochar was tested as an amendment for contaminated soils to reduce the trace element (TE) contents in crops. A DOR sample was transformed into biochar at 350 °C and 500 °C, and a pot experiment was conducted, where spring wheat was grown. Moreover, the mutual effect of biochar application and arbuscular mycorrhizal fungi (AMF) inoculation was assessed. The results showed the decreasing extractable proportions of Cd in the treated soils, whereas an ambiguous effect of DOR biochar on the mobility of As, Pb, and Zn in soil was observed. The changes in TE in the treated soils were related to enhanced soil pH due to the biochar application. Stepwise increases in extractable soil potassium (K) proportions were determined because of the high content of K in DOR. The element contents in wheat plants were affected by an interaction of the soil element contents and pH, and biochar pyrolysis temperature. The AMF inoculation did not affect the biochar-induced changes in element fate in the soils. The results proved the ability of DOR-based biochar to serve as the source of nutrients, especially K. However, further research is necessary to test a wider range the application rates of biochar, as well as the long-term fate of biochar in the treated soils.

Keywords Dry olive residue · Trace elements · Mobility · Nutrient availability · Contaminated soil

1 Introduction

Soil is the basis of life; it is used to produce our food, filter our water and air, and it is the platform for building our cities and houses. Soils are susceptible to chemical and physical disturbances, induced predominantly by anthropogenic activities. Vegetation, such as agricultural crops, grown on contaminated soils with high concentrations of bioavailable trace elements (TE) is prone to the uptake of these pollutants. This can have severe consequences on the plant's health and ability to grow and on the health of humans who consume products with high levels of TE (McLaughlin et al. 2000).

In the Mediterranean regions, mining and smelting had been going on for hundreds of years, leading to a release of large quantities of TE into the environment (Costagliola et al. 2008; Rodríguez et al. 2009). The severity of soil contamination has brought forward a variety of remediation technologies that help to prevent further contamination and to mitigate deleterious effects of TE. In situ chemical immobilisation decreases the concentration of dissolved contaminants by sorption or precipitation (Basta and McGowen 2004). For this purpose, biochar from carbonaceous waste materials has gained growing interest in the past few years (Ahmad et al. 2014). The mechanism of TE immobilisation on the surface of

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biochars may include the following: (i) ion exchange, (ii) electrostatic attraction, (iii) surface complexation, (iv) physical adsorption, or (v) surface precipitation and co-precipitation, especially with phosphate and carbonate species (Tan et al. 2015). Moreover, there are indirect immobilising effects; biochar usually shifts the soil pH upward when applied to the soil, thus contributing to the stabilisation of cationic TE in soils (Houben et al. 2013). Interactions between the biochar and contaminants depend on the pyrolysis temperature and nature of the parent material (Ding et al. 2014; Jindo et al. 2014; Gusiatin et al. 2016).

Hmid et al. (2015) used BC450 from olive mill waste to remediate acidic multi-contaminated soil situated near a zinc smelter. The application of biochar at 5% (w/w) decreased the content of Cd, Pb, and Zn in the leaves of bean (*Phaseolus vulgaris*) by 92, 30, and 53% as compared with the untreated soil. Ibrahim et al. (2017) amended acidic multi-contaminated soil with BC550 derived from peanut shell at a rate of 4% (w/w). The contents of Cd, Pb, Zn, and As in the fruits of *P. vulgaris* were significantly reduced by 79, 53, 23, and 41%, respectively. Sewage sludge–derived BC550 in the same experiment showed a lower ameliorative efficiency for all TE, and it even increased the Zn content in the fruits by 21%. Rees et al. (2015) cultivated ryegrass (*Lolium perenne*) in a pot experiment in heavily contaminated soils collected near Pb and Zn smelters. The addition of 5% (w/w) BC450 derived from wood chips significantly decreased Cd, Pb, and Zn contents in the shoots (up to 58, 84, and 57%, respectively). Moreover, the effective elimination of the risk element toxicity symptoms in the biochar-treated plants was reported (Yildiztugay et al. 2019).

Besides soil TE remediation, biochar is also known as a source of calcium, magnesium, potassium, and phosphorus, which are essential for plant growth (Xu et al. 2013; Amini et al. 2016). Application of biochar to agricultural soils has been reported to increase plant growth (Brantley et al. 2016; Ibrahim et al. 2017) and soil water holding capacity (Laird et al. 2010a), to decrease the occurrence of crop diseases (Elad et al. 2010), and to reduce greenhouse gas emission (Ibrahim et al. 2017) and nutrient leaching (Laird et al. 2010b).

The economies of Mediterranean areas thrive on olive (*Olea europaea* L.) cultivation. The world production of olive oil reached 3.3 million tons in 2017/2018 (International Olive Council 2018). The commonly applied two-phase extraction system generates "dry olive residue" (DOR) as a final by-product produced in large quantities (Siles et al. 2014). DOR on its own is harmful to plants and microorganisms because of its high number of phenolic compounds (Sampedro et al. 2009). However, the fungal transformation or composting of DOR, as well as the formation of DOR-based biochar, can reduce the unfavourable properties of this material. The biotransformed DOR has been found to improve the chemical characteristics of soil and bacterial and fungal communities (Sampedro et al. 2009). Hovorka et al. (2016) conducted an experiment to compare the effects of various DORs on their sorption abilities of cadmium, lead, and zinc in soil that were transformed by the following four species of fungi: *Penicillium chrysogenum*, *Coriolopsis floccos*, *Bjerkhandera adusta*, and *Chondrostereum purpureum*. The results of the experiment proved that DOR had good potential for the sorption of lead and less potential for the sorption of cadmium and zinc. Biotransformed DOR was shown to have better sorption characteristics. On the other hand, the desorption experiment showed certain instabilities of the elements bound to the DOR. The transformation of the DOR biomass to biochar could lead to better stability of the TE bound in the soil.

Příbram is a Czech town located approximately 60 km southwest of the country's capital, Prague. It has a vast history of mining and smelting of Pb and Ag. The emissions from primary and secondary lead smelters have led to high concentrations of TE (especially Pb, Cd, and Zn, and to a lesser extent, As) in soils. Vaněk et al. (2005) observed extremely high concentrations of Pb, Zn, and Cd in soils, reaching up to 4500, 8700, and 68 mg kg^{-1}, respectively. High spatial variability of the TE concentrations, especially Pb, Cd, and Zn, in the soils was reported (Šichorová et al. 2004). For this experiment, three soils differing in their TE levels from this location were sampled to assess the ability of DOR-based biochar on the mobility and plant availability of these TEs.

We hypothesise that the DOR-based biochar will reduce availability of TE in the abovementioned soils, and, simultaneously, improve the nutrient status of soils. Additionally, arbuscular mycorrhizal fungi (AMF) inoculation can improve the ability of wheat plants to grow in extremely contaminated soils treated by biotransformed DOR (García-Sánchez et al. 2017). The potential role of AMF inoculation in combination with DOR biochar treatment of soil in their remediation effectivity in TE-contaminated soils has not been investigated yet. Therefore, the possible mutual effect of AMF and biochar application was taken into account as well. The three main objectives of this study are defined as follows: (i) to assess the effect of the DOR biochar on the potential immobilisation of TE in smelter-contaminated soil under wheat cultivation using a single chemical extraction; (ii) to verify the ability of DOR biochar to reduce the TE content in wheat plants from the contaminated soil; and (iii) to demonstrate the DOR biochar as a source of available nutrients for plants growing in the contaminated soil.

2 Materials and Methods

2.1 Soils

Three soils with different TE contents were chosen, sampled, and labelled "low", "medium", and "high" according to their As, Cd, Pb, and Zn levels. As mentioned above, the

contamination of the soils originated from both long-term mining of Pb and Ag ores, and emissions caused by smelting of these ores, and, more recently, by the recycling of the Pb-bearing materials. The locations chosen were the following: soil "low" (49° 43′ 15.730″ N; 13° 58′ 33.126″ E), soil "medium" (49° 42′ 43.450″ N; 13° 59′ 7.615″ E), and soil "high" (49° 43′ 9.353″ N; 14° 0′49.828″ E). Soils were sampled at a depth of 0–20 cm and immediately homogenised, sieved through a 5-mm diameter mesh, and stored at room temperature prior to the experiment. The soil pH, cation exchange capacity (CEC), potential bioavailability of essential elements and TE, and pseudo-total contents of TE were determined prior to the experiment (Table 1).

2.2 Biochar Preparation

The DOR used for the experiment was obtained from the manufacturing company Sierra Sur S.L. (Granada, Spain). Before pyrolysis, the DOR was sterilised by an autoclave three times (121 °C for 20 min) and subsequently frozen at − 20 °C until use. For biochar production in the laboratory conditions, the pyrolytic furnace Carbolite 301 (Carbolite Gero, Great Britain) was used. The pyrolysis was performed in an electrically heated quartz tube for 25 min at the target temperatures of 350 °C and 500 °C in the presence of nitrogen (N_2 flow of 4.5 L per min). The biochar was homogenised, and the nutrient and TE content was determined prior to the experiment (Table 2).

Table 1 The pseudo-total element contents in soil extractable with aqua regia (mg kg^{-1}) and Mehlich III (mg kg^{-1}) and the main soil characteristics at the start of the pot experiment; data are presented as mean ± standard deviation; $n = 3$

Soil type	Soil contamination level		
	Low Cambisol	Medium Cambisol	High Fluvisol
As_t (mg kg^{-1})	21.3 ± 0.68	34 ± 1.16	344 ± 20
Ca_t (mg kg^{-1})	3930 ± 47.4	3430 ± 211	2880 ± 52
Cd_t (mg kg^{-1})	1.29 ± 0.11	2.48 ± 0.18	23.8 ± 2.71
K_t (mg kg^{-1})	9230 ± 54.7	6010 ± 61.3	5180 ± 622
Mg_t (mg kg^{-1})	4350 ± 36	4170 ± 181	4330 ± 34
P_t (mg kg^{-1})	993 ± 38	572 ± 19	617 ± 54
Pb_t (mg kg^{-1})	332 ± 21.4	643 ± 21	2110 ± 299
S_t (mg kg^{-1})	335 ± 16	290 ± 9	481 ± 62
Zn_t (mg kg^{-1})	145 ± 1.65	372 ± 44	3340 ± 112
Ca_M (mg kg^{-1})	1195 ± 21	2333 ± 0	1369 ± 10
K_M (mg kg^{-1})	298 ± 10	288 ± 3	196 ± 7
Mg_M (mg kg^{-1})	86.9 ± 2.0	271 ± 2	188 ± 6
P_M (mg kg^{-1})	85.6 ± 4.2	37.4 ± 0.0	39.6 ± 1.1
S_M (mg kg^{-1})	6.43 ± 0.24	11.3 ± 0.2	16.1 ± 0.4
C (%)	2.66 ± 0.42	2.86 ± 0.14	2.62 ± 0.04
H (%)	0.56 ± 0.01	0.76 ± 0.05	0.55 ± 0.05
N (%)	0.24 ± 0.03	0.23 ± 0.00	0.16 ± 0.00
C/N ratio	11.2 ± 0.1	12.7 ± 0.9	16.6 ± 0.3
C/H ratio	4.80 ± 0.87	3.79 ± 0.43	4.80 ± 0.37
pH	6.45 ± 0.01	6.41 ± 0.03	5.73 ± 0.03
CEC ($mmol_+$ kg^{-} 1)	102 ± 1	136 ± 1	77.8 ± 3.7

[t] Pseudo-total element content

[M] Mehlich III extractable element content (Mehlich 1984)

2.3 Arbuscular Mycorrhizal Fungi Inocula and Quantification

The AM fungus used in this experiment was *Funneliformis mosseae*, former *Glomus mosseae*. The mycorrhizal inoculum was obtained using trap-pot cultures of *Medicago sativa* L., consisting of soil, spores, mycelia, and colonised root fragments (10 sporocarps/g, with 1–5 spores per sporocarp). The percentage of mycorrhizal fungi root length infected was estimated using the method of Giovannetti and Mosse (1980) after the root system was cleared and stained (Phillips and Hayman 1970).

2.4 Experimental Design

The model pot experiment was set up to evaluate dry olive residue (DOR)-based biochar as a potential immobilising agent for arsenic, lead, cadmium, and zinc and improving the nutrient status of the soil. The experiment was set up in a series of identical 0.3-L polypropylene pots containing 300 g of the studied contaminated soils and was conducted by Estación Experimental Del Zaidín, Granada, Spain. The experimental design consisted in a randomised factorial system

Table 2 Total element contents in DOR before pyrolysis and in the biochars; data are presented as mean ± standard deviation; $n = 3$

	DOR	Biochar 350 °C	Biochar 500 °C
As (mg kg^{-1})	< 0.3	< 0.3	< 0.3
Ca (mg kg^{-1})	4540 ± 39	7460 ± 883	12,700 ± 114
Cd (mg kg^{-1})	< 0.03	< 0.03	< 0.03
K (mg g^{-1})	18,670 ± 1080	36,200 ± 2974	54,560 ± 650
Mg (mg kg^{-1})	134 ± 53	2110 ± 332	3530 ± 7
P (mg kg^{-1})	1370 ± 155	2450 ± 266	3910 ± 32
Pb (mg kg^{-1})	< 0.2	< 0.2	0.92 ± 0.07
S (mg kg^{-1})	1245 ± 89	937 ± 91	1152 ± 30
Zn (mg kg^{-1})	39.8 ± 14.0	55.5 ± 11.2	103 ± 2
C (%)	47.3 ± 0.26	63.6 ± 0.56	66.7 ± 0.27
H (%)	6.55 ± 0.07	5.83 ± 0.11	3.88 ± 0.14
N (%)	1.60 ± 0.02	1.98 ± 0.01	1.68 ± 0.02
C/N ratio	29.6 ± 0.61	32.2 ± 0.48	39.8 ± 0.59
C/H ratio	7.22 ± 0.11	10.9 ± 0.30	17.2 ± 0.02
pH	4.85 ± 0.02	8.21 ± 0.03	8.45 ± 0.01

<Data under detection limit

with four factors of variation. The first experimental factor was the soil contamination level, where the soils labelled "low", "medium", and "high" differed in their As, Cd, Pb, and Zn levels (Table 1). The second factor was the DOR biochar type and comprised three levels: control without biochar, biochar prepared at the target temperature 350 °C, and biochar prepared at the target temperature 500 °C. The third factor was the DOR biochar rate and comprised also three levels: control without biochar, biochar from 350 °C to 500 °C with a 2% (w/w) application rate, and biochar from 350 °C to 500 °C with a 5% (w/w) application rate. The whole experiment was established with inoculation or not of *F. mosseae* as the fourth experimental factor.

The DOR biochars were applied and mixed manually with the soil to reach concentrations of 2% and 5% (w/w). Four replicates were established per each treatment. Besides, *F. mosseae* was inoculated by adding 8 g of inoculum as suggested García-Sánchez et al. (2014), and soil samples non-inoculated by AM fungus received the same weight of inoculum filtrate (Whatman no. 1 filter paper) containing soil microbiota free of AM fungal propagules. The moisture of the soil was brought to 60% of the water holding capacity of the soil (checked by weight during the experiment). Four replicates were established per each treatment.

One 15-day-old wheat plant (*Triticum aestivum*) was planted in each pot. The experiment was run in greenhouse conditions (supplementary light, 25/19 °C, and 50% relative humidity, García-Sánchez et al. 2017), and the plants were regularly watered in order to maintain the same initial moisture conditions. After 90 days, the plants were harvested. The plant samples were separated into roots, shoots, and grain. The roots were washed after harvest in order to eliminate the soil by using an ultrasonic bath, and all the plant parts were dried at 60 °C for 48 h and homogenised before analysis. Soil samples from each pot were homogenised, sieved (2-mm mesh), and air-dried at room temperature for chemical analysis.

2.5 Analytical Methods

The soil pH was determined in 0.01 mol L^{-1} $CaCl_2$ extracts using a 1:5 (w/v) ratio of soil to solution by employing a pH meter (pH 315i/SET, WTW Wissenschaftlich-Technische Werkstätten, Germany). For total soil carbon and nitrogen determination, a CHNS vario MACRO cube (Elementar Analysensysteme GmbH, Germany) analyser was used, where C and N were determined via a thermal conductivity detector. Soil CEC was calculated as the sum of Ca, Mg, K, Na, Fe, Mn, and Al extractable in 0.1 mol L^{-1} $BaCl_2$ (w/v = 1:20 for 2 h) (ISO 1994).

The pseudo-total concentration of TE was determined by the digestion of 0.5 g of air-dried soil that was extracted in digestion vessels with 10 mL of aqua regia and a mixture of 65% nitric acid and 30% hydrochloric acid in a ratio of 1:3. The mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave-assisted wet digestion system for 35 min at 210 °C. Similarly, for the determination of total element concentration in plant biomass (root, shoot, and grain), 0.5 g of sample was weighed into digestion vessels, and 8 mL of 65% nitric acid and 2 mL of 30% hydrogen peroxide were added. The mixture was subjected to microwave-assisted wet digestion for 30 min at 220 °C. After cooling, the digests were transferred to 20-mL glass tubes and filled to the mark using deionised water. Each sample was prepared in triplicate. The total concentration of elements in the DOR biochar was also determined following the same procedure as the digestion of plant biomass. The Mehlich III extraction procedure was performed by shaking the extractant (0.2 mol L^{-1} CH_3COOH, 0.25 mol L^{-1} NH_4NO_3, 0.013 mol L^{-1} HNO_3, 0.015 mol L^{-1} NH_4F, and 0.001 mol L^{-1} EDTA at a ratio 1:10 (w/v)), and soil was shaken for 10 min (Mehlich 1984) for the determination of the available nutrient status of soils before incubation. The bioavailable fraction of elements was determined by using 0.11 mol L^{-1} acetic acid (HOAc, 1:20 w/v) extraction (Liu et al. 2019).

Inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA), equipped with a two-channel peristaltic pump, a Sturman-Masters spray chamber, and a V-groove pneumatic nebuliser made of inert material, was used to determine As, Cd, Pb, Zn, and P concentrations of the soil and plant digests, as well as soil extracts. Flame atomic absorption spectrometry (F-AAS, Varian 280FS, Varian, Australia) was used to determine the Ca, Mg, and K concentrations of the solutions. The low concentrations of As, Cd, and Pb in the plant digests were measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA) using a collision cell pressurised with helium to reduce potential polyatomic interferences.

2.6 Data Processing

All statistical analyses were performed using the Statistica 12.0 software (www.StatSoft.com). A Shapiro-Wilk's *W* test was applied to test the normality of the data. A one-way analysis of variance (ANOVA) at $p < 0.05$ followed by the Tukey's test was applied to assess the effect of the individual treatments. The interactions of the treatment and other variables (e.g. biochar rate, pyrolysis temperature, and part of the plant investigated) were analysed by factorial ANOVA (Lindeman et al. 1980), where the significance was assessed at $p < 0.05$, $p < 0.01$, and $p < 0.001$. Correlation analysis was used for the assessment of relationships between variables, where Pearson's correlation was used with $p < 0.05$ as the criterion for significance.

3 Results

3.1 Impact of Biochar Amendment on TE and Nutrient Mobility in Soil

The pseudo-total contents of the elements investigated in the soils are summarised in Table 1. The national regulation (Ministry of the Environment of the Czech Republic 2016) defining the protection of agricultural soil quality in the Czech Republic was chosen for a rough risk assessment of TE contamination level in the soils. The maximum levels for Cd and Pb exceeded the indicative values; these soil element contents show the potential crop contamination risk (i.e. 40 mg kg^{-1} for As, 2 mg kg^{-1} for Cd, and 300 mg kg^{-1} for Pb). The Zn level in the soil "high" represents a risk to plant growth and soil biological value (i.e. exceeds the indicative value 400 mg kg^{-1} for Zn), and As, Cd, and Pb levels can directly threaten human and animal health (i.e. exceeds the indicative values 40 mg kg^{-1} for As, 20 mg/kg for Cd, 400 mg kg^{-1} for Pb). In the case of Pb, this indicative value was exceeded even in the "medium" soil, confirming Pb as the predominant contaminant in the area. The soil pH was comparable in all the experimental soils and can be considered slightly acidic. The nutrient status of the soils determined by using the Mehlich III extraction procedure (Table 1) showed acceptable (soil "low") and low (soils "medium" and "high") supply of P, good supply of K, low (soil "low") and good (soils "medium" and "high") supply of Mg, acceptable supply of Ca, and low supply of S (Ministry of the Agriculture of the Czech Republic 1998; ÚKZÚZ 2018).

The elemental composition of DOR and DOR biochars is summarised in Table 2. The contents of As, Cd, and Pb were below the procedural detection limits (ICP-OES); therefore, the addition of DOR biochar at a rate of 5% (w/w) cannot be considered a significant contributor to the existing soil contamination with these TEs. Similarly, soil enrichment in Zn is not expected to exacerbate the soil burden following a single application of the biochar.

The experiment proved significant increase of pH levels in treated "low" and "medium" soils, and in lesser extent in the soil "high". Thus, paradoxically, the lowest liming effect of biochar appeared in the soil with the lowest initial pH level (Fig. 1). Although not statistically proven, the soils treated by the DOR biochar pyrolysed at 500 °C tended to higher pH compared with those treated by the DOR biochar pyrolysed at 350 °C. Moreover, the AMF inoculation resulted in the significant ($p < 0.05$) increase of soil pH regardless of the treatment, where the DOR biochar application resulted in further enhancement of the soil pH similarly as in the case of the non-inoculated variants.

It shows the effect of the DOR biochar on the extractability of both TE and nutrients as determined using 0.11 mol L^{-1} HOAc. There was some tendency toward reduced extractability of Cd, Zn, and Pb (up to 13, 11, and 9%, respectively, as compared with that of the control), observed only in the "low" and "medium" soils. AMF inoculation significantly decreased ($p < 0.05$) the mobile proportions of Cd, Pb, and Zn compared with those of the non-inoculated variants, regardless of the treatment. On the contrary, AMF inoculation resulted in an increase in the mobile proportions of As, where the increase was significant ($p < 0.05$) for the soil "high". AMF inoculation significantly increased ($p < 0.05$) the mobility of Ca and Mg, whereas the K mobility tended to increase in all soils, but the difference was significant ($p < 0.05$) only in the case of soil "medium". On the contrary, the mobility of P decreased significantly ($p < 0.05$) in the soil "medium".

3.2 Impact of Soil Treatment on Trace Element and Nutrient Content in Plants

The response of wheat dry biomass yield to the application of DOR biochar is provided in Fig. 2. The nutrient contents in the individual plant parts can be found in Tables 3, 4, 5, 6, 7 and 8. The highest abundance of significant differences among soil treatments induced by biochar was observed for the aboveground biomass grown in the "high" soil. As suggested by the results of soil chemical extraction (HOAc), K and P were the nutrients whose content in the plants increased most frequently (up to 42 and 116%, respectively, in the grain), especially when the soils were treated with biochar at 5% (w/w). Biochar applied to "high" soil significantly ($p < 0.05$) decreased the Ca content in the grain and shoot (up to 51 and 48%, respectively), and there was also a decreasing tendency in the root. The higher supply of P, K, and Mg through the biochar application (at 5% w/w) significantly ($p < 0.05$) increased the contents of these elements in the grain, but the amount of Ca translocated to the grain decreased at the soil "high", and "medium" soils did not clearly reflect the increase in the extractable portion of this element in soil as a result of high K content in biochar. Among the biochar treatments, the effect of pyrolysis temperature on the wheat nutrient content was of minor importance and rather ambiguous (Tables 3, 4, 5, 6, 7 and 8).

The effect of DOR biochar, specifically its application rate and pyrolysis temperature, on the TE contents in wheat plants can be observed in Tables 3, 4, 5, 6, 7 and 8. Generally, the TE levels in the individual parts of plants decreased in order roots > shots > grain. As a consequence of biochar application to "low" and "medium" soils, the root and shoot As contents tended to decrease, but these trends were not statistically proven. On the contrary, in the "high" soil As enrichments up to 48% (root) and 29% (shoot) were observed. However, an opposite pattern was observed in the AMF-inoculated variants. The storage of As in the grain decreased significantly ($p < 0.05$) in the "low" and "medium" soils (up to 45 and 53%, respectively) as compared with those of the control. The decrease in As achieved in the "high" soil was still

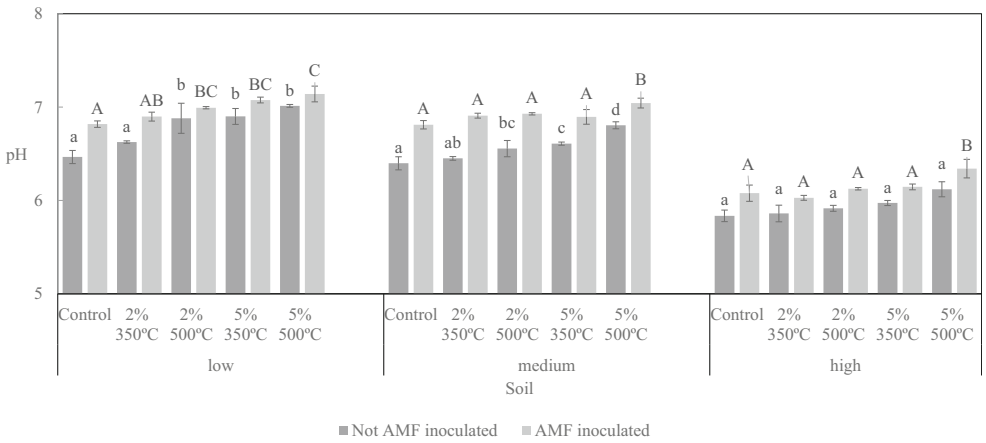

Fig. 1 The effects of the individual treatments on soil pH at the end of the experiment; data expressed as mean (bar) and standard deviation (line segment). The bars marked by the same letter did not significantly differ at $p < 0.05$ within individual soils; small case letters for not AMF-inoculated variants, capital letters for AMF-inoculated variants. "Low", "medium", and "high" indicate low, medium, and high levels of elements in soil, respectively

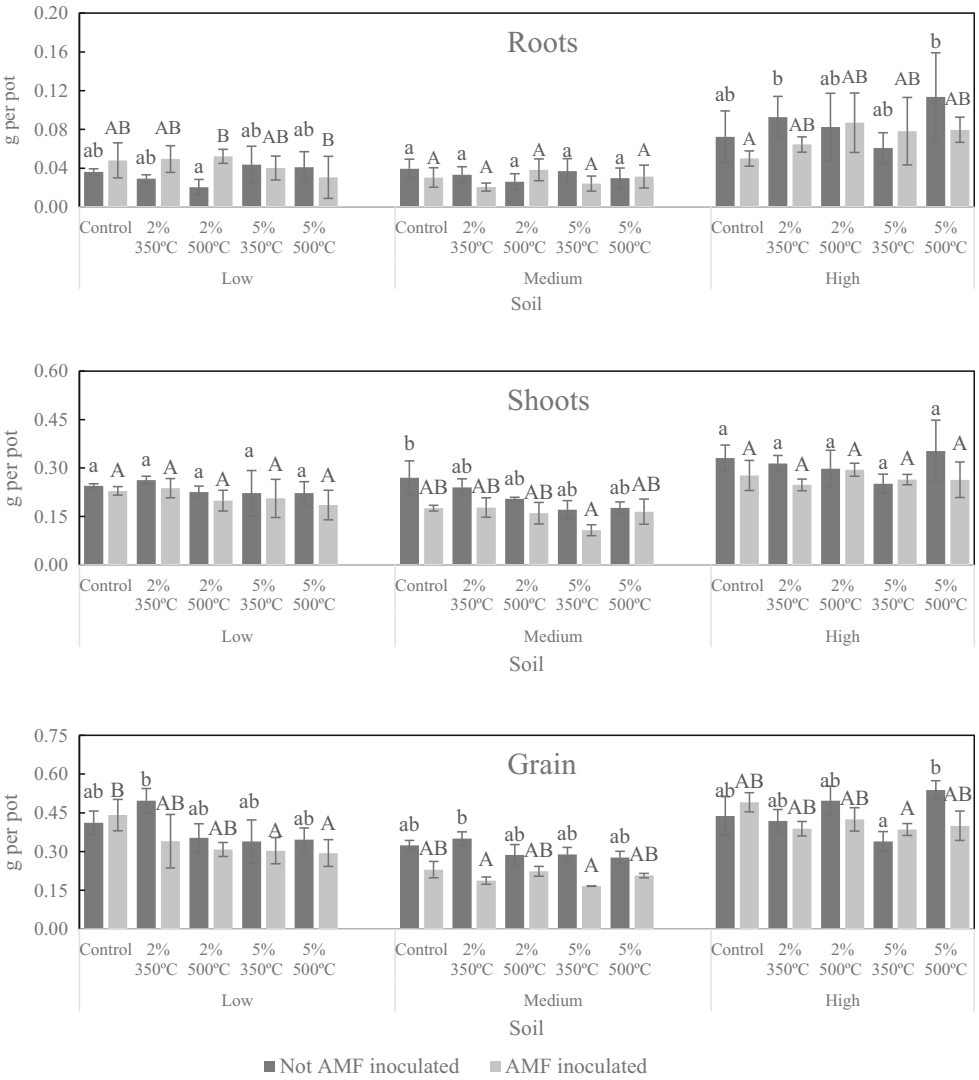

Fig. 2 The effects of the individual treatments on yield of wheat biomass; data expressed as mean (bar) and standard deviation (line segment). The bars marked by the same letter did not significantly differ at $p < 0.05$ within individual soils (small case letters for not AMF-inoculated variants, capital letters for AMF-inoculated variants). "Low", "medium", and "high" indicate low, medium, and high levels of elements in soil, respectively

Table 3 The element concentrations in the wheat roots (mg kg^{-1}) in the end of the experiment (90th day of cultivation)—not AMF-inoculated; The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual columns; data are presented as mean ± standard deviation, $n = 4$

	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Ca (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	P (mg kg^{-1})
Not AMF-inoculated								
Low level of elements in soil								
Control	5.72 ± 1.32^{a}	4.26 ± 0.91^{b}	61.4 ± 7.4^{a}	114 ± 12^{bc}	3420 ± 187^{a}	5916 ± 526^{b}	1385 ± 218^{a}	1569 ± 159^{a}
DOR biochar 2% 350 °C	3.87 ± 0.92^{a}	2.42 ± 0.26^{a}	50.5 ± 5.4^{a}	69.8 ± 5.1^{a}	2761 ± 262^{a}	2627 ± 406^{a}	1666 ± 128^{a}	1286 ± 184^{a}
DOR biochar 2% 500 °C	4.11 ± 1.22^{a}	2.19 ± 0.15^{a}	46.4 ± 8.0^{a}	83.2 ± 7.9^{ab}	3360 ± 386^{a}	2532 ± 533^{a}	1884 ± 153^{a}	1794 ± 241^{a}
DOR biochar 5% 350 °C	4.97 ± 0.80^{a}	2.62 ± 0.64^{a}	46.7 ± 14.7^{a}	87.1 ± 2.6^{ab}	3362 ± 899^{a}	8624 ± 923^{c}	1517 ± 500^{a}	1569 ± 292^{a}
DOR biochar 5% 500 °C	5.22 ± 0.95^{a}	2.50 ± 0.47^{a}	65.1 ± 15.5^{a}	131 ± 29^{c}	4012 ± 379^{a}	7728 ± 599^{bc}	1819 ± 394^{a}	1622 ± 350^{a}
Medium level of elements in soil								
Control	9.09 ± 0.91^{a}	7.40 ± 0.14^{b}	136 ± 13^{a}	114 ± 15^{b}	3221 ± 473^{a}	4912 ± 821^{a}	1491 ± 181^{a}	1537 ± 263^{a}
DOR biochar 2% 350 °C	8.52 ± 0.82^{a}	6.73 ± 1.00^{b}	128 ± 16^{a}	102 ± 9^{ab}	2068 ± 194^{a}	5189 ± 295^{a}	1536 ± 242^{a}	1470 ± 351^{a}
DOR biochar 2% 500 °C	6.84 ± 1.19^{a}	4.98 ± 1.23^{a}	83.2 ± 13.8^{a}	77.9 ± 8.1^{ab}	3141 ± 510^{a}	4019 ± 1045^{a}	1309 ± 214^{a}	1262 ± 188^{a}
DOR biochar 5% 350 °C	7.16 ± 1.34^{a}	6.00 ± 1.54^{b}	106 ± 33^{a}	94.9 ± 2.9^{ab}	3539 ± 914^{a}	5450 ± 488^{a}	1453 ± 305^{a}	1685 ± 434^{a}
DOR biochar 5% 500 °C	7.00 ± 1.20^{a}	4.66 ± 1.09^{a}	92.7 ± 7.0^{a}	80.4 ± 21.6^{a}	3712 ± 939^{a}	6204 ± 947^{a}	1824 ± 525^{a}	1402 ± 172^{a}
High level of elements in soil								
Control	51.7 ± 10.6^{a}	48.0 ± 9.3^{a}	367 ± 76^{a}	1456 ± 230^{a}	4312 ± 313^{a}	1863 ± 243^{a}	1410 ± 280^{a}	523 ± 50^{a}
DOR biochar 2% 350 °C	76.5 ± 14.0^{b}	39.4 ± 6.6^{a}	496 ± 141^{a}	1488 ± 131^{a}	3082 ± 409^{a}	2508 ± 205^{ab}	1670 ± 410^{a}	630 ± 50^{ab}
DOR biochar 2% 500 °C	54.1 ± 11.1^{ab}	47.4 ± 12.5^{a}	391 ± 71^{a}	1417 ± 150^{a}	3420 ± 895^{a}	2865 ± 410^{b}	1254 ± 196^{a}	582 ± 79^{ab}
DOR biochar 5% 350 °C	55.8 ± 9.7^{ab}	40.6 ± 5.5^{a}	512 ± 159^{a}	1470 ± 319^{a}	3632 ± 426^{a}	2486 ± 349^{ab}	1575 ± 159^{a}	755 ± 114^{b}
DOR biochar 5% 500 °C	61.9 ± 7.0^{ab}	35.3 ± 1.1^{a}	547 ± 172^{a}	1260 ± 162^{a}	2704 ± 359^{a}	2555 ± 368^{ab}	1722 ± 933^{a}	657 ± 88^{ab}

considerable (up to 34%). The application of BC350 at 5% (w/w) was unambiguously the most efficient amendment reducing As content in the grain in the non-AMF–inoculated variants.

Cadmium content decreased in all plant compartments when grown in biochar-amended "low" and "medium" soils (up to 47% and 49% in the grain, respectively) compared with that of the control. In the "high" soil, the Cd content tended to decrease in the root (up to 26%), whereas it significantly increased in the shoots and grain (up to 46% and 106%, respectively).

The effect of biochar on the Pb content in wheat biomass showed only a few clear tendencies, and, in most cases, it was insignificant ($p > 0.05$). The higher application rate of biochar, especially that of BC500, seemed to slightly promote Pb storage in the root and shoots. All biochar treatments showed great potential to reduce Pb content in the grain for "low" soil (up to 63%). Pb translocation in the other two soils was rather ambiguous.

Table 4 The element concentrations in the wheat roots (mg kg^{-1}) in the end of the experiment (90th day of cultivation)—AMF-inoculated; The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual columns; data are presented as mean ± standard deviation, $n = 4$

	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Ca (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	P (mg kg^{-1})
AMF-inoculated								
Low level of elements in soil								
Control	4.66 ± 0.60^{a}	2.66 ± 0.33^{ab}	65.8 ± 12.4^{b}	121 ± 20^{b}	4098 ± 1084^{a}	4460 ± 3023^{a}	1477 ± 838^{a}	1049 ± 32^{a}
DOR biochar 2% 350 °C	4.50 ± 1.09^{a}	3.09 ± 0.58^{b}	61.4 ± 6.0^{b}	135 ± 29^{b}	5219 ± 1250^{a}	7773 ± 1092^{a}	2162 ± 516^{a}	1485 ± 235^{bc}
DOR biochar 2% 500 °C	4.07 ± 0.35^{a}	2.55 ± 0.19^{ab}	52.9 ± 9.9^{ab}	102 ± 15^{ab}	4238 ± 687^{a}	7099 ± 801^{a}	1732 ± 491^{a}	1328 ± 121^{ab}
DOR biochar 5% 350 °C	4.94 ± 1.73^{a}	2.78 ± 0.31^{ab}	55.1 ± 9.7^{ab}	112 ± 17^{ab}	3958 ± 836^{a}	5376 ± 2891^{a}	1817 ± 85^{a}	1802 ± 258^{c}
DOR biochar 5% 500 °C	4.32 ± 2.10^{a}	2.04 ± 0.64^{a}	34.6 ± 7.8^{a}	78.0 ± 8.5^{a}	3977 ± 567^{a}	4236 ± 1528^{a}	1822 ± 51^{a}	1301 ± 240^{ab}
Medium level of elements in soil								
Control	6.94 ± 0.98^{a}	6.04 ± 0.83^{a}	89.3 ± 7.4^{a}	87.5 ± 13.2^{b}	3341 ± 532^{ab}	4537 ± 948^{ab}	1502 ± 43^{a}	1407 ± 213^{a}
DOR biochar 2% 350 °C	5.93 ± 1.09^{a}	6.06 ± 1.00^{a}	69.6 ± 9.4^{a}	93.8 ± 11.2^{b}	4380 ± 753^{c}	5437 ± 656^{b}	1611 ± 477^{a}	1569 ± 302^{a}
DOR biochar 2% 500 °C	6.97 ± 1.00^{a}	5.34 ± 1.13^{a}	87.7 ± 14.1^{a}	88.1 ± 13.4^{b}	4268 ± 698^{bc}	6184 ± 572^{b}	1910 ± 503^{a}	1274 ± 69^{a}
DOR biochar 5% 350 °C	5.47 ± 0.43^{a}	4.08 ± 1.43^{a}	72.6 ± 10.8^{a}	85.7 ± 6.8^{b}	2833 ± 504^{a}	7034 ± 1359^{b}	1727 ± 208^{a}	1734 ± 368^{a}
DOR biochar 5% 500 °C	5.35 ± 0. 76^{a}	4.12 ± 1.43^{a}	71.9 ± 14.7^{a}	61.6 ± 10.0^{a}	3293 ± 841^{ab}	2673 ± 272^{a}	1424 ± 68^{a}	1369 ± 157^{a}
High level of elements in soil								
Control	55.3 ± 6.7^{a}	48.3 ± 5.1^{a}	354 ± 110^{a}	1353 ± 169^{a}	3596 ± 1501^{a}	2284 ± 203^{a}	1153 ± 112^{a}	598 ± 42^{a}
DOR biochar 2% 350 °C	61.8 ± 16.1^{a}	42.2 ± 9.4^{a}	453 ± 98^{a}	1390 ± 146^{a}	4316 ± 1669^{a}	2696 ± 486^{ab}	1423 ± 212^{ab}	701 ± 122^{ab}
DOR biochar 2% 500 °C	66.0 ± 18.7^{a}	33.4 ± 3.6^{a}	438 ± 141^{a}	1315 ± 186^{a}	4680 ± 620^{a}	3147 ± 625^{ab}	1792 ± 460^{b}	664 ± 95^{ab}
DOR biochar 5% 350 °C	39.6 ± 8.2^{a}	50.6 ± 4.6^{a}	267 ± 38^{a}	1412 ± 230^{a}	1742 ± 382^{a}	6230 ± 1408^{b}	1304 ± 149^{ab}	860 ± 62^{b}
DOR biochar 5% 500 °C	41.8 ± 11.8^{a}	43.4 ± 12.9^{a}	288 ± 48^{a}	1150 ± 174^{a}	2319 ± 872^{a}	3523 ± 1348^{ab}	1253 ± 208^{ab}	723 ± 175^{ab}

Table 5 The element concentrations in the wheat shoots (mg kg^{-1}) in the end of the experiment (90th day of cultivation)—not AMF-inoculated; The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual columns; data are presented as mean ± standard deviation, $n = 4$

	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Ca (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	P (mg kg^{-1})
Not AMF-inoculated								
Low level of elements in soil								
Control	0.949 ± 0.174^{b}	0.551 ± 0.050^{a}	1.39 ± 0.48^{bc}	64.1 ± 12.0^{a}	3481 ± 458^{a}	23,177 ± 2022^{a}	1297 ± 271^{a}	905 ± 90^{a}
DOR biochar 2% 350 °C	0.509 ± 0.049^{a}	0.408 ± 0.093^{a}	0.656 ± 0.275^{a}	42.4 ± 9.1^{a}	2509 ± 281^{a}	24,036 ± 4079^{a}	1060 ± 74^{a}	851 ± 80^{a}
DOR biochar 2% 500 °C	0.948 ± 0.259^{b}	0.374 ± 0.105^{a}	0.893 ± 0.205^{ab}	43.8 ± 6.4^{a}	3937 ± 986^{a}	26,628 ± 2241^{a}	1094 ± 65^{a}	931 ± 84^{a}
DOR biochar 5% 350 °C	0.654 ± 0.179^{ab}	0.392 ± 0.117^{a}	1.12 ± 0.32^{abc}	60.4 ± 15.0^{a}	3528 ± 807^{a}	25,550 ± 2585^{a}	1414 ± 209^{a}	2311 ± 577^{b}
DOR biochar 5% 500 °C	0.718 ± 0.144^{ab}	0.389 ± 0.081^{a}	1.68 ± 0.20^{c}	51.2 ± 2.9^{a}	2925 ± 66^{a}	28,100 ± 1860^{a}	1334 ± 228^{a}	1842 ± 537^{b}
Medium level of elements in soil								
Control	0.985 ± 0.182^{a}	2.23 ± 0.18^{c}	2.01 ± 0.66^{a}	78.8 ± 7.1^{a}	4796 ± 875^{a}	18,021 ± 4000^{a}	1606 ± 149^{a}	931 ± 116^{a}
DOR biochar 2% 350 °C	0.855 ± 0.325^{a}	1.35 ± 0.07^{b}	2.78 ± 0.57^{a}	65.3 ± 11.2^{a}	5036 ± 432^{a}	22,912 ± 3077^{a}	1679 ± 335^{a}	1128 ± 154^{a}
DOR biochar 2% 500 °C	0.680 ± 0.155^{a}	1.48 ± 0.30^{b}	2.20 ± 0.82^{a}	59.4 ± 11.1^{a}	4617 ± 1012^{a}	22,905 ± 4237^{a}	1772 ± 420^{a}	915 ± 58^{a}
DOR biochar 5% 350 °C	0.636 ± 0.125^{a}	1.13 ± 0.15^{ab}	2.35 ± 0.78^{a}	64.8 ± 14.3^{a}	5421 ± 592^{a}	25,880 ± 4204^{a}	1895 ± 548^{a}	1558 ± 153^{b}
DOR biochar 5% 500 °C	0.682 ± 0.131^{a}	0.916 ± 0.152^{a}	3.31 ± 1.40^{a}	57.8 ± 8.2^{a}	4351 ± 360^{a}	18,637 ± 3319^{a}	1719 ± 183^{a}	894 ± 74^{a}
High level of elements in soil								
Control	12.6 ± 1.7^{a}	4.39 ± 0.21^{a}	3.40 ± 1.42^{a}	694 ± 45^{a}	4821 ± 750^{b}	23,436 ± 3712^{a}	1313 ± 246^{b}	117 ± 13^{a}
DOR biochar 2% 350 °C	14.7 ± 1.8^{a}	4.97 ± 0.80^{ab}	3.40 ± 1.13^{a}	554 ± 58^{a}	3947 ± 467^{ab}	24,271 ± 3658^{a}	1120 ± 46^{b}	163 ± 28^{a}
DOR biochar 2% 500 °C	14.4 ± 0.3^{a}	5.32 ± 0.42^{ab}	3.67 ± 1.14^{a}	589 ± 35^{a}	3990 ± 764^{ab}	24,344 ± 885^{a}	1197 ± 184^{b}	179 ± 50^{a}
DOR biochar 5% 350 °C	13.1 ± 2.0^{a}	6.40 ± 0.30^{b}	3.42 ± 1.37^{a}	530 ± 99^{a}	2765 ± 337^{ab}	34,046 ± 2735^{b}	733 ± 89^{a}	426 ± 66^{b}
DOR biochar 5% 500 °C	16.2 ± 4.4^{a}	5.38 ± 0.98^{ab}	3.05 ± 1.34^{a}	607 ± 116^{a}	2526 ± 698^{a}	37,205 ± 1096^{b}	987 ± 146^{ab}	278 ± 58^{a}

The highest reduction in Zn content in the root and shoot was found in the "low" soil (up to 39 and 34%, respectively) when BC350 was applied at 2% (w/w). The ameliorative effect of biochar clearly weakened in the order of "low" > "medium" > "high" soil. As for wheat grain, its Zn content slightly decreased in the amended "low" and "medium" soils (up to 24 and 20%, respectively). The addition of biochar at 5% to the "high" soil increased the grain Zn content by up to 25%.

For Cd and Pb, the response of the element contents in the wheat plants in the AMF inoculation was ambiguous. The addition of BC500 at 5% led to a significant decrease in the Pb concentration in the wheat root. Regardless of pyrolysis temperature, the significant effect of BC addition at 5% on the wheat grain Pb concentration was observed, too. On the other hand, the AMF-inoculated variants showed a trend of decreasing As accumulation in the roots and increasing accumulation of this element in the

Table 6 The element concentrations in the wheat shoots (mg kg^{-1}) in the end of the experiment (90th day of cultivation)—AMF-inoculated; The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual columns; data are presented as mean ± standard deviation, $n = 4$

	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Ca (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	P (mg kg^{-1})
AMF-inoculated								
Low level of elements in soil								
Control	0.894 ± 0.132^{a}	0.375 ± 0.118^{a}	1.33 ± 0.20^{ab}	60.6 ± 9.7^{a}	4291 ± 702^{a}	26,193 ± 958^{a}	1540 ± 315^{a}	1580 ± 358^{a}
DOR biochar 2% 350 °C	0.797 ± 0.109^{a}	0.482 ± 0.141^{a}	1.59 ± 0.23^{b}	68.8 ± 4.3^{a}	5078 ± 816^{a}	28,112 ± 2324^{ab}	1647 ± 145^{a}	1721 ± 314^{a}
DOR biochar 2% 500 °C	0.773 ± 0.119^{a}	0.381 ± 0.051^{a}	1.36 ± 0.31^{ab}	63.3 ± 6.7^{a}	4671 ± 458^{a}	26,573 ± 2939^{a}	1585 ± 475^{a}	1896 ± 187^{a}
DOR biochar 5% 350 °C	0.715 ± 0.063^{a}	0.417 ± 0.064^{a}	1.20 ± 0.47^{ab}	56.6 ± 2.1^{a}	5106 ± 742^{a}	31,435 ± 4057^{ab}	1485 ± 123^{a}	2234 ± 293^{a}
DOR biochar 5% 500 °C	0.729 ± 0.071^{a}	0.352 ± 0.083^{a}	1.11 ± 0.52^{a}	54.1 ± 11.1^{a}	3998 ± 64^{a}	33,864 ± 2778^{b}	1323 ± 91^{a}	1762 ± 412^{a}
Medium level of elements in soil								
Control	0.665 ± 0.134^{a}	2.03 ± 0.43^{b}	1.73 ± 0.22^{a}	99.2 ± 12.2^{b}	3632 ± 487^{a}	23,787 ± 2284^{a}	1591 ± 248^{a}	1021 ± 156^{a}
DOR biochar 2% 350 °C	1.02 ± 0.35^{a}	1.42 ± 0.29^{ab}	3.72 ± 1.69^{a}	83.4 ± 16.4^{ab}	5281 ± 691^{a}	21,405 ± 2009^{a}	1997 ± 213^{a}	1017 ± 227^{a}
DOR biochar 2% 500 °C	0.969 ± 0.228^{a}	1.54 ± 0.31^{ab}	5.06 ± 0.87^{a}	91.2 ± 13.1^{ab}	4977 ± 683^{a}	24,069 ± 2873^{a}	1784 ± 511^{a}	972 ± 43^{a}
DOR biochar 5% 350 °C	0.887 ± 0.166^{a}	1.37 ± 0.23^{ab}	3.86 ± 1.54^{a}	74.8 ± 23.6^{ab}	4501 ± 492^{a}	26,132 ± 4032^{a}	1707 ± 204^{a}	1252 ± 251^{a}
DOR biochar 5% 500 °C	0.862 ± 0.124^{a}	1.25 ± 0.30^{a}	3.97 ± 1.81^{a}	57.6 ± 7.6^{ab}	4842 ± 440^{a}	28,932 ± 5548^{a}	1881 ± 520^{a}	1069 ± 129^{a}
High level of elements in soil								
Control	17.3 ± 3.8^{ab}	5.57 ± 0.48^{a}	3.43 ± 1.89^{ab}	664 ± 58^{a}	5692 ± 859^{d}	27,869 ± 1417^{a}	1389 ± 242^{b}	149 ± 38^{a}
DOR biochar 2% 350 °C	19.4 ± 3.5^{b}	5.21 ± 0.72^{a}	2.72 ± 0.66^{ab}	648 ± 142^{a}	4317 ± 430^{cd}	30,363 ± 3951^{a}	975 ± 84^{a}	174 ± 48^{a}
DOR biochar 2% 500 °C	16.1 ± 3.5^{ab}	5.04 ± 1.39^{a}	2.06 ± 1.17^{a}	530 ± 95^{a}	3622 ± 926^{bc}	30,717 ± 2160^{a}	867 ± 90^{a}	209 ± 66^{a}
DOR biochar 5% 350 °C	12.3 ± 0.8^{a}	6.70 ± 0.70^{a}	4.83 ± 1.44^{b}	700 ± 69^{a}	2713 ± 377^{ab}	34,428 ± 3118^{ab}	856 ± 86^{a}	558 ± 233^{b}
DOR biochar 5% 500 °C	11.7 ± 1.5^{a}	6.36 ± 0.91^{a}	2.92 ± 0.11^{ab}	660 ± 157^{a}	1864 ± 35^{a}	38,088 ± 4141^{b}	834 ± 132^{a}	606 ± 222^{b}

Table 7 The element concentrations in the wheat grain (mg kg^{-1}) in the end of the experiment (90th day of cultivation)—not AMF-inoculated; The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual columns; data are presented as mean ± standard deviation, $n = 4$

	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Ca (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	P (mg kg^{-1})
Not AMF-inoculated								
Low level of elements in soil								
Control	0.173 ± 0.019^{c}	0.467 ± 0.111^{a}	0.213 ± 0.004^{b}	74.3 ± 3.3^{a}	697 ± 16^{a}	8995 ± 824^{a}	1554 ± 62^{a}	4067 ± 244^{a}
DOR biochar 2% 350 °C	0.142 ± 0.031^{ab}	0.313 ± 0.026^{a}	0.103 ± 0.014^{ab}	56.8 ± 8.1^{a}	592 ± 61^{a}	8442 ± 1017^{a}	1454 ± 150^{a}	4033 ± 450^{a}
DOR biochar 2% 500 °C	0.134 ± 0.033^{bc}	0.250 ± 0.044^{a}	0.113 ± 0.034^{ab}	59.4 ± 7.9^{a}	716 ± 141^{a}	9040 ± 1302^{a}	1554 ± 117^{a}	4212 ± 419^{a}
DOR biochar 5% 350 °C	0.095 ± 0.023^{a}	0.248 ± 0.073^{a}	0.078 ± 0.019^{a}	58.6 ± 7.1^{a}	679 ± 188^{a}	8471 ± 878^{a}	1437 ± 253^{a}	4429 ± 842^{a}
DOR biochar 5% 500 °C	0.111 ± 0.023^{abc}	0.298 ± 0.035^{a}	0.124 ± 0.029^{ab}	59.4 ± 4.4^{a}	590 ± 152^{a}	9301 ± 369^{a}	1588 ± 222^{a}	4309 ± 422^{a}
Medium level of elements in soil								
Control	0.149 ± 0.029^{b}	1.07 ± 0.19^{b}	0.301 ± 0.100^{a}	58.6 ± 6.1^{a}	719 ± 47^{a}	11,733 ± 2449^{ab}	1381 ± 32^{a}	3838 ± 325^{a}
DOR biochar 2% 350 °C	0.098 ± 0.043^{ab}	0.887 ± 0.055^{b}	0.364 ± 0.092^{a}	46.6 ± 7.9^{a}	666 ± 67^{a}	9913 ± 1187^{a}	1306 ± 210^{a}	4030 ± 402^{a}
DOR biochar 2% 500 °C	0.095 ± 0.014^{ab}	0.970 ± 0.205^{b}	0.458 ± 0.135^{a}	55.1 ± 7.0^{a}	610 ± 101^{a}	12,264 ± 3341^{ab}	1331 ± 120^{a}	3690 ± 113^{a}
DOR biochar 5% 350 °C	0.070 ± 0.019^{a}	0.570 ± 0.112^{a}	0.294 ± 0.058^{a}	54.7 ± 6.3^{a}	859 ± 154^{a}	10,281 ± 909^{a}	1453 ± 88^{a}	4685 ± 189^{b}
DOR biochar 5% 500 °C	0.093 ± 0.027^{ab}	0.542 ± 0.079^{a}	0.221 ± 0.071^{a}	45.7 ± 5.3^{a}	725 ± 56^{a}	16,639 ± 3637^{b}	1252 ± 129^{a}	4170 ± 354^{b}
High level of elements in soil								
Control	3.18 ± 0.72^{a}	1.55 ± 0.15^{a}	0.711 ± 0.310^{a}	171 ± 25^{a}	1234 ± 181^{c}	9357 ± 814^{a}	888 ± 66^{a}	1310 ± 18^{a}
DOR biochar 2% 350 °C	2.38 ± 0.20^{a}	2.15 ± 0.19^{b}	0.536 ± 0.072^{a}	159 ± 12^{a}	981 ± 90^{b}	9769 ± 566^{a}	878 ± 19^{a}	1956 ± 188^{b}
DOR biochar 2% 500 °C	2.85 ± 0.44^{a}	2.08 ± 0.08^{b}	0.390 ± 0.047^{a}	158 ± 14^{a}	1003 ± 88^{bc}	11,942 ± 665^{ab}	897 ± 53^{a}	1716 ± 399^{ab}
DOR biochar 5% 350 °C	2.10 ± 0.42^{a}	3.20 ± 0.23^{c}	0.804 ± 0.100^{a}	213 ± 46^{b}	777 ± 70^{ab}	12,823 ± 1116^{b}	1074 ± 81^{b}	2825 ± 237^{c}
DOR biochar 5% 500 °C	2.72 ± 0.70^{a}	2.20 ± 0.23^{b}	0.819 ± 0.243^{a}	196 ± 9^{ab}	604 ± 90^{a}	13,198 ± 1475^{b}	1124 ± 55^{b}	2806 ± 111^{c}

shoots, but these trends were not statistically proven. For Zn in the shoots, this trend was observed only for the soils "medium" and "high", where the differences were significant in wheat shoots growing in the soil "medium". In the "low" and "medium" soils, the addition of BC500 at 5% had led to a significant decrease in the Zn concentration of wheat roots. Moreover, the BC350 addition at 5% resulted in the significant increase of Zn concentration of wheat grain.

4 Discussion

The contents of essential elements and nutrients in the biochar increased markedly in the following order: DOR < BC350 < BC500, with the exception of N, H, and S (Table 2), which exhibited their own pattern. Enrichment or loss of an element in the biochar is attributed to the carbonisation and ashing processes, both increasing with pyrolysis temperature (Chen et al. 2008). Based explicitly on the HOAc extraction data, the

Table 8 The element concentrations in the wheat grain (mg kg^{-1}) in the end of the experiment (90th day of cultivation)—AMF-inoculated; The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual columns; data are presented as mean ± standard deviation, $n = 4$

	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Ca (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	P (mg kg^{-1})
AMF-inoculated								
Low level of elements in soil								
Control	0.136 ± 0.022^{a}	0.240 ± 0.101^{a}	0.255 ± 0.064^{b}	54.4 ± 14.2^{a}	624 ± 27^{a}	8524 ± 1088^{a}	1558 ± 165^{a}	4438 ± 598^{a}
DOR biochar 2% 350 °C	0.105 ± 0.024^{a}	0.307 ± 0.109^{a}	0.167 ± 0.023^{ab}	65.0 ± 16.1^{a}	603 ± 32^{a}	9103 ± 709^{a}	1688 ± 184^{a}	4931 ± 108^{ab}
DOR biochar 2% 500 °C	0.103 ± 0.023^{a}	0.269 ± 0.079^{a}	0.145 ± 0.045^{a}	73.1 ± 12.0^{a}	643 ± 58^{a}	7890 ± 322^{a}	1780 ± 130^{a}	4907 ± 179^{ab}
DOR biochar 5% 350 °C	0.132 ± 0.030^{a}	0.202 ± 0.062^{a}	0.151 ± 0.049^{a}	64.8 ± 14.3^{a}	587 ± 65^{a}	9057 ± 754^{a}	1496 ± 250^{a}	4886 ± 45^{ab}
DOR biochar 5% 500 °C	0.089 ± 0.023^{a}	0.154 ± 0.036^{a}	0.105 ± 0.015^{a}	58.3 ± 5.9^{a}	616 ± 62^{a}	9160 ± 362^{a}	1523 ± 139^{a}	5175 ± 103^{b}
Medium level of elements in soil								
Control	0.105 ± 0.020^{a}	1.17 ± 0.31^{a}	0.205 ± 0.034^{a}	77.3 ± 8.7^{a}	1046 ± 108^{a}	9450 ± 362^{a}	1733 ± 160^{a}	3862 ± 152^{a}
DOR biochar 2% 350 °C	0.108 ± 0.035^{a}	1.08 ± 0.37^{a}	0.274 ± 0.081^{a}	64.5 ± 7.4^{a}	1069 ± 254^{a}	10,289 ± 920^{a}	1580 ± 275^{a}	3787 ± 209^{a}
DOR biochar 2% 500 °C	0.128 ± 0.019^{a}	0.953 ± 0.093^{a}	0.321 ± 0.114^{a}	67.6 ± 7.3^{a}	1033 ± 113^{a}	10,741 ± 3208^{a}	1549 ± 205^{a}	3619 ± 320^{a}
DOR biochar 5% 350 °C	0.091 ± 0.028^{a}	0.715 ± 0.097^{a}	0.348 ± 0.064^{a}	58.1 ± 6.5^{a}	1142 ± 198^{a}	14,420 ± 1504^{a}	1627 ± 168^{a}	4250 ± 402^{a}
DOR biochar 5% 500 °C	0.107 ± 0.017^{a}	0.676 ± 0.163^{a}	0.292 ± 0.071^{a}	71.7 ± 7.6^{a}	931 ± 166^{a}	10,388 ± 1672^{a}	1707 ± 303^{a}	4389 ± 759^{a}
High level of elements in soil								
Control	2.86 ± 0.04^{ab}	2.26 ± 0.28^{a}	0.825 ± 0.234^{a}	178 ± 10^{a}	1396 ± 38^{c}	13,164 ± 838^{ab}	1154 ± 102^{ab}	1940 ± 290^{a}
DOR biochar 2% 350 °C	3.36 ± 1.19^{b}	2.32 ± 0.24^{a}	0.618 ± 0.086^{a}	157 ± 14^{a}	1150 ± 108^{c}	11,950 ± 946^{ab}	1020 ± 94^{a}	2147 ± 515^{a}
DOR biochar 2% 500 °C	2.33 ± 0.45^{a}	2.49 ± 0.57^{a}	0.773 ± 0.160^{a}	155 ± 44^{a}	854 ± 44^{b}	11,125 ± 889^{a}	1080 ± 112^{a}	2492 ± 422^{ab}
DOR biochar 5% 350 °C	2.06 ± 0.25^{a}	3.59 ± 0.43^{b}	0.844 ± 0.108^{a}	227 ± 24^{b}	834 ± 92^{b}	14,125 ± 1334^{b}	1157 ± 60^{ab}	3140 ± 171^{bc}
DOR biochar 5% 500 °C	2.00 ± 0.27^{a}	2.86 ± 0.23^{ab}	0.743 ± 0.138^{a}	208 ± 31^{ab}	490 ± 100^{a}	14,523 ± 1566^{b}	1320 ± 107^{b}	3537 ± 244^{c}

efficiency of the TE immobilisation would be insignificant both in practice and statistically ($p < 0.05$). Recently, Liu et al. (2019) found soil extraction with diluted HOAc to be a satisfactory strategy for the estimation of the plant-available portion of Cd in soil. It consists of releasing soluble, cation-exchangeable, and carbonate-bound TE (Rodríguez et al. 2009). When browsing the TE-immobilisation performance achieved in other studies, biochars with a substantial ability to decrease TE extractability in contaminated soils have often been reported (Hmid et al. 2015; Gusiatin et al. 2016; Yang et al. 2016). However, in these studies, the chemical extractants (such as $Ca(NO_3)_2$ and $CaCl_2$) had no acidifying effect. Soil extraction with HOAc used in this study decreases the pH of the soil suspension; thus, the net surface charge turns more positive, and carbonates, (hydr)oxides, and phosphates dissolve, inevitably releasing the cationic TE back to the solution. Such a pH-dependent TE remobilisation from biochar (Qian et al. 2019) or biochar-amended soil (Houben et al. 2013) was reported by employing various acid-leaching tests. This behaviour may partially mimic the acidifying effect of low-molecular-weight acids excreted in the rhizosphere. Ding et al. (2014) suggested that intraparticle diffusion might play a dominant role in cation immobilisation on high-temperature biochars, while cation exchange was shown to be the major immobilising mechanism for low-temperature biochars (250–400 °C).

At higher temperatures, acidic functional surface groups begin disappearing simultaneously with a decrease in sorption capacity (Lehmann and Joseph 2015). The best immobilisation performance for cationic TE in the "low" soil was achieved when BC500 was applied at 2% (w/w). The higher stabilisation potential of BC500, at least in the short term, may be explained by a difference in desorption kinetics (simple cation exchange vs. intraparticle diffusion). In our experiment, biochar hardly exhibited any positive effect on the stabilisation of As in all treated soils. Moreover, the mobility of this anionic TE in the soils could even be enhanced, similarly to the results reported by Beesley et al. (2010) and Kloss et al. (2014a). Biochar addition increased the soil pH consistently with the application rate and temperature of pyrolysis (Fig. 1), as commonly described elsewhere (Houben et al. 2013; Hmid et al. 2015; Gusiatin et al. 2016; Paneque et al. 2016). The significant ($p < 0.05$) Pearson's correlation coefficients between the soil pH and the individual TE, varying between $r = -0.64$ and $r = -0.88$ (data not shown), indicated decreasing Cd, Pb, and Zn mobility with increasing soil pH. Moreover, the AMF inoculation resulted in the significant ($p < 0.05$) increase in soil pH regardless of the treatment, where the DOR biochar application resulted in further enhancement of the soil pH, similar as in the case of the non-inoculated variants. García-Sánchez et al. (2017) suggest that the increase of pH in the AMF-inoculated soil compared with the non-inoculated variants could be due to the changed composition of the root exudates.

Only HOAc-extractable macronutrient K positively responded to the biochar amendments. The amount of extracted Ca, P, and Mg did not reflect either the pyrolysis temperature or biochar ratio, i.e. the factors increasing the total input of nutrients into soil with biochar application. Enhanced extractability of K, P, Ca, Mg, and S was frequently observed after biochar application (Xu et al. 2013; Kloss et al. 2014b; Brantley et al. 2016), although soil nutrient enrichment strongly depends on the pyrolysis conditions and biochar feedstock.

An insignificant effect of AMF inoculation on TE mobility in both untreated and biochar-treated soils was reported by Qiao et al. (2015), indicating low interaction of biochar with the mycorrhizal fungi. García-Sánchez et al. (2017) tested the effect of the soil application of the fungal-transformed DOR on the TE and nutrient mobility in soil. They found that the mobility of TE significantly decreased, Ca and Mg mobility increased, and soil pH was the main factor affecting the mobile proportions of elements in the treated soils. Although the changes of the Ca and Mg mobility were not proven by this study, the role of pH was confirmed in this case. These statements are supported by the results of the multivariate analysis of the data. These results confirmed the predominant role of soil TE/nutrient contents in the differences in mobile proportions of these elements and the effect of pyrolysis temperature of the DOR biochar rather than the DOR biochar rate in interaction with these soils, as expected. However, AMF inoculation affected the TE/nutrient mobility regardless of the biochar application, where no mutual effect of these treatments was indicated by the multivariate analysis.

As apparent from Fig. 2, commonly reported benefits of biochar application, such as an increase in soil pH and a higher availability of macronutrients, micronutrients, and soil water (Brantley et al. 2016; Khan et al. 2017; Fischer et al. 2019), were not the influential factors increasing the crop yield in our investigation. Moreover, the results did not indicate any unambiguous changes of the plant yield as compared with those of the control (Fig. 2) or as compared with other risk element uptake–reducing amendments (Maghsoudi et al. 2019). Rajkovich et al. (2012) and Kloss et al. (2014b) discussed higher N immobilisation, salinity, mutual nutrient interactions, and micronutrient deficiency or toxicity as potential causes of reduced crop yield after biochar amendment. Both research groups have shown that the extent of plant growth regulation depends on the plant species, soil type, and the origin and rate of the biochar.

As indicated by Tables 3, 4, 5, 6, 7 and 8, the increased contents of nutrients in wheat plants grown on the biochar-treated soils were observed for K, due to the high loads of this element via application of the K-rich biochar, and also P. The soil pH values were ≤ 7 in all treatments, implying that

production of phytic acid salts, the major storage form of P in wheat grain, should be taken into account in this case. The order of contents $K^+ > Mg^{2+} > Ca^{2+}$ determined in phytate globoids isolated from wheat bran (Bohn et al. 2007) may help to explain the observed Ca, Mg, and K contents in the wheat grain grown in the "high" soil (Tables 7 and 8), but this statement remains speculative at the present time.

Unfortunately, biochar's feedstock, processing, and application rate (Namgay et al. 2010; Hmid et al. 2015; Ibrahim et al. 2017; Xiao et al. 2019), soil physicochemical parameters (Kloss et al. 2014a; Rees et al. 2015; Xiao et al. 2019), TE of interest along with its total level in soil (Namgay et al. 2010; Xiao et al. 2019), plant species, and compartment under investigation (Rees et al. 2015; Ibrahim et al. 2017) are the influential factors that make each plant growth experiment and its conclusions unique and difficult to generalise. Significant reduction of the Cd, Pb, Zn, and As contents was reported in various crops cultivated in the biochar-treated soils. The significant effects were observed in the soils with TE contents similar to our "low" (Ibrahim et al. 2017), "medium" (Hmid et al. 2015), and even "high" (Rees et al. 2015) soils. The extraordinary increase in Cd uptake and translocation to wheat grain at the soil "high" (Tables 7 and 8) is likely related to the almost 10-fold and 20-fold higher pseudo-total Cd contents in the soil as compared with the "low" and "medium" soil, respectively. Both cations and anions could be released from biochar to interact with soil particles in Cd-contaminated soils (e.g. K^+, through cation exchange for Cd^{2+}, and Cl^-, through the formation of aqueous complexes with Cd^{2+}), thus impacting Cd plant availability.

Successful soil remediation using biochar may be conditionally limited to only a few elements, as observed by Namgay et al. (2010). These researchers investigated the effect of wood BC550 applied at 1.5% (w/w) to an artificially contaminated slightly acidic sandy soil on the availability of TE to maize (*Zea mays*). The contents of Cd and As in the shoots were significantly reduced (by approximately 60 and 30%, respectively), but the protection of shoot biomass against Pb and Zn uptake was inconsistent and prevailingly unsatisfactory. Xiao et al. (2019) treated smelter-contaminated soils with multiple BC500 at 2.5% (w/w) produced from plant straw of different origins. The achieved low remediation ability for Cd and Zn was assigned to the alkaline soil pH and the severely contaminated status of both experimental soils. When the pyrolysis temperature was modified (BC300 and BC700), the TE content in ryegrass (*Lolium multiflorum*) shoots varied depending on the soil and element, where BC300 had a lower remediation potential.

As for pyrolysis temperature, Zhang et al. (2016) reported that the TE content in *Lolium multiflorum* was frequently at its minimum after various low-temperature crop residue biochars had been applied to a low-polluted soil at 2.5% (w/w). The decrease in Cd and Zn shoot content approached 70 and 90%, respectively, when BC250 was added. In our study, examination of the results of a 4-way ANOVA indicated no significant impact of the pyrolysis temperature on the TE contents in wheat for the majority of the TE. The same conclusion can be drawn for the biochar application rate. Instead, the level of soil contamination and plant tissue under investigation were the predominant factors affecting the wheat TE contents, but significant interactions of these factors with biochar rate and pyrolysis temperature were also determined. Limited interactions of the AMF inoculation and biochar application were obtained as well. The importance of the change in soil pH induced by the DOR biochar can be documented by significant ($p < 0.05$) correlation coefficients varying between $r = -0.37$ and -0.64 (data not shown). Moreover, increasing K contents in the soil can result in the reduction of plant-available TE pool, as presented by Chen et al. (2007). The negative significant ($p < 0.05$) correlation between the TE and K contents in the plants (r values between -0.36 and -0.51) indicated that the K content in the biochar belongs to the factors that are able to alter the TE availability in soils within this investigation.

5 Conclusions

The application of dry olive residue (DOR) biochar to smelter-contaminated soils hardly impacted the amount of chemically extractable (0.11 mol L^{-1} acetic acid) nutrients (Ca, Mg, and P) and trace elements (TE) such as As, Cd, Pb, and Zn, but it significantly changed the nutrient and TE availability to wheat plants. We often failed to demonstrate the biochar pyrolysis temperature and application rate as the major variables that consistently affect the TE immobilisation and content in wheat plants, especially when all experimental soils were evaluated together. Moreover, the arbuscular mycorrhizal fungi (AMF) inoculation affected the mobility of TE and nutrients, but did not show any ability to affect the biochar-derived changes. Therefore, any potential mutual effect of the biochar application and AMF inoculation was not observed in this experiment. Despite the DOR biochars supplying the soils with K, P, Ca, and Mg for wheat growth, an increase in the grain yield was scarcely observed most probably due to sufficient nutrient supply of the experimental soils. Thus, the potential increase of the nutrient status of the crops was apparent only in the case of the extremely high contents of K in the biochar due to the high content of this element in the feedstock (DOR). The inputs of the nutrients to soils could secondarily alter the mobility and plant availability of TE, thus further complicating the prediction of biochar remediation performance. Although the application of DOR biochar to the "low" and "medium" contaminated soils efficiently decreased the contents of TE in wheat grain, Cd and Pb contents still frequently exceeded the maximum level (0.2 mg kg^{-1} in wet weight), forbidding the

use of the grain as a food ingredient (ES 2006). In addition to a considerably lower remediation efficiency achieved in the "high" contaminated soil, we noticed that biochar treatments of this soil may substantially promote undesirable translocation of Cd to the grain. This finding emphasises the importance of chemical speciation and geochemical fractionation of TE in a particular soil. Rather than increasing the application rates, further studies could examine the use of the DOR biochar combined with other amendments.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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