



Combined effects of carbonaceous-immobilizing agents and subsequent sulphur application on maize phytoextraction efficiency in highly contaminated soil

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Abstract

The establishment of phytoextraction crops on highly contaminated soils can be limited by metal toxicity. A recent proposal has suggested establishing support crops during the critical initial phase by metal immobilization through soil amendments followed by subsequent mobilization using elemental sulphur to enhance phytoextraction efficiency. This ‘combined phytoremediation’ approach is tested for the first time in a pot experiment with a highly contaminated soil. During a 14-week period, relatively metal-tolerant maize was grown in a greenhouse under immobilization (before sulphur (S) application) and mobilization (after S application) conditions with soil containing Cd, Pb and Zn contaminants. Apart from the control (C) sample, the soil was amended with activated carbon (AC), lignite (Lig) or vermicompost (VC) all in two different doses (dose 1~45 g additive kg⁻¹ soil and dose 2~90 g additive kg⁻¹ soil). Elemental S was added as a mobilization agent in these samples after 9 weeks. Biomass production, nutrient and metal bioavailability in the soil were determined, along with their uptake by plants and the resulting remediation factors. Before S application, Cd and Zn mobility was reduced in all the AC, Lig and VC treatments, while Pb mobility was increased only in the Lig1 and VC1 treatments. Upon sulphur application, Fe, Mn, Cd, Pb and Zn mobility was not significantly affected in the C, AC and VC treatments, nor total Cd, Pb and Zn contents in maize shoots. Increased sulphate, Mn, Cd, Pb and Zn mobilities in soil together with related higher total S, Mn, Pb and Zn contents in shoots were observed in investigated treatments in the last sampling period. The highest biomass production and the lowest metal toxicity were seen in the VC treatments. These results were associated with effective metal immobilization and showed the trend of steady release of some nutrients. The highest remediation factors and total elemental content in maize shoots were recorded in the VC treatments. This increased phytoremediation efficiency by 400% for Cd and by 100% for Zn compared to the control. Considering the extreme metal load of the soil, it might be interesting to use highly metal-tolerant plants in future research. Future investigations could also explore the effect of carbonaceous additives on S oxidation, focusing on the specific microorganisms and redox reactions in the soil. In addition, the homogeneous distribution of the S rate in the soil should be considered, as well as longer observation times.

Keywords Activated carbon · Lignite · Heavy metals · Vermicompost · *Zea mays*

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Introduction

Highly metal(loid)-contaminated soils are a global issue and lead to the poor and irregular development of vegetation or even the total disappearance of plant cover on affected sites (Gray et al. 2006). Although novel, gentle technologies for plant microbe-based bioremediation (phytoremediation) and in situ immobilization using soil amendments have been developed and widely tested on slightly to moderately contaminated soils (Mench et al. 2009; Vangronsveld et al. 2009), there is little information concerning their performance at extremely high, toxic metal levels. Remedial action at such sites

faces a number of problems, including enhanced metal leaching from the root zone or deeper soil layers and problems with crop establishment due to metal toxicity (Nagajyoti et al. 2010; Iqbal et al. 2012). In situ immobilization is only a temporary solution; i.e. contaminants still remain in the environment (Wuana and Okieimen 2011).

Recently, a novel soil remediation option combining enhanced phytoextraction with prior in situ immobilization of metals using immobilization agents has been proposed (Iqbal et al. 2012). Initial metal(loid) immobilization helps reduce the metal(loid) mobility and toxicity to plants, as well as leaching metal(loid)s from the root zone and thus facilitates the development of seedlings or cuttings of the metal-accumulating crops, mainly during the critical initial growth stages. Since the immobilizing agents are expected to limit phytoextraction, it has been suggested they support the phytoextraction process by the subsequent controlled addition of metal-mobilizing agents. Easily biodegraded chelating agents, including naturally occurring substances, come to the fore in enhanced phytoextraction (Iqbal et al. 2012; Ali et al. 2013).

Some studies have tested the feasibility of using acidifying compounds such as elemental sulphur (Wang et al. 2007; Iqbal et al. 2012; Amoakwah et al. 2014; Hoefler et al. 2015). The chemical fate of sulphur in soil (oxidation or reduction) depends on many factors, such as the presence of oxygen and its content (Edwards 1998). The application of elemental sulphur induces slow and steady soil acidification, mainly confined to the rhizosphere, which is considered to limit leaching while rendering metal contaminants available to phytoextraction (Iqbal et al. 2012). Under aerobic conditions, this effect is ascribed to the oxidation of sulphur to sulphuric acid by *Thiobacillus* bacteria domiciled in the soil matrix and thus a subsequent decrease in soil pH through the release of SO_4^{2-} and H^+ ions (Van Ranst 2006). Under occasional anaerobic conditions, S-oxidizing bacteria may use Mn and Fe oxides as terminal electron acceptors. This leads to a reduction and dissolution of these oxides and greatly contributes to metal desorption (Iqbal et al. 2012). Under anaerobic conditions, S is reduced mainly to hydrogen sulphide and these species can be quickly volatilized, reoxidized to elemental S or precipitated with metals (Edwards 1998). Wenger et al. (2002) described the duration (i.e. a range of 4 weeks to 7 weeks) required for the sulphur-containing amendments to mobilize substantial amounts of the metals investigated. Several authors (Cui et al. 2004; Iqbal et al. 2012; Hoefler et al. 2015; Azeez et al. 2016) have verified the efficiency of elemental sulphur application on metal-tolerant plants, for example maize, sunflower or willow, and confirmed an increased plant uptake of the metals.

Following on from the experiments of Iqbal et al. (2012) and Hoefler et al. (2015), our study tests the feasibility and efficiency of sulphur treatments following immobilization of

Cd, Zn and Pb in a highly contaminated soil. Whereas in previous work immobilization was achieved using oxidic (red mud) and silicatic (gravel sludge) additives, the focus here is on carbonaceous materials, namely activated carbon (Břendová et al. 2016; Zemanová et al. 2017), lignite (Havelcová et al. 2009; Uzinger et al. 2014) and vermicompost (Jadia and Fulekar 2008; Hoehne et al. 2016). The aim is metal stabilization/immobilization via processes such as adsorption, cation exchange, surface complexation and/or transformation on the sorbent surface (Kumpiene et al. 2008; Tica et al. 2011), as well as providing nutrients to support plant growth in the contaminated soil.

The objectives of this research are (i) to test the efficiency of the combined immobilization/mobilization technology (i.e. before and after S application) using relatively metal-tolerant maize in pot conditions and (ii) to investigate the solubility of bioavailable nutrients (P, S) and metals (Mn, Fe, Cd, Pb, Zn), maize growth, element uptake by the maize and the resulting remediation factors and efficiencies of the different treatments.

Materials and methods

Experimental design

The experimental soil was sampled from the topsoil of a Gleyic Fluvisol (FAO 2014), long-term, highly Cd/Pb/Zn-contaminated soil (54 mg Cd kg^{-1} , 3305 mg Pb kg^{-1} , 6172 mg Zn kg^{-1} as pseudo-total concentrations extracted with aqua regia) from the alluvium of the Litavka River. Anthropogenic soil contamination originates from two main sources: metallurgical industry emission outputs and floods of contaminated water from metallurgical setting pits, the barriers of which have repeatedly been damaged (Borůvka and Vácha 2006). The specific characteristics of the experimental soil are given in Vondráčková et al. (2014).

Four kilograms of air-dried and sieved (≤ 2 mm) soil in 5-L plastic double pots (each inner pot with three holes, 20 cm in diameter and depth) was mixed with nutrients at the rate of 0.1 g N kg^{-1} (NH_4NO_3), 0.032 g P kg^{-1} and 0.08 g K kg^{-1} (both in the form of K_2HPO_4) of soil. After 1 month of plant growth, a supplementary application of nutrient solution containing only nitrogen and phosphorous in the same forms and rates as in the initial application was repeated due to visible deficiencies. Another potassium application was omitted to prevent further cation mobilization. The immobilizing agents (applied in two different doses: dose 1 (~45 g additive per kg of soil) and dose 2 (~90 g additive per kg of soil)), including activated carbon (Erspol Ltd., Czech Republic), lignite (Mibrag GmbH, Germany) and vermicompost (Oekovermes, Germany), were sieved (≤ 2 mm) without any drying and thoroughly mixed with the soil immediately after the first

application of nutrient solution. The agents and characteristics were as follows: activated carbon from coconut shells ($\text{pH}_{\text{H}_2\text{O}} = 7.6$, 647 g C kg^{-1} , 4.3 g N kg^{-1} , 1.6 g S kg^{-1} , $8.3 \text{ mg Zn kg}^{-1}$), lignite from sedimented organic substances ($\text{pH}_{\text{H}_2\text{O}} = 3.4$, 629 g C kg^{-1} , 5.3 g N kg^{-1} , 28.7 g S kg^{-1} , $2.8 \text{ mg Zn kg}^{-1}$) and vermicompost from horse manure ($\text{pH}_{\text{H}_2\text{O}} = 5.5$, 247 g C kg^{-1} , 23.3 g N kg^{-1} , 11.2 g S kg^{-1} , $231 \text{ mg Zn kg}^{-1}$). Total Cd and Pb concentrations of the additives were below the limit of detection ($< 0.1 \text{ mg kg}^{-1}$ and 2 mg kg^{-1} , respectively).

High-yielding *Zea mays* (variety Colisee, KWS Saat AG), with a known potential for phytoremediation (Mojiri 2011), was seeded (five individuals per pot) immediately after the initial application of nutrient solution and immobilizing agents. Only 3 maize plants of similar height were left after 10 days of growth. In the vicinity of the roots, 10-cm-long Rhizon samplers (Rhizosphere Research Products, Wageningen, Netherlands) were inserted at a soil depth of 10 cm into individual pots to collect soil pore water (i.e. soil solution).

Nine weeks after sowing, elemental sulphur (Central Chem, Prague, Czech Republic) was applied as a mobilization agent to the soil in all pots at a rate of 0.5 g S kg^{-1} . The S application was very difficult due to its low aqueous solubility. Therefore, a suspension of S and demineralized water was applied via five holes drilled into the uppermost 7 cm of each pot. All soil treatments (untreated control soil with maize plants (C), activated carbon (AC), lignite (Lig) and vermicompost (VC) at dose 1 ($\sim 45 \text{ g}$ additive per kg of soil) and dose 2 ($\sim 90 \text{ g}$ additive per kg of soil)) were replicated 9 times for a total of 63 pots. These high rates were necessary to alleviate the extreme Cd, Pb and Zn concentrations of the Litavka soil.

The plants were grown for 14 weeks in an outdoor university department vegetation hall in Prague–Suchbát under natural temperature and light conditions. Plants were harvested at several intervals (always with three replications)—after 9 weeks (before S application), 12 weeks (19 days after S application) and finally 14 weeks (33 days after S application)—and were separated into roots and shoots. Concurrently, soil samples (approximately 30 g of soil taken from the top 10 cm of soil) were collected at 3 weeks, 9 weeks (before S), 12 weeks (19 days after S) and 14 weeks (33 days after S) after planting and soil solutions after 9 weeks (before S) and 12 weeks (19 days after S) using Rhizons. Soil leachates were collected from the bottom of the outer pot after 14 weeks, i.e. 33 days after S application.

Amendments and soil and plant analysis

The pH (EN 13037 1999) of the amendments was measured, and elemental analysis of C, N and S was undertaken using a Thermo Scientific Flash 2000 NCS Analyser (Germany).

Total elemental (Cd, Pb, Zn) concentrations in the amendments were determined by neutron activation analysis (Kubešová and Kučera 2012).

Soil material was collected from each pot, air-dried and analysed for pH in a 1:5 (*w/v*) suspension of soil and 0.01 M CaCl_2 after 1 h of shaking (ISO 10390 2011). Concurrently, the plant-available elements (P, Fe, Mn, Cd, Pb, Zn) and major inorganic (NO_3^- and SO_4^{2-}) and organic (oxalate) soil anions were measured. The measurements were performed after extraction with 0.01 M CaCl_2 (soil:solution ratio 1:10, 6 h shaking; Tlustoš et al. 1994) and determined using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Varian Vista Pro, Varian, Australia) for elements or by means of an ICS 90 ion chromatograph equipped with IonPac AS14A (Dionex, USA) for anions, respectively. For further details of this method, see Tejnecký et al. (2013). After collection, the soil solutions and leachates were stored in vials, immediately cooled (4°C) and analysed within 12 h for pH, elemental concentrations and major inorganic and organic anion concentrations using the same procedure as for the soil samples. The soil samples were analysed 3 weeks after maize sowing to determine nutrient and toxic metal bioavailability. To determine dissolved organic carbon (DOC) and N form (total, NO_3^- , NH_4^+), analysis was undertaken using a segmented flow analyser (Skalar San++ system, Netherlands). Plant-available and exchangeable fractions of selected elements were analysed using ICP-OES (for P, Cd, Pb and Zn) and flame atomic absorption spectroscopy (FAAS; Varian SpectrAA-280, Australia; for K, Mg) according to the extraction methods of Tlustoš et al. (1994; plant-available fraction) and of Pierzynski (2000; exchangeable fractions, for P).

All plant materials were harvested and dried in an oven at 60°C to constant weight on sampling dates (i.e. before S application, 19 days and 33 days after S), and the dry weight was recorded before grinding in a stainless steel mill. The roots were washed in an ultrasonic-assisted bath filled with deionized water to remove any soil particles adhering to the roots (Elmasonic S30, Elma Ultrasonic Technology). The total elemental concentrations (P, S, Fe, Mn, Cd, Pb, Zn) in plant parts (roots and shoots) were determined by ICP-OES after microwave-assisted high-pressure acid digestion (65% HNO_3 :30% H_2O_2 4:1, Ethos 1, MLS GmbH, Germany). Certified reference material (CTA-OTL-1 oriental tobacco leaves) was used for quality control. The remediation factor (RF, %) was calculated as the ratio between the total metal content in a plant (g per pot) and the total metal content in soil (g per pot) multiplied by 100.

Data analysis

All statistical analyses were performed using Statistica 12.0 software (www.statsoft.com). All data were checked for normality and homogeneity of variance (Shapiro–Wilk and

Table 1 Dissolved organic carbon (DOC), dissolved organic nitrogen (DON), nutrient and toxic element bioavailability (mg kg^{-1} , mean \pm standard error of the mean (SEM), $n = 3$) in soil 3 weeks after maize sowing

Treatment	DOC	Total DON	NO_3^-	NH_4^+	P	Mg	K	Cd	Pb	Zn
C	71 \pm 8a	1621 \pm 312a	1542 \pm 299a	13.1 \pm 1.5a	23 \pm 2b	222 \pm 35a	317 \pm 19b	9.1 \pm 1.3a	< 0.2b	379 \pm 59a
AC1	20 \pm 4d	261 \pm 43b	267 \pm 37b	4.9 \pm 0.3b	26 \pm 1b	128 \pm 8b	158 \pm 3 cd	4.3 \pm 0.3bc	< 0.2b	135 \pm 12bc
AC2	10 \pm 1d	115 \pm 34b	104 \pm 32b	2.6 \pm 0.3b	28 \pm 3b	114 \pm 9b	143 \pm 37d	2.4 \pm 0.5c	< 0.2b	62 \pm 17c
Lig1	19 \pm 4d	146 \pm 2b	142 \pm 2b	2.3 \pm 0.4b	24 \pm 1b	103 \pm 6b	102 \pm 8d	5.0 \pm 0.3bc	< 0.2b	240 \pm 10b
Lig2	21 \pm 2 cd	59 \pm 16b	51 \pm 14b	1.9 \pm 0.2b	23 \pm 1b	127 \pm 5b	69 \pm 4d	3.8 \pm 0.1bc	< 0.2b	209 \pm 5b
VC1	43 \pm 3bc	171 \pm 39b	161 \pm 43b	3.9 \pm 1.5b	57 \pm 8ab	173 \pm 15ab	276 \pm 34bc	5.6 \pm 0.4b	< 0.2b	261 \pm 16ab
VC2	55 \pm 7ab	561 \pm 3b	537 \pm 7b	4.0 \pm 0.1b	86 \pm 19a	257 \pm 24a	497 \pm 48a	5.2 \pm 0.4bc	0.3 \pm 0.02a	254 \pm 24ab

Significant differences among the treatment means at $p < 0.05$ are indicated with letters. Extraction method: Olsen P, CaCl_2 and the others; detection limit: Pb, 0.2 mg kg^{-1}

C untreated control soil, AC1 activated carbon in dose 1, AC2 activated carbon in dose 2, Lig1 lignite in dose 1, Lig2 lignite in dose 2, VC1 vermicompost in dose 1, VC2 vermicompost in dose 2

Levene tests). A one-way analysis of variance (ANOVA), followed by a Tukey HSD test ($\alpha = 0.05$), was used to identify significant differences among treatments before S application and after S application for soil and plant data. The relationship between selected data was analysed by linear regression using Pearson coefficients.

Results and discussion

Nutrient and toxic metal bioavailability after maize sowing

Three weeks after sowing, the AC and Lig treatments showed the lowest CaCl_2 -extractable concentrations of DOC, NO_3^- , NH_4^+ , Mg, K and Zn (Table 1). This can be attributed to the adsorption and/or complexation of cations to the large specific surface of AC and Lig (Qi et al. 2011; Břendová et al. 2016). The highest concentrations of P and K were recorded in the VC2 treatment, which can be explained by the release of nutrients from vermicompost (our measured nutrient contents in vermicompost were 12.5 g K kg^{-1} and 11 g P kg^{-1} ; Uz et al.

2016) and by the release of Pb from dissolved organic matter in the soil (Jordan et al. 1997). According to Pierzynski (2000), 10 mg P kg^{-1} (Olsen’s method, i.e. extraction with 0.5 M NaHCO_3 and then adjustment of the pH to 8.5) can be considered the optimum for plants grown in agricultural soils. The lowest measured extractable P concentration was 23 mg kg^{-1} in the C and Lig2 treatments but did not indicate a low P availability for plants. However, this result was in contrast to the reddish colour of maize leaves in the C, AC and Lig treatments, indicating P deficiency (Nagajyoti et al. 2010). This inconsistency was probably related to the high level of soil contamination (i.e. the Olsen extraction method can overestimate plant-available P because metal phosphate formation may be blocked by the alkaline pH associated with this method). Higher NO_3^- concentrations compared to NH_4^+ supported the nitrification activity, as expected in aerated soil.

Effects of immobilization agents and sulphur on soil pH

The soil pH, measured with both methods (CaCl_2 extraction and soil solution obtained by suction cups), followed a similar

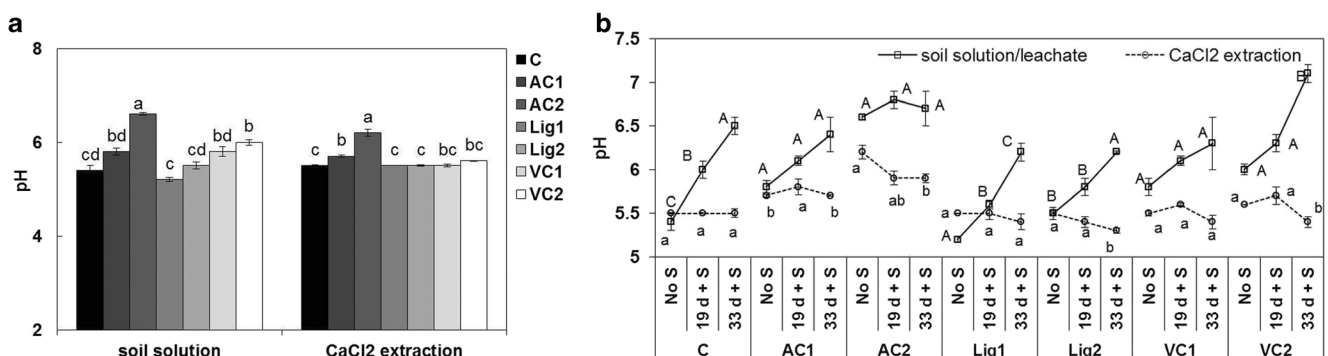


Fig. 1 Soil solution and extracted CaCl_2 pH (mean \pm SEM, $n = 3$) **a** before S application and **b** after S application. Significant differences among the treatment and sulphur application means at $p < 0.05$ are

indicated with letters. Soil leachate applies only for day 33. See Table 1 for more details about the treatments

Table 2 CaCl₂-extractable element concentration (mg kg⁻¹, mean ± SEM, *n* = 3) for the different treatments and periods

Element	Treatment	Before S application	After S application	
			19 days	33 days
P	C	3.9 ± 0.2cA	3.7 ± 0.3A	4.4 ± 0.0A
	AC1	6.6 ± 0.5abA	3.6 ± 0.1B	3.9 ± 0.4B
	AC2	3.0 ± 0.05cB	4.1 ± 0.1A	4.2 ± 0.3A
	Lig1	4.3 ± 0.3bcA	3.6 ± 0.1A	3.8 ± 0.3A
	Lig2	4.0 ± 0.2bcB	3.3 ± 0.04AB	5.0 ± 0.6A
	VC1	7.6 ± 1.2aA	5.4 ± 0.4A	6.8 ± 0.4A
	VC2	8.0 ± 0.05aB	8.7 ± 0.1AB	10.0 ± 0.5A
Fe	C	4.3 ± 0.02cdA	4.0 ± 0.3A	6.6 ± 1.3A
	AC1	5.4 ± 0.4bcdA	4.5 ± 0.2A	5.5 ± 0.3A
	AC2	4.2 ± 0.3dA	4.1 ± 0.2A	7.2 ± 2.3A
	Lig1	5.7 ± 0.2bcA	5.7 ± 0.5A	5.5 ± 0.2A
	Lig2	5.3 ± 0.5bcdA	6.0 ± 0.4A	5.9 ± 0.1A
	VC1	9.6 ± 0.3aA	6.8 ± 0.3A	9.0 ± 1.3A
	VC2	6.1 ± 0.2bA	6.9 ± 0.5A	5.9 ± 0.6A
Mn	C	35.1 ± 0.8aA	23.6 ± 1.4B	9.7 ± 1.7C
	AC1	20.7 ± 0.5dA	9.0 ± 0.4B	11.6 ± 4.3AB
	AC2	6.3 ± 0.5eA	7.0 ± 0.4A	9.7 ± 2.9A
	Lig1	24.4 ± 0.8cAB	22.6 ± 0.04B	33.6 ± 4.2A
	Lig2	29.3 ± 0.4bB	28.8 ± 3.2B	52.0 ± 4.9A
	VC1	24.9 ± 0.4cA	21.4 ± 0.8A	47.5 ± 17.8A
	VC2	25.7 ± 0.4cA	22.7 ± 0.8A	25.7 ± 1.7A
Cd	C	10.0 ± 0.02aA	9.9 ± 0.1A	10.0 ± 0.6A
	AC1	6.6 ± 0.2bcA	5.2 ± 0.2A	7.7 ± 1.1A
	AC2	2.9 ± 0.1eB	4.1 ± 0.2AB	5.3 ± 0.6A
	Lig1	6.2 ± 0.3cB	6.3 ± 0.3B	8.4 ± 0.4A
	Lig2	4.5 ± 0.001 dB	5.2 ± 0.2B	6.8 ± 0.3A
	VC1	7.4 ± 0.4bA	7.2 ± 0.4A	9.9 ± 2.2A
	VC2	5.6 ± 0.05cA	3.6 ± 0.2B	6.7 ± 0.5A
Pb	C	1.1 ± 0.01cA	1.2 ± 0.03A	1.2 ± 0.2A
	AC1	0.9 ± 0.06dAB	0.7 ± 0.04B	1.1 ± 0.1A
	AC2	0.5 ± 0.02eA	0.6 ± 0.03A	1.0 ± 0.3A
	Lig1	1.3 ± 0.01bA	1.2 ± 0.05A	1.5 ± 0.2A
	Lig2	1.1 ± 0.04cC	1.4 ± 0.04B	1.7 ± 0.1A
	VC1	1.6 ± 0.02aA	1.3 ± 0.02A	2.2 ± 0.5A
	VC2	1.1 ± 0.02cB	1.1 ± 0.04B	1.4 ± 0.1A
Zn	C	507 ± 2aA	534 ± 19A	505 ± 64A
	AC1	291 ± 14cdA	200 ± 12A	363 ± 103A
	AC2	79 ± 5eB	148 ± 13.5AB	225 ± 39A
	Lig1	328 ± 22cB	339 ± 7B	580 ± 42A
	Lig2	272 ± 6 dB	318 ± 10B	553 ± 29A
	VC1	388 ± 10bA	442 ± 68A	680 ± 185A
	VC2	292 ± 4cdB	263 ± 9B	420 ± 37A

Significant differences among the treatment (before S application, indicated by lowercase letters) and sulphur application (after S application, indicated by uppercase letters) means at $p < 0.05$ are indicated. See Table 1 for more details about the treatments

pattern of changes before S application (Fig. 1a). The pH_{CaCl₂}/soil solution increased from 5.5/5.4 in the control to 6.2/6.6

in the AC treatments. This increase can be attributed to the alkalinity of activated carbon and a corresponding liming

Table 3 CaCl₂-extractable inorganic and organic anion concentration (mg kg⁻¹, mean ± SEM, *n* = 3) for the different treatments and periods

Element	Treatment	Before S application	After S application	
			19 days	33 days
SO ₄ ²⁻	C	401 ± 44aA	405 ± 7A	574 ± 209A
	AC1	489 ± 188aA	415 ± 26A	674 ± 280A
	AC2	466 ± 17aA	876 ± 27A	1215 ± 308A
	Lig1	145 ± 21aB	442 ± 78B	1022 ± 126A
	Lig2	331 ± 78aB	934 ± 4B	1772 ± 265A
	VC1	181 ± 25aA	458 ± 35A	1499 ± 602A
	VC2	371 ± 5aA	492 ± 45A	936 ± 333A
	Oxalate	C	48 ± 2.5aB	49 ± 3B
AC1		33 ± 3bA	30 ± 1A	38 ± 2A
AC2		25 ± 1cB	29 ± 0.4A	26 ± 1B
Lig1		18 ± 1cdA	18 ± 0.5A	21 ± 0.1A
Lig2		16 ± 1dA	15 ± 1A	14 ± 2A
VC1		14 ± 1dA	15 ± 0.3A	27 ± 10A
VC2		13 ± 0.5dA	13 ± 0.4A	15 ± 1A
NO ₃ ⁻		C	755 ± 27aA	722 ± 10A
	AC1	802 ± 144aA	391 ± 5B	567 ± 46AB
	AC2	550 ± 21abA	446 ± 28A	321 ± 27B
	Lig1	210 ± 49bcB	462 ± 57AB	469 ± 68A
	Lig2	378 ± 66bcA	516 ± 68A	815 ± 194A
	VC1	145 ± 1cB	580 ± 99A	758 ± 58A
	VC2	340 ± 96bcA	423 ± 35A	618 ± 222A

Significant differences among the treatment (before S application, indicated by lowercase letters) and sulphur application (after S application, indicated by uppercase letters) means at *p* < 0.05 are indicated. See Table 1 for more details about the treatments

effect (Břendová et al. 2016). In the remaining treatments (except VC2 in soil solution), soil pH did not change. Before S application, there was no difference between the pH measurement in CaCl₂ and soil solution extraction as observed previously by Amoakwah et al. (2013).

In general, only a few changes in pH_{CaCl₂} were recorded between before and after S application (Fig. 1b). This is supported by the linear regression results for CaCl₂-extractable metal concentrations (Cd, Pb, Zn) and pH before and after S application (Cd: before S, *r* = -0.62, *p* < 0.01; 33 days after S, *r* = -0.49, *p* = 0.02; Pb: before S, *r* = -0.79, *p* < 0.01; 33 days after S, *r* = -0.49, *p* = 0.03; Zn: before S, *r* = -0.78, *p* < 0.01; 33 days after S, *r* = -0.63, *p* < 0.01). These results contrast with those of other studies (Iqbal et al. 2012; Hoefler et al. 2015) in which S application had strong effects on the correlation between pH and metals. Therefore, we can speculate that pH did not decrease after S application in the whole profile (pH of soil solution) but only in the top 10 cm of soil (pH_{CaCl₂}) at the end of the experiment. The S oxidation was limited in most treatments after the application in the pot experiment. However, significant increases in soil solution pH recorded after S application were also related to the absence of an acidifying rhizosphere effect because the last collection

was extracted from soil leachate (i.e. bulk soil) and not from soil solution (i.e. root zone). Similar results were observed in the study of Azimzadeh et al. (2016).

Effects of immobilization agents and sulphur on nutrient in soil and toxic metal soil mobility

More substantial changes in CaCl₂-extractable nutrients and metals were recorded in soil samples compared to soil solution before and after S application (data for soil solution not shown). A tendency for higher CaCl₂-extractable concentrations of P and Fe was recorded in all treatments before S application (Table 2). The highest increase was recorded in VC treatments (i.e. 105% (VC2) and 123% (VC1) increase over control for P and Fe, respectively). Significantly higher CaCl₂-extractable Pb was recorded in the VC1 and Lig1 treatments compared to the control. A decrease of the DOC was found in all amendment treatments compared to the control, showing the stability of applied amendments. The highest immobilization for Mn, Cd, Pb and Zn in the AC treatments was caused by an alkaline pH, as demonstrated by the linear regression (Mn: *r* = -0.94, *p* < 0.01; Cd: *r* = -0.95, *p* < 0.01; Pb: *r* = -0.91, *p* < 0.01; Zn: *r* = -0.94, *p* < 0.01). This was

probably also caused by the corresponding metal adsorption to the large specific surface of activated carbon (Břendová et al. 2016). Before S application, the oxalate concentrations decreased considerably in all the amended treatments (Table 3). We speculate that the lower oxalate concentration is related to lower Zn and other metal toxicity (i.e. lower CaCl_2 -extractable metal concentrations from the soil solution) in response to the amendments. Therefore, the root exudation of oxalate, i.e. a potential detoxification mechanism due to the formation of metal complexes, was downregulated. Li et al. (2012) recorded a similar result, i.e. increased exudation of oxalic acids by Chinese cabbage grown in a Zn-polluted soil.

As mentioned above, only a few pH and metal concentration changes were recorded in CaCl_2 extracts after S application (Fig. 1b, Table 2). We can speculate that the S oxidation and therefore the metal mobilization (Fe, Mn, Cd, Pb, Zn) in the C, AC and VC treatments were inhibited. Generally, S oxidation is performed by numerous microbial species (Hagedorn 2010) within a wide pH range of 1.9 to 8.5, but it is also affected by various other environmental conditions. The inhomogeneity of S application in the pots, the S rate (higher S rate, i.e. 1.0 g kg^{-1} , with a more pronounced pH response and metal solubilization; Mohnke 2014; Hoefler et al. 2015), the use of organic immobilization agents (lowering the redox potential) and/or more time for observation are possible factors limiting S oxidation.

The CaCl_2 -extractable Mn, Cd, Pb, Zn and sulphate concentrations increased and $\text{pH}_{\text{CaCl}_2}$ decreased after S application in investigated treatments in the last sampling period (Fig. 1b, Tables 2 and 3). This may be also related to the S forms of amendments: inorganic forms easily accessed by microbes (mainly sulphate and pyritic S) and organic forms (bound to carbon and esters) greatly depend on the coal integrity, i.e. structure, and the S release from ester sulphates using extracellular enzymatic hydrolysis (Ruamsap and Akaracharanya 2002; Hagedorn 2010; Blum et al. 2013). It could also be linked to its preparation: the milling of amendments before mixing with soil

could have increased the microbial availability of the organic S form since its structure was destroyed (Ruamsap and Akaracharanya 2002). Blum et al. (2013) showed that microbial S transformation in soil is greatly affected by the composition of different S forms of the source material (i.e. amendments and soil). Therefore, we speculate that easily accessible S forms in Lig and, to a lesser extent, in other amendments functioned as a driver for S oxidation and thus stimulated S-oxidizing bacteria, probably before S was applied to the soil. This could explain the tendency for increasing CaCl_2 -extractable sulphate concentrations in the AC2, Lig and VC treatments, and no notable change was determined in the control after S addition (Table 3).

A tendency for decreasing CaCl_2 -extractable nitrate concentrations was only recorded in the C and AC2 treatments after S application, which did not correspond with low biomass yield and is in line with the findings of Hoefler et al. (2015). Hailegnaw et al. (2019) also found immobilization of soil nitrates due to decline of negatively charged groups at a high rate of high temperature-produced biochar. In contrast, the CaCl_2 -extractable nitrate concentration in the AC1, Lig and VC treatments increased after S application despite higher plant uptake due to the higher biomass production compared to the C and AC treatments. The VC and Lig applications may have influenced the N mineralization, leading to a sustained release from organic compounds. This agrees with the results of Murugan and Swarnam (2013), who found slow but steady N mineralization in vermicompost-treated soil. The oxalate concentrations significantly increased in the control treatment after S application, probably due to increasing root exudation of organic acids.

Effects of immobilization agents and sulphur on maize growth

Maize biomass increased for the treatments in the order of $C \approx AC \approx \text{Lig} < VC$ after S application (Fig. 2a). This is consistent with Tury et al. (2008), who reported positive

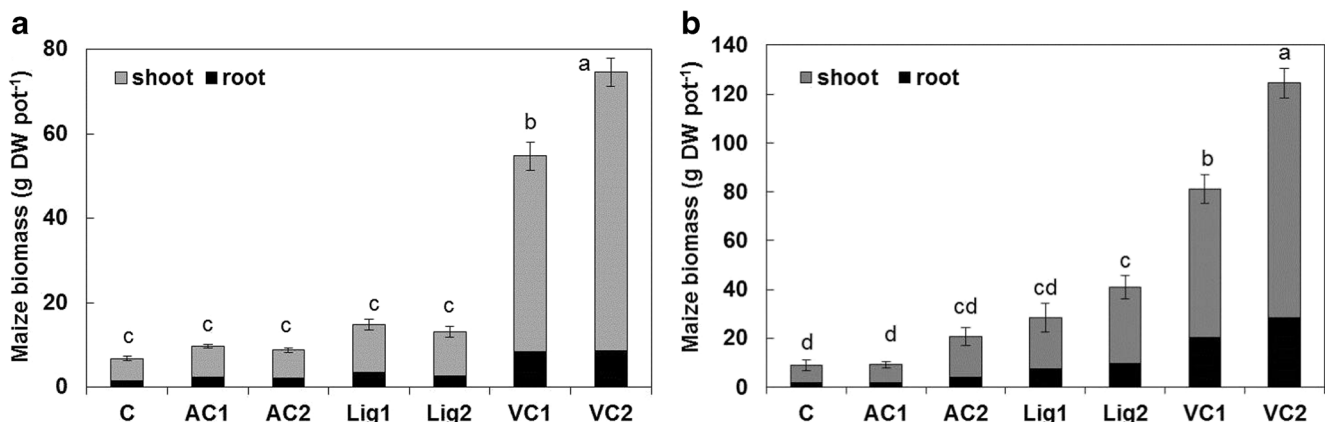


Fig. 2 Maize biomass (dry weight, DW) measured **a** before S application and **b** after S application. Significant differences among the treatment means at $p < 0.05$ are indicated with letters. Error bars indicate the standard error of the mean ($n = 3$). See Table 1 for more details about the treatments

Table 4 Element concentrations in maize roots (mg kg^{-1} , mean \pm SEM, $n = 3$) for the different treatments and periods

Element	Treatment	Before S application	After S application	
			19 days	33 days
P	C	546 \pm 9bA	477 \pm 79A	615 \pm 50A
	AC1	573 \pm 12bA	394 \pm 30B	430 \pm 32B
	AC2	467 \pm 43bA	422 \pm 9A	478 \pm 44A
	Lig1	482 \pm 30bA	595 \pm 34A	558 \pm 34A
	Lig2	539 \pm 23bA	344 \pm 148A	589 \pm 18A
	VC1	1113 \pm 202aA	750 \pm 105A	871 \pm 41A
S	VC2	586 \pm 46bC	842 \pm 11B	1046 \pm 21A
	C	4453 \pm 63aA	3527 \pm 271A	4292 \pm 250A
	AC1	5149 \pm 267aA	4780 \pm 39A	4270 \pm 252A
	AC2	4759 \pm 346aA	4481 \pm 12A	4682 \pm 1203A
	Lig1	4099 \pm 109aB	3502 \pm 397B	5744 \pm 242A
	Lig2	5094 \pm 5aA	3133 \pm 1546A	6582 \pm 624A
Fe	VC1	5058 \pm 353aA	4131 \pm 236A	4048 \pm 443A
	VC2	4490 \pm 129aA	3460 \pm 1114A	4875 \pm 257A
	C	1585 \pm 62bA	2975 \pm 415A	2687 \pm 1286A
	AC1	3127 \pm 533bA	4865 \pm 647A	3193 \pm 860A
	AC2	1241 \pm 88bA	2538 \pm 203A	6664 \pm 2455A
	Lig1	1154 \pm 59bA	12,250 \pm 5442A	10,652 \pm 2291A
Mn	Lig2	3425 \pm 1241bA	10,041 \pm 5118A	9905 \pm 3940A
	VC1	10,977 \pm 2031aA	7916 \pm 2981A	15,985 \pm 2912A
	VC2	2455 \pm 1022bB	877 \pm 344B	18,292 \pm 2621A
	C	99 \pm 2bA	300 \pm 11A	476 \pm 254A
	AC1	235 \pm 67bA	479 \pm 103A	330 \pm 98A
	AC2	141 \pm 43bA	216 \pm 28A	918 \pm 502A
Cd	Lig1	82 \pm 13bA	2053 \pm 847A	1669 \pm 89A
	Lig2	657 \pm 299abA	1556 \pm 726A	1603 \pm 613A
	VC1	1441 \pm 330aA	1618 \pm 745A	2810 \pm 571A
	VC2	212 \pm 92bB	80 \pm 21B	3201 \pm 593A
	C	193 \pm 14abA	176 \pm 26A	142 \pm 18A
	AC1	252 \pm 37aA	126 \pm 1B	131 \pm 16B
Pb	AC2	114 \pm 13bA	106 \pm 0.2A	113 \pm 15A
	Lig1	154 \pm 21bA	129 \pm 14AB	84 \pm 3B
	Lig2	104 \pm 14bA	40 \pm 17B	74 \pm 5AB
	VC1	192 \pm 13abA	195 \pm 28A	128 \pm 36A
	VC2	121 \pm 4bA	117 \pm 62A	128 \pm 6A
	C	248 \pm 6bA	365 \pm 51A	424 \pm 166A
Zn	AC1	368 \pm 69bA	570 \pm 81A	446 \pm 111A
	AC2	190 \pm 40bA	279 \pm 12A	888 \pm 421A
	Lig1	159 \pm 17bA	2034 \pm 915A	1496 \pm 216A
	Lig2	605 \pm 238bA	1430 \pm 719A	1479 \pm 567A
	VC1	1367 \pm 308aA	1304 \pm 533A	2305 \pm 317A
	VC2	267 \pm 105bB	109 \pm 47B	2598 \pm 406A
Zn	C	7067 \pm 141aA	6422 \pm 228AB	5802 \pm 149B
	AC1	4769 \pm 156bcA	4028 \pm 215AB	3374 \pm 439B
	AC2	1976 \pm 125 dB	2476 \pm 199AB	3154 \pm 369A
	Lig1	3673 \pm 523bcdA	4946 \pm 397A	4945 \pm 310A
	Lig2	3219 \pm 480cdA	2888 \pm 1366A	4077 \pm 838A
	VC1	5309 \pm 525bA	3485 \pm 503A	5394 \pm 229A
VC2	2377 \pm 276 dB	1602 \pm 609B	5407 \pm 331A	

Significant differences among the treatment (before S application, indicated by lowercase letters) and sulphur application (after S application, indicated by uppercase letters) means at $p < 0.05$ are indicated. See Table 1 for more details about the treatments

effects of compost on plant growth in contaminated soils. After S application, the final maize biomass was also significantly increased in the Lig2 treatment (Fig. 2b). Despite presenting the lowest concentrations of CaCl_2 -extractable Cd, Pb and Zn (Table 2), the AC treatments showed a substantially lower (up to 14 times) biomass production than the VC treatments. The NO_3^- immobilization was

only pronounced in the AC2 treatment. Therefore, we can conclude that the VC treatment—and to a lesser extent the Lig application—showed the trend of steady release of some nutrients, while in the AC treatment, sorption mechanisms limited the availability of nutrients, during the whole experimental period and thus limited plant growth.

Effects of immobilization agents and sulphur on total metal uptake and toxic metal remediation factors

Large amounts of metals (Mn, Fe, Cd, Pb, Zn) were taken up by the maize roots due to the high metal concentrations (both total and CaCl₂-extractable) in the Litavka soil (Table 4). Before S application, majority of treatments did not show

statistical differences in the root concentrations, higher values recorded for the VC1 treatment; could be caused by some impurity coming from soil; and were not determined in other samplings. Only the mobile elements like Zn and, up to some extent, Cd decreased root concentrations in treated soils before S application, presenting a stronger effect at treatments with higher rates of amendments. Generally, metal (Fe, Mn, Cd,

Table 5 Element concentrations in maize shoots (mg kg⁻¹, mean ± SEM, *n* = 3) for the different treatments and periods

Element	Treatment	Before S application	After S application	
			19 days	33 days
P	C	1046 ± 18cA	718 ± 28B	809 ± 100AB
	AC1	1095 ± 6bcA	666 ± 5B	591 ± 20C
	AC2	1176 ± 20bcA	781 ± 1.5A	797 ± 173A
	Lig1	1151 ± 8bcA	855 ± 1.5B	709 ± 43C
	Lig2	1172 ± 3bcA	848 ± 23B	653 ± 21C
	VC1	1221 ± 45bA	791 ± 11B	609 ± 16C
	VC2	1503 ± 72aA	836 ± 14B	642 ± 22C
S	C	2124 ± 6bcA	3576 ± 153A	5417 ± 1796A
	AC1	2269 ± 8bB	2690 ± 338AB	4931 ± 932A
	AC2	2840 ± 155aA	4441 ± 175A	3845 ± 1806A
	Lig1	1788 ± 87cdA	2190 ± 195A	3314 ± 684A
	Lig2	2022 ± 56bcdB	1908 ± 97B	2780 ± 165A
	VC1	1692 ± 14dAB	1887 ± 21A	1462 ± 100B
	VC2	1841 ± 72cdA	1425 ± 61B	1354 ± 50B
Fe	C	2317 ± 795aA	1600 ± 306A	4406 ± 2103A
	AC1	432 ± 70bA	555 ± 165A	1747 ± 641A
	AC2	638 ± 77bA	830 ± 83A	731 ± 471A
	Lig1	338 ± 14bA	491 ± 137A	508 ± 158A
	Lig2	268 ± 31bA	280 ± 16A	439 ± 91A
	VC1	381 ± 12bA	373 ± 1A	157 ± 18B
	VC2	324 ± 9bAB	576 ± 171A	140 ± 22B
Mn	C	405 ± 144aA	286 ± 37A	821 ± 515A
	AC1	42 ± 6bA	56 ± 17A	239 ± 80A
	AC2	56 ± 9bA	137 ± 27A	101 ± 68A
	Lig1	33 ± 3bA	35 ± 1A	89 ± 26A
	Lig2	37 ± 3bB	30 ± 1B	70 ± 9A
	VC1	35 ± 1bB	46 ± 0.4A	24 ± 2C
	VC2	50 ± 4bA	41 ± 2AB	34 ± 4B
Cd	C	46 ± 2aA	48 ± 3A	47 ± 10A
	AC1	22 ± 1bAB	16 ± 1B	28 ± 4A
	AC2	22 ± 1bA	19 ± 0.1A	14 ± 4A
	Lig1	12 ± 1cA	13 ± 1A	15 ± 3A
	Lig2	13 ± 1cA	6.5 ± 0.4B	10 ± 1AB
	VC1	14 ± 1cB	18 ± 0.01A	12 ± 1B
	VC2	16 ± 1cA	10 ± 0.5B	12 ± 1B
Pb	C	385 ± 165aA	232 ± 58A	679 ± 403A
	AC1	33 ± 5bA	48 ± 17A	203 ± 76A
	AC2	50 ± 9bA	106 ± 17A	84 ± 62A
	Lig1	24 ± 3bA	21 ± 2A	61 ± 20A
	Lig2	25 ± 3bB	21 ± 1B	47 ± 8A
	VC1	20 ± 1bB	29 ± 2A	13 ± 2C
	VC2	17 ± 3bA	21 ± 4A	12 ± 3A
Zn	C	3255 ± 247aA	3907 ± 164A	4479 ± 907A
	AC1	1171 ± 16bB	1052 ± 240B	2193 ± 175A
	AC2	1099 ± 17bA	1279 ± 20A	868 ± 364A
	Lig1	1422 ± 42bA	1134 ± 43A	1437 ± 241A
	Lig2	1298 ± 43bA	1034 ± 12B	1118 ± 36B
	VC1	1202 ± 73bA	1206 ± 27A	983 ± 3B
	VC2	1056 ± 0.5bA	692 ± 83B	520 ± 13B

Significant differences among the treatment (before S application, indicated by lowercase letters) and sulphur application (after S application, indicated by uppercase letters) means at *p* < 0.05 are indicated. See Table 1 for more details about the treatments

Pb, Zn) concentrations in the maize shoots were largest in the control, and partly at AC1 treatment with low biomass production, and lowest in the treatments with high biomass production before and after S application (Fig. 2, Table 5). The opposite trend was found for roots: high biomass yield corresponded with high Fe, Mn and Pb concentrations, because of relatively high availability, and root uptake, but limited translocation to aboveground tissues (Siedlecka 1995). Hence, we conclude that biomass production caused a dilution effect for the respective metal concentrations only in the maize shoots. Vondráčková et al. (2014) recorded similar results. Another reason could be related to an increase in biomass over time after S application. Moreover, the plant membrane selectivity during stress due to toxicity or deficiency can deteriorate and lead to an uncontrolled uptake of toxic metals (Hall 2002). Pourrut et al. (2011) described the breakdown of the Casparian stripe under toxicity stress. This could explain the excessive uptake of less mobile Pb and other metals (Cd, Zn, Fe and Mn) into the shoots in the control (Table 5). There were no considerable changes in root or shoot concentrations of elements after S application.

In general, distinguishing between toxicity symptoms and deficiencies was rather difficult throughout the experiment. Certainly, a combination of both occurred. We can speculate that P and K deficiencies might have reduced plant growth at treatments with high C/N ratio. The high CaCl₂-extractable concentrations of Cd and Zn might have induced plant stress and a poor performance (i.e. tapering leaves and a reduction in the leaf area) in the C treatment. Other authors have recorded similar symptoms (Reichmann 2002; White 2012; Sherameti and Varma 2015). These findings can be supported by comparing the concentrations of nutrients and metals measured in the maize shoots with the sufficiency ranges recommended in the literature (White 2012). The Cd, Pb and Zn concentrations in the maize shoots in all treatments were often higher than the toxicity range (5–10 mg Cd kg⁻¹; 10–20 mg Pb kg⁻¹; 100–700 mg Zn kg⁻¹). The concentrations in the control were always the highest. The content of Fe and Mn in shoots lay within the sufficiency range (50–250 mg Fe kg⁻¹; 10–20 mg Mn kg⁻¹) in both VC treatments, but within the toxicity range (> 500 mg Fe kg⁻¹; 200–5300 mg Mn kg⁻¹) in the control. The content of P in the shoots indicates a deficiency for the plants in all treatments (sufficiency range 3000–5000 mg kg⁻¹; Marschner 1995).

The highest total Cd and Zn uptake in the maize shoots was recorded in the VC treatments (Fig. 3a, c) and increased phytoremediation efficiency by 400% for Cd and 100% for Zn in comparison with the control. Phytoremediation efficiency could be substantially increased in the VC and, to a lesser extent, in the Lig treatments, since the total Cd and Zn uptake in the maize shoots was higher than that in the control. While the total Cd and Zn uptake by the maize shoots from the VC treatments was not significantly affected by S application, the

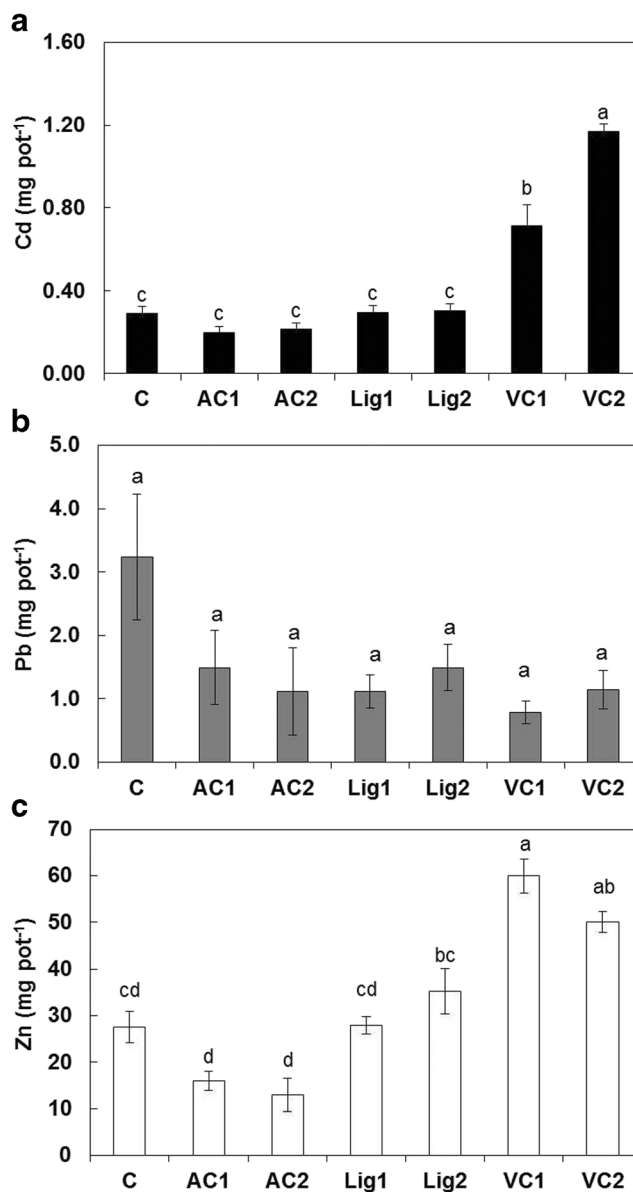
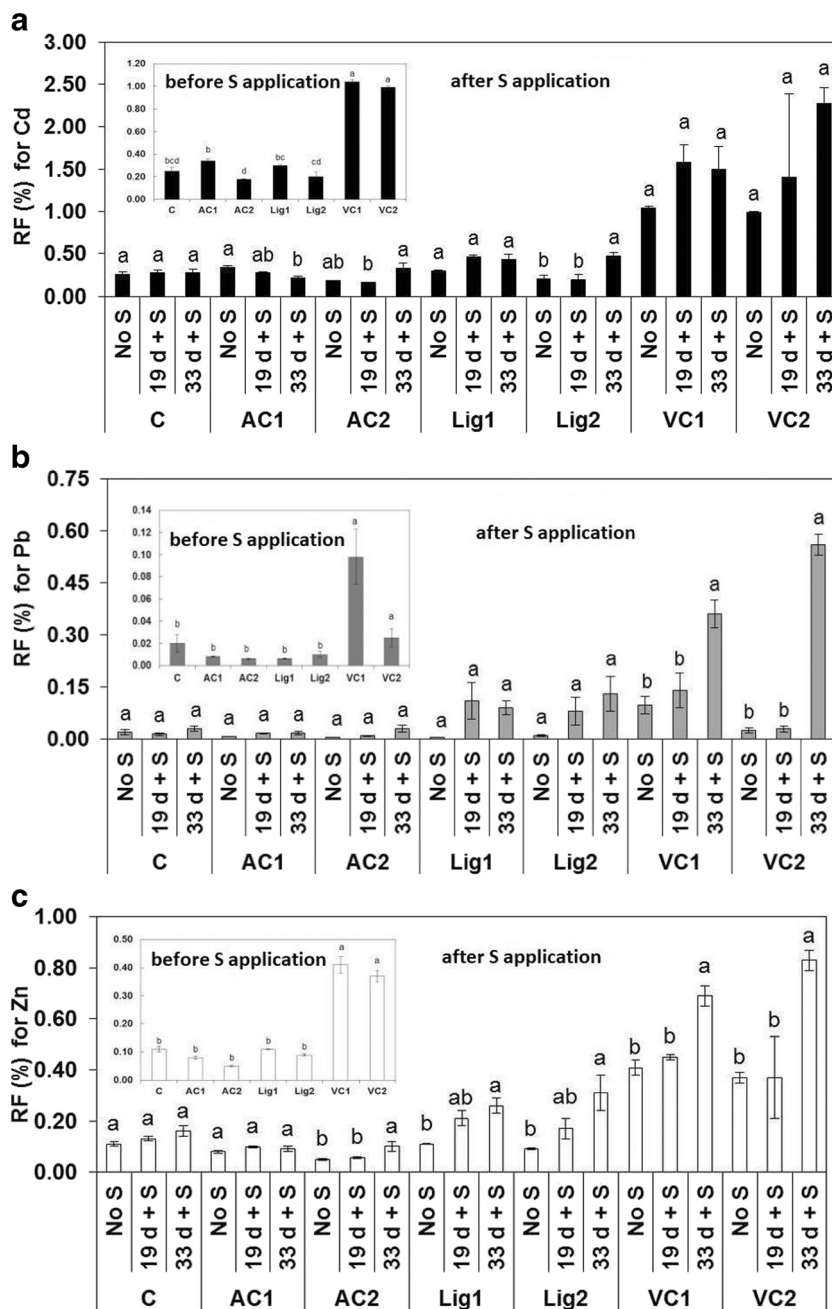


Fig. 3 a–c Total metal content (biomass multiplied by the metal concentration, mg pot⁻¹) in maize shoots after S application. Significant differences among the treatment means at *p* < 0.05 are indicated with letters. Error bars indicate the standard error of the mean (*n* = 3). See Table 1 for more details about the treatments

content in the Lig2 treatment increased significantly, but the values were more than 40% (for Zn) to 70% (for Cd) lower than those in the VC treatments. The phytoremediation efficiency in VC treatments was mostly driven by higher shoot biomass. The Cd and Zn concentration in shoot decreased after S application. The VC treatments did not show higher nutrient availability than the other treatments (Table 3). Therefore, the higher Cd and Zn removal is only related to the high shoot growth, very likely due to the effect of vermicompost on plant growth. Alternatively, a high metal-accumulating plant (i.e. willow instead of relatively metal-tolerant maize) could be used in further experiments to enable

Fig. 4 Proportion of **a** Cd, **b** Pb and **c** Zn removed from the soil by maize biomass before S application (treatment) and after S application (sulphur application) periods as indicated by the remediation factor (RF, %). Significant differences among the treatment and sulphur application means at $p < 0.05$ are indicated with letters. Error bars indicate the standard error of the mean ($n = 3$). See Table 1 for more details about the treatments



larger amounts of Cd, Pb and Zn to be taken up without suffering from toxicity (Mohnke 2014).

Before S application, the remediation factors for Cd, Pb and Zn increased considerably in the VC treatments by approximately three, five and four times, respectively (Fig. 4a–c). For Cd and Zn, this can be explained by higher total biomass, as confirmed by linear regression (Cd: $r = 0.94$, $p < 0.01$; Zn: $r = 0.94$, $p < 0.01$). In contrast, the higher remediation factor for Pb can be better explained by higher Pb concentrations in the root ($r = 0.89$, $p < 0.01$) than by higher biomass. Moreover, after S application, the remediation factors also increased in the Lig treatment for Cd and Zn. The increased removal of toxic metals is due to the higher plant growth.

Conclusions

Before S application, AC, Lig and VC applications reduced the CaCl_2 -extractable Cd and Zn concentrations, while the concentrations of Pb increased in the Lig1 and VC1 treatments compared to the control. After S application, the metal concentrations (Fe, Mn, Cd, Pb, Zn) in the soil solution and the CaCl_2 extraction in the C, AC and VC treatments were not significantly affected by S application. Similarly, the Cd, Pb and Zn concentrations in the maize shoots did not significantly increase in the respective treatments. Several processes (heterogeneous S application or the use of organic agents;

Edwards 1998) might have decreased the redox potential and thus inhibited S oxidation. The CaCl_2 -extractable concentrations of Mn, Cd, Pb, Zn and sulphate increased significantly, and the pH decreased after S addition in investigated treatments in the last sampling period. We suppose that easily accessible S forms in amendments functioned as a driver for microbial S oxidation, probably even before S was applied to soil. During the whole experimental period, the VC treatment and, to a lesser extent, the Lig treatment showed the trend of steady release of some nutrients, while in AC treatment, sorption mechanisms limited the availability of nutrients. Consequently, the highest biomass production and the lowest toxicity and deficiency symptoms were recorded in the VC and Lig treatments, while the AC treatments produced even less biomass than the control. Severe toxicity symptoms were found over the duration of the experiment there. The maize biomass production at the final harvest was about five times higher in the Lig2 treatment and fourteen times higher in the VC treatment compared to the control. The remediation factors for Cd, Pb and Zn increased mainly in the VC treatments because of higher total biomass (for Cd and Zn) and higher metal concentration in the roots (for Pb).

In future research, redox potential changes in the plant rhizosphere should be regularly monitored to test the possibility of microbial sulphate reduction when using Litavka soil and organic amendments. Using different plants (hyperaccumulators or fast-growing, metal-accumulating trees such as *Salix smithiana*) that are able to take up large amounts of toxic metals in highly contaminated soils might also be productive. The subsequent S application should be improved to guarantee a more homogeneous distribution in the soil, as well as a higher S application rate. Alternatively, longer observation times could be used to obtain statistical significance.

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References

- Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. *Chemosphere* 91:869–881
- Amoakwah E, Van Slycken S, Tack FMG, Essumang D (2013) Assessing the extraction efficiency of CaCl_2 and Rhizon extraction methods after the application of organic matter and CaCl_2 as soil amendments to enhance the mobility of Cd and Zn. *J Environ Anal Toxicol* 3(2)
- Amoakwah E, Van Slycken S, Essumang DK (2014) Comparison of the solubilizing efficiencies of some pH lowering (sulphur and $(\text{NH}_4)_2\text{SO}_4$) amendments on Cd and Zn mobility in soils. *Bull Environ Contam Toxicol* 93:187–191
- Azeez JO, Adeleye OA, Oyinlola RO (2016) The effect of sulfur and urea on the heavy metal extraction by african wild sunflower (*Tithonia diversifolia*) in an artificially contaminated soil. *Commun Soil Sci Plant Anal* 47:1940–1949
- Azimzadeh Y, Shirvani M, Shariatmadari H (2016) Rhizosphere and green manure effects on soil chemical attributes and metal bioavailability as a function of the distance from plant roots in mono and mixed corn and canola cultures. *Arch Agron Soil Sci* 62:1066–1081
- Blum SC, Lehmann J, Solomon D, Caires EF, Alleoni LRF (2013) Sulfur forms in organic substrates affecting S mineralization in soil. *Geoderma* 200:156–164
- Borůvka L, Vácha R (2006) Litavka river alluvium as a model area heavily polluted with potentially risk elements. In: *Phytoremediation of metal-contaminated soils*. Springer, Dordrecht, pp 267–298
- Břendová K, Zemanová V, Pavlíková D, Tlustoš P (2016) Utilization of biochar and activated carbon to reduce Cd, Pb and Zn phytoavailability and phytotoxicity for plants. *J Environ Manag* 181:637–645
- Cui Y, Dong Y, Li H, Wang Q (2004) Effect of elemental sulphur on solubility of soil heavy metals and their uptake by maize. *Environ Int* 30:323–328
- Edwards PJ (1998) Sulfur cycling, retention, and mobility in soils: a review. US Department of Agriculture, Forest Service, Radnor
- EN 13037 (1999) Soils improvers and growing media—determination of pH. CEN, Brussels
- Food and Agriculture Organization of the United Nations Rome (2014) World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps. FAO, Rome
- Gray CW, Dunham SJ, Dennis PG, Zhao FJ, McGrath SP (2006) Field evaluation of in situ remediation of a heavy metal contaminated soil using lime and red-mud. *Environ Pollut* 142:530–539
- Hagedorn C (2010) Environmental microbiology: sulfur oxidation. URL http://filebox.vt.edu/users/chagedor/biol_4684/Cycles/Soxidat.html (accessed 16 Oct 2013)
- Hailegnaw NS, Mercl F, Pračke K, Száková J, Tlustoš P (2019) High temperature-produced biochar can be efficient in nitrate loss prevention and carbon sequestration. *Geoderma* 338:48–55
- Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. *J Exp Bot* 53:1–11
- Havelcová M, Mizera J, Sýkorová I, Pekař M (2009) Sorption of metal ions on lignite and the derived humic substances. *J Hazard Mater* 161:559–564
- Hofer C, Santner J, Puschenreiter M, Wenzel WW (2015) Localized metal solubilisation in the rhizosphere of *Salix smithiana* upon sulphur application. *Environ Sci Technol* 49:4522–4529
- Hoehne L, de Lima CVS, Martini MC, Altmayer T, Brietzke DT, Finatto J, Goncalves TE, Granada CE (2016) Addition of vermicompost to heavy metal-contaminated soil increases the ability of black oat (*Avena strigosa* Schreb) plants to remove Cd, Cr, and Pb. *Water Air Soil Pollut* 227:443
- Iqbal M, Puschenreiter M, Oburger E, Santner J, Wenzel WW (2012) Sulfur-aided phytoextraction of Cd and Zn by *Salix smithiana* combined with in situ metal immobilization by gravel sludge and red mud. *Environ Pollut* 170:222–231
- ISO 10390 (2011) Soil quality—determination of pH. Czech Standardization Organization
- Jadia CD, Fulekar MH (2008) Phytoremediation: the application of vermicompost to remove zinc, cadmium, copper, nickel and lead by sunflower plant. *Environ Eng Manag J* 7:547–558
- Jordan R, Yonge DR, Hathhorn WE (1997) Enhanced mobility of Pb in the presence of dissolved natural organic matter. *J Contam Hydrol* 29:59–80
- Kubešová M, Kučera J (2012) How to calculate uncertainties of neutron flux parameters and uncertainties of analysis results in k0-NAA? *J Radioanal Nucl Chem* 293:87–94
- Kumpiemi J, Lagerkvist A, Maurice C (2008) Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments—a review. *Waste Manag* 28:215–225

- Li X, Chen X, Cui X (2012) Zinc chemical forms and organic acid exudation in non-heading Chinese cabbages under zinc stress. *Agric Sci China* 3:562–566
- Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London
- Mench M, Schwitzguébel JP, Schroeder P, Bert V, Gawronski S, Gupta S (2009) Assessment of successful experiments and limitations of phytotechnologies: contaminant uptake, detoxification, and sequestration, and consequences to food safety. *Environ Sci Pollut Res* 16: 876–900
- Mohnke S (2014) Combined chemical immobilization and subsequent remobilization of metallic pollutants in enhanced phytoextraction. Master thesis. BOKU, Vienna. CULS, Prague, p 109
- Mojiri A (2011) The potential of corn (*Zea mays*) for phytoremediation of soil contaminated with cadmium and lead. *J Biol Environ Sci* 5:17–22
- Murugan AV, Swamam TP (2013) Nitrogen release pattern from organic manures applied to an acid soil. *J Agri Sci* 5(6):174
- Nagajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. *Environ Chem Lett* 8:199–216
- Pierzynski GM (2000) Methods of phosphorus analysis for soils, sediments, residuals, and waters. North Carolina State University, Southern Cooperative Series Bulletin No. 396. North Carolina, pp 1–102
- Pourrut B, Shahid M, Dumat C, Winterton P, Pinelli E (2011) Lead uptake, toxicity, and detoxification in plants. In: Whitacre DM (ed) *Reviews of environmental contamination and toxicology*, vol 213. Springer, New York, pp 113–136
- Qi Y, Hoadley FA, Chaffee AL, Garnier G (2011) Characterisation of lignite as an industrial absorbent. *Fuel* 90:1567–1574
- Reichmann SM (2002) The response of plants to metal toxicity. The Australian Minerals & Energy Environment Foundation, Melbourne
- Ruamsap N, Akaracharanya A (2002) Pyritic sulfur removal from lignite by thiobacillus ferrooxidans: optimization of a bioleaching process. *J Sci Res Chula Univ* 27(2):155–162
- Sherameti I, Varma A (2015) Heavy metal contamination of soils: monitoring and remediation. *Soil biology* 44. Springer International, Cham
- Siedlecka A (1995) Some aspects of interactions between heavy metals and plant mineral nutrients. *Acta Soc Bot Pol* 64:265–272
- Tejnecký V, Bradová M, Borůvka L, Němeček K, Šebek O, Nikodem A (2013) Profile distribution and temporal changes of sulphate and nitrate contents and related soil properties under beech and spruce forests. *Sci Total Environ* 442:165–171
- Tica D, Udovic M, Lestan D (2011) Immobilization of potentially toxic metals using different soil amendments. *Chemosphere* 85:577–583
- Tlustoš P, van Dijk D, Száková J, Pavlíková D (1994) Cd and Zn release through the selected extractants. *Rostl Vyr* 40:1107–1121
- Tury R, Szakal P, Szegedi L (2008) A tavaszi árpa (*Hordeum vulgare*) növekedése és nehézfémakkumulációja a gyöngyöSOROSZI bányameddőn különböző kezelések hatására. *Talajvédelem, Talajtani vándorgyűlés* 29–29
- Uz I, Sonmez S, Tavali IE, Citak S, Uras DS, Citak S (2016) Effect of vermicompost on chemical and biological properties of an alkaline soil with high lime content during celery (*Apium graveolens* L. var. *dulce* Mill.) production. *Not Bot Horti Agrobot Cluj-Na* 44:280–290
- Uzinger N, Rekasi M, Draskovits E, Anton A (2014) Stabilization of Cr, Pb, and Zn in soil using lignite. *Soil Sediment Contam* 23:270–286
- Van Ranst E (2006) Properties and management of soils in the tropics. Chapter 2:31–32
- Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnevajova E, Lelie D, Mench M (2009) Phytoremediation of contaminated soils and groundwater: lessons from the field. *Environ Sci Pollut Res* 16:765–794
- Vondráčková S, Hejman M, Száková J, Müllerová V, Tlustoš P (2014) Soil chemical properties affect the concentration of elements (N, P, K, Ca, Mg, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) and their distribution between organs of *Rumex obtusifolius*. *Plant Soil* 379: 231–245
- Wang G, Koopmans GF, Song J, Temminghoff EJM, Luo Y, Zhao Q, Japenga J (2007) Mobilization of heavy metals from contaminated paddy soil by EDDS, EDTA, and elemental sulfur. *Environ Geochem Health* 29:221–235
- Wenger K, Kayser A, Gupta SK, Furrer G, Schulin R (2002) Comparison of NTA and elemental sulphur as potential soil amendments in phytoremediation. *Soil Sediment Contam* 11:655–672
- White PJ (2012) Heavy metal toxicity in plants. In: Shabala S (ed) *Plant stress physiology*. Cabi, Cambridge, pp 210–235
- Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *ISRN Ecology* 2011:1–20
- Zemanová V, Břendová K, Pavlíková D, Kubátová P, Tlustoš P (2017) Effect of biochar application on the content of nutrients (Ca, Fe, K, Mg, Na, P) and amino acids in subsequently growing spinach and mustard. *Plant Soil Environ* 63:322–327

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