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Application of Biochar for the Restoration of Metal(loid)s Contaminated Soils

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Abstract: Biochar has recently aroused great interest for the restoration of contaminated soils since it improves soil properties and induces the immobilization of pollutants. This study evaluates the use of biochar from plant pruning, applied as an amendment, for immobilizing metal(loid)s in a highly contaminated soil as well as for reducing the phytotoxicity of these pollutants by promoting natural revegetation. For this purpose, a bioassay with *Trifolium pratense* L. was used to test the effectiveness of the soil amendment in greenhouse conditions. Three treatments were carried out including soil contaminated with metal(loid)s (RA), and this soil was amended with biochar at different dosage: 4% (RA4B) and 8% (RA8B). A non-contaminated soil (NC) from a nearby area not affected by contamination was used as a control. The results show that biochar increased soil pH by several units depending on the dose used, 8% being the most effective one. Biochar treatments also reduced soluble and bioavailable forms of Zn and Cu. Likewise, phytotoxicity was significantly reduced, promoting seed germination and biomass with plant growth values similar to the non-polluted soil. In light of the results obtained, the evaluation of the bioremediation potential of biochar under field conditions can be considered.

Keywords: biochar; phytotoxicity; metal immobilization; *Trifolium pratense*; soil bioremediation

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1. Introduction

Soil pollution has become a great concern all over the world, and is considered the third threaten for the soil function by the Intergovernmental Technical Panel on Soils [1]. The main sources of organic and inorganic pollutants are related to industrial, urban, and agricultural activities. In the last few decades, mining has become the primary entrance of metal(loid)s into the environment with important harmful implications for human health [2] due to the immobile nature of these compounds and the lack of biodegradation processes, which makes them highly persistent pollutants [3]. In this context, there is an increasing interest in new and sustainable solutions for the prevention, mitigation, or remediation of soil contamination caused by metal(loid)s [4]. Assisted natural remediation (ANR) is a novel concept that consists in enhancing natural processes by, for example, adding to the soil amendments that favor the immobilization of contaminants and the improvement of soil properties [5], and therefore promoting the natural restoration of vegetation. Calcium carbonate, iron, and organic matter rich amendments are the most used to reduce metal(loid)s mobility and toxicity by precipitation, complexation, and adsorption, with proved and high efficiency at field scale [6,7].

Biochar is a carbon rich material obtained from the pyrolysis of organic wastes at low temperatures (from 400 to 700 °C) under O reducing conditions [8]. This process stabilizes the organic carbon and makes it more resistant to chemical and biological degradation [9]. Biochar is characterized by its high content of aromatic compound, high porosity, and surface area [9]. It can be produced from different biomasses such as agro-industrial, forestry and urban wastes, manure, and sewage sludge [10]. The pyrolysis conditions and the nature of the biomass determine the physical and chemical properties of the biochar [11–13]. The main and most spread application of biochar is as soil amendment [9], since it improves the physical and chemical soil properties and consequently the crop yield [14,15]. Biochar has been shown to increase the carbon holding capacity of soils, reduce the emission of greenhouse gases [9,16,17], promote the soil permeability and the water holding capacity [15], favor the liming of acid soils [14], and supply essential nutrients such as phosphorous [18,19]. In addition, several studies indicated the ability of biochar to immobilize organic and inorganic contaminants, decreasing their bioavailability and toxicity [20,21]. The adsorption capacity of biochar is a consequence of its porous structure and the presence of different surface functional groups [22,23]. Besides adsorption capacity, biochar can increase pH values in acid soils and has high cationic exchange and buffer capacity [11,24]. These features make biochar a feasible amendment for contaminated soils, especially for those with metal(loid)s [6,25]. Different studies analysed the different mechanisms that govern the sorption of metal(loid)s into biochar depending on the type of elements and the biochar and soil properties [26–28]. However, more information is still needed to determine efficiency of biochar application in soil remediation practices.

In this study we explore the effect of biochar as an organic amendment to immobilize metal(loid)s and promote the vegetation restoration of a highly contaminated soil from the Guadiamar Green Corridor (Seville, Spain), an area affected by the Aznalcollar′s pyrite mining spill where residual pollution is still detected more than twenty years after the accident [29] due to the oxidation of the remaining tailing in the soil. For this purpose, a pot experiment under greenhouse conditions was carried out. The total concentration of metal(loid)s and their soluble and bioavailable fractions were analyzed in amended and non-amended soil to assess the effect of biochar on metal(loid)s mobility and bioavailability. Phytotoxicity to *Trifolium pratense* was also evaluated. Germination rate, root and shoot biomass, and plant uptake were measured to infer the changes in metal(loid)s phytotoxicity caused by the addition of biochar to contaminated soil.

2. Materials and Methods

2.1. Soils and Biochar

Two soils were used for this study: a soil with high concentration of metal(loid)s (RA) to be amended and a non-contaminated soil (NC) used as control.

The RA soil was collected from the area known as Guadiamar Green Corridor (37°29′42″ N 6°13′12″ W), near to the pyrite mine of Aznacóllar (Seville, Spain) (Figure 1A), where highly metal(loid)s contaminated soils exist due to the mining accident which occurred in 1998 [30]. Despite the efforts for restoring the area, metal(loid)s contamination persists more than 20 years after the accident. This soil is classified as eutric Regosol [31] and characterized by the absence of vegetation (Figure 1C) due to the presence of residual pollution [29,32].

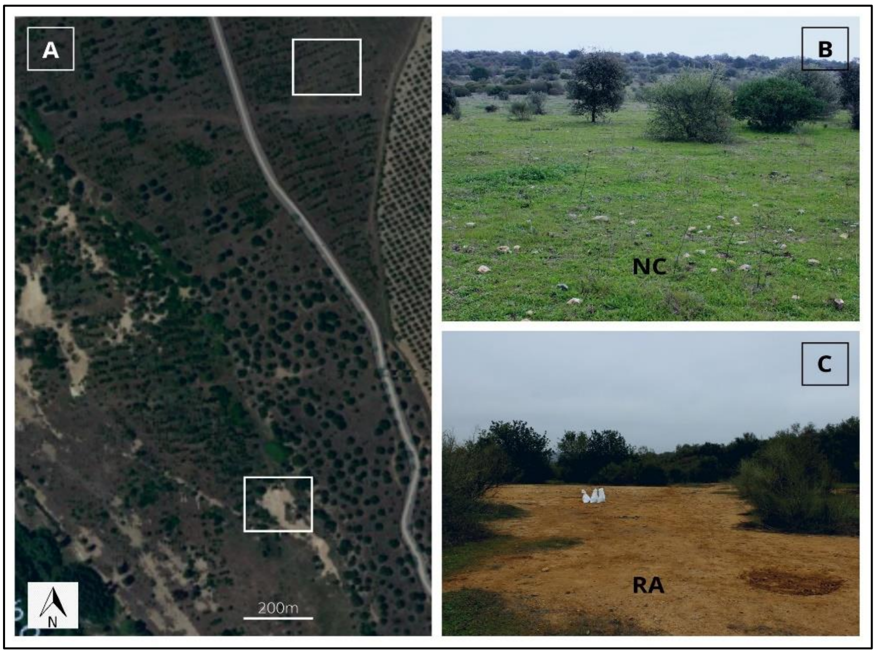

Figure 1. (**A**) Satellite image of the nearest area to Aznalcollar's mine in the Guadiamar Green Corridor (Seville, Spain). The light spots in the left image (**A**) represent highly contaminated soils. White squares indicate the sampling sites of the two soils used in this study. (**B**) Non contaminated soil (NC). (**C**) Soil contaminated with metal(loid)s (RA).

The NC soil was collected from an area close to the mining spill but non-affected by metal(loid)s contamination (37°30′00″ N 6°13′11″ W) (Figure 1A). This soil is classified also as eutric Regosol and covered with vegetation as seen in Figure 1B.

Soil samples were collected from the surface layer (0–10 cm) using the methodology described in Aguilar et al. [33]. Briefly, five soil subsamples from a square area of 1 × 1 m were obtained, one subsample from each vertex and one from the center of the square. The subsamples were homogenized by manual mixing and then, sieved through 2 mm sieve to obtain a representative sample of each soil. The main soil properties, pH, electrical conductivity (EC) and organic carbon (OC) content of the two soils and their total content of different metal(loid)s were analyzed (Table 1).

Table 1. pH, electrical conductivity (EC), organic carbon (OC) content and total content of different metal(loid)s in non-contaminated (NC), metal contaminated (RA) soils, biochar (BS), and contaminated soil amended with 4% (RA4B) and 8% (RA8B) of biochar (mean ± standard error).

	RA	**NC**	**BS**	**RA4B**	**RA8B**
pH	3.67 ± 0.02	7.86 ± 0.02	9.83 ± 0.02	4.75 ± 0.06	5.69 ± 0.12
EC (dS/m)	3.49 ± 0.02	0.69 ± 0.01	1.39 ± 0.01	3.20 ± 0.03	3.28 ± 0.03
OC (%)	0.51 ± 0.03	0.51 ± 0.04	82.50 ± 0.01	3.81 ± 0.04	7.11 ± 0.05
Pb (mg/kg)	446.55 ± 7.51	103.85 ± 0.63	1.88 ± 0.33	463.00 ± 7.64	473.93 ± 34.73
As (mg/kg)	244.56 ± 1.49	28.50 ± 0.77	0.05 ± 0.02	246.34 ± 2.14	232.96 ± 9.92
Zn (mg/kg)	161.15 ± 0.77	189.44 ± 3.71	16.84 ± 1.57	172.84 ± 2.15	166.80 ± 2.78
Cu (mg/kg)	112.17 ± 4.92	83.65 ± 1.99	10.28 ± 0.32	133.18 ± 2.77	110.54 ± 1.31

The biochar (BS) was supplied by Swiss-biochar (Belmont-sur-Laussanne, Switzerland) and was elaborated from the pyrolysis of oak pruning at 650 °C. The specific surface area of the biochar was 250–350 m^2 g^{-1}. Other physical and chemical properties of the biochar, determined as described below, are included in Table 1. The biochar was ground and sieved by 500 µm before use.

2.2. Bioassay with *Trifolium pratense*

A short-term pot experiment was conducted in the greenhouse to evaluate the effect of biochar on the bioavailability and phytotoxicity of metal(loid)s in the contaminated soil. Three treatments with RA soil were prepared by adding biochar at different doses: 0 (RA), 4 (RA4B) and 8% (RA8B) of biochar. The mixture of soil and biochar was homogenized by manual agitation. In addition, another treatment with non-amended unpolluted soil (NC) was used as a control. The main physical and chemical properties of the amended soil were included in Table 1.

Each treatment was placed in a plastic tray and incubated at 60% of its water holding capacity during one week. Thereafter, an aliquot of 400 g of each soil was placed in 12 cells pot-in-frame. For each treatment, 12 pot replicates were prepared (i.e., 144 cells in total). In each cell, five seeds of *Trifolium pratense* were placed at 0.5 cm depth. The moisture content of the samples was adjusted to 60% of their water holding capacity. Then, samples were placed in the greenhouse at natural sunlight and temperature between 10 and 35 °C. Moisture content was maintained by weighing and adding distilled water when necessary.

After two months, soil samples for each pot were air dried at room temperature, sieved through a 2 mm sieve, and stored at 4 °C before analysis. Plants of each pot were collected and divided in root and shoot, thoroughly washed with distilled water, and dried in an oven (Memmert 100–800, Memmert GmbH, Schwabach, Germany) at 70 °C for 48 h. Thereafter, the root and shoot biomass was weighted in a precision weighing balance (Gram precision STA-310S, Gram group, Barcelona, Spain). Finally, to obtain data on metals plant uptake, root and shoot biomass were attacked by acid digestion and measured in ICP-OES as described below.

2.3. Sample Extraction and Analysis

Electrical conductivity (EC) and pH analyses were carried out following official methods [34]. Both parameters were measured in a 1:2.5 soil: distilled water ratio with a pH/conductometer (Metrohm AG, Herisau, Swiss) at the initial (t0) and final (tf) time of the bioassay.

For the metal(loid)s analysis, only As, Pb, Cu, and Zn were considered since a previous study indicated those were the most dominant metal(loid)s in the contaminated soil studied [32]. A portable X-ray fluorescence analyzer (NITON XL3t-980 GOLDD+, ThermoFisher, Waltham, MA, USA) was used for directly measuring the total concentration of each metal(loid) in finely ground soil samples.

To extract the metal(loid)s soluble fraction, soil was mixed with distillated water at a 1:2.5 ratio and agitated in an end-over-end shaker overnight. Then, samples were centrifugated at 3000 rpm for 10 min and 5 mL of the supernatant were added with 0.2 mL of nitric acid following the protocol described in Sposito et al. [35]. Samples were kept at 4 °C until analysis.

The extraction of the metal(loid)s bioavailable fraction was carried as described by Quevauviller et al. [36]. Briefly, soil samples (5 g) were mixed with EDTA (50 mL) 0.05 M and pH = 7 and the mixture was agitated in an end-over-end shaker for 1 h. Then, samples were centrifugated at 3000 rpm for 10 min and the supernatant kept at 4 °C until analysis.

The extracts containing soluble and bioavailable fractions were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, PE SCIEX ELAN-5000, Perkin Elmer Inc., Walthman, MA, USA). The ICP method precision was evaluated by the six repetitive analysis of the reference material SRM 2711. The average recovery of the reference material ranged from 91 and 105% for the metal(loid)s studied [32].

To determinate metal(loid)s concentration in shoot (SC) and root (RC) biomass the protocol used was based on EPA METHOD 3050B (successive attacks with nitric acid and hydrogen peroxide) [37], and then the extracts were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES).

2.4. Plant Germination and Growth Indicators

To analyze the effect of the soil treatments on the plant germination and growth, different indicators were used. Two weeks after sowing, the germination rate (Ge) in each pot was calculated as the number of germinated seeds in relation to the total number of seeds sowed and expressed as percentage. At the end of the experiment (two months), the plant survival (PI) was calculated as the number of plants at final time in relation to the total number of seeds sowed and expressed as percentage. The survival rate was calculated as the percentage of PI in relation to Ge.

In relation to plant growth the following indicators were calculated: (i) plant coefficient (Pc), calculated by the ratio between shoot biomass to total biomass; (ii) root coefficient (Rc) calculated by the ratio between root biomass to total biomass; (iii) and shoot/root ratio (PR), the ratio between the aboveground biomass to the belowground biomass.

In all cases, biomass refers to dry weight (mg) and total biomass was calculated as the sum of root and shoot biomass.

In relation to plant uptake of metal(oid)s, metal shoot concentration (SC) was calculated as the amount of metal (mg) measured in shoot tissue divided by shoot biomass (kg). Similarly, metal root concentration (RC) was calculated as the amount of metal (mg) measured in roots divided by shoot biomass (kg). SC/RC coefficient was calculated in order to evaluate the metal traslocation potential. The bioaccumulation factor (BAF) was calculated by dividing metal(loid)s concentrations in plants (mg kg^{-1} dry weight) by bioavailable fraction (extracted by EDTA) in all treatments (mg kg^{-1} dry soil) as described in García Carmona et al. [38].

2.5. Statistical Analysis

Descriptive statistics were calculated from each measurement. The data were analyzed to verify normality and homoscedasticity through graphical exploration of the residuals [39]. If the requirements were fulfilled, significance of differences between treatments was analyzed by the one-way analysis of variance (ANOVA) test. Tukey's test was used to find means that were significantly different from each other at a confidence level of 95%. If the requirements were not fulfilled, two non-parametric analyses (Kruscal-Wallis and Mann Whitney-U tests) were used for comparison purposes. Spearman's correlation analysis was conducted to analyse the relationships among soil properties, metal(loid)s forms, and plant growth variables. In addition, a principal component analysis was carried out to stablish the relation between the different variables studied and thus, determine the soil properties and the metal(loid)s that affect plant toxicity.

All of the statistical analyses were conducted at a 95% confidence level using SPSS 24.0 (IBM Corp., New York, NY, USA) and the software R 4.0.3 (R Core Team, Los Angeles, CA, USA).

3. Results

3.1. Effects of Biochar on Soil Properties

The NC soil showed neutral pH values (7.86 ± 0.02) and low EC (0.69 ± 0.01 dS m^{-1}), while the RA soil had acidic pH (3.67 ± 0.02) and high EC (3.49 ± 0.02 dS m^{-1}) at the initial time of the experiment (Table 1).

The addition of biochar to soil increased the soil pH and decreased soil EC (Figure 2). The values of pH increased with the biochar dosage. Thus, pH values were 3.81 ± 0.01, 4.40 ± 0.01 and 5.41 ± 0.01 for RA, RA4B, and RA8B, respectively. In contrast, no significant differences were observed between EC values of amended soils (RA4B and RA8B), which reflects the absence of influence of the dose of biochar on the soil EC. Moreover, the EC of amended RA soil is still five times higher than that of the NC soil (Figure 2).

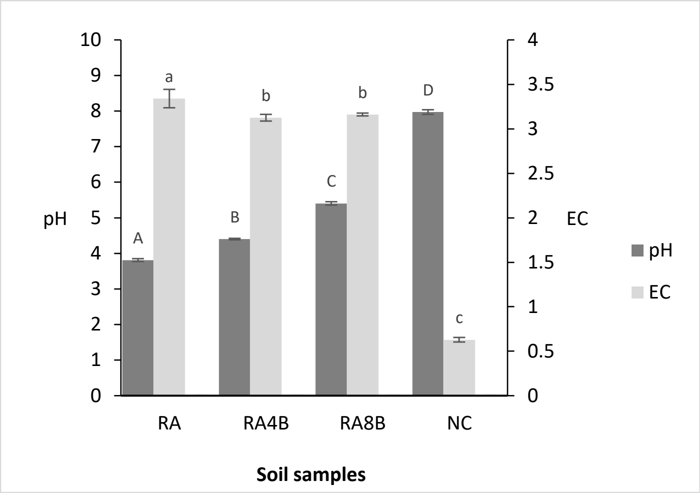

Figure 2. pH and electrical conductivity (EC, dS/m) of non-contaminated soil (NC) unamended contaminated soil (RA) and RA soil amended with biochar at 4% (RA4B) and 8% (RA8B) at the final time of the experiment. Error bars represent the standard deviation. Different letters for each parameter represent statistically significant differences ($p < 0.05$) according to Tukey's post-hoc test.

3.2. Effects of Biochar on Metal(loid)-Mobility

High concentrations of metals are present in the contaminated soils compared to the uncontaminated soil tested (Table 1). The total concentration of metals present in the analysed soils does not vary significantly between the beginning and the end of the experiment, since it is a closed-box experiment carried out for a limited period. However, differences in the content of the different form of metal(loid)s analysed were observed between treatments.

Soluble and Bioavailable Forms

Distribution of the soluble and bioavailable forms of the metal(loid)s varied between RA and NC soils depending on the element studied. Thus, concentration of the soluble and bioavailable forms of As and Pb were lower in RA than in NC, unlike Zn and Cu which showed higher concentrations in RA, especially in the case of the soluble Zn (Table 2).

Table 2. Soluble (_s) and bioavailable (_b) metal(loid) s content (mean ± SE) in the non-contaminated soil (NC), in the unamended contaminated soil (RA) and in soil amended with biochar at 4% (RA4B) and 8% (RA8B) at the end of the experiment. Different letters indicate significant difference ($p < 0.05$) between treatments according to Tukey's post-hoc test.

	RA	**RA4B**	**RA8B**	**NC**
Pb_s (µg/kg)	0.54 ± 0.27 a	0.88 ± 0.62 a	0.27 ± 0.18 a	16.53 ± 3.84 b
As_s (µg/kg)	4.83 ± 0.29 a	6.33 ± 1.99 a	5.57 ± 2.76 a	6.67 ± 0.25 a
Zn_s (mg/kg)	5.37 ± 0.17 d	3.39 ± 0.06 c	0.41 ± 0.01 b	0.02 ± 0.01 a
Cu_s (mg/kg)	0.33 ± 0.02 c	0.07 ± 0.01 b	0.02 ± 0.01 a	0.02 ± 0.01 a
Pb_b (µg/kg)	31.50 ± 1.09 a	40.50 ± 2.35 b	59.92 ± 5.03 c	6063 ± 63.12 d
As_b (µg/kg)	57.42 ± 0.66 a	56.92 ± 1.30 a	94.58 ± 2.35 c	84.17 ± 0.78 b
Zn_b (mg/kg)	8.97 ± 0.40 d	6.96 ± 0.18 c	4.19 ± 0.06 b	3.26 ± 0.04 a
Cu_b (mg/kg)	6.01 ± 0.06 d	5.27 ± 0.05 b	5.64 ± 0.03 c	2.64 ± 0.03 a

In the amended soils, the soluble fraction of Zn significantly decreased with respect to the unamended contaminated soil, especially in the 8% treatment where it dropped up to 13-fold. The bioavailable fraction of Zn in the amended soils also decreased significantly with respect to the RA, dropping by half in RA8B, thus reducing its potential toxicity (Table 2). In both cases, however, soluble and bioavailable concentrations were higher than in NC. In the case of Cu, its concentration decreased significantly both in the soluble fraction, where RA8B treatment reached similar values to the non-contaminated soil, and in the bioavailable fraction, although to a lesser extent since concentrations in RA8B treatment were higher than NC. In contrast, the soluble fraction of Pb showed no significant differences between amended and contaminated soil, while the bioavailable fraction increases with the percentage of amendment. However, the highest values of mobile forms of Pb were found in the non-contaminated soil. In the case of As, the soluble fraction did not show any significant difference between RA, RA4B, and RA8B (or with NC). However, the bioavailable fraction showed no differences between RA and RA4B, with lower content than the NC, but increased in the RA8B treatment, even exceeding the concentration present in NC (Table 2).

3.3. Plant Response

3.3.1. Plant Germination

The germination of *Trifolium pratense* in the unamended contaminated soil (RA) was four times lower than that of the unamended soil (NC) used as a control (Figure 3). In RA soil, the rate of the number of plants that grew two months after sowing (Pl) was lower than the germination rate (Ge) observed at two weeks, although the difference was not significant. This decrease indicates that during this period there was either no new germination and some plant died or the rate of new germination was lower than the death rate.

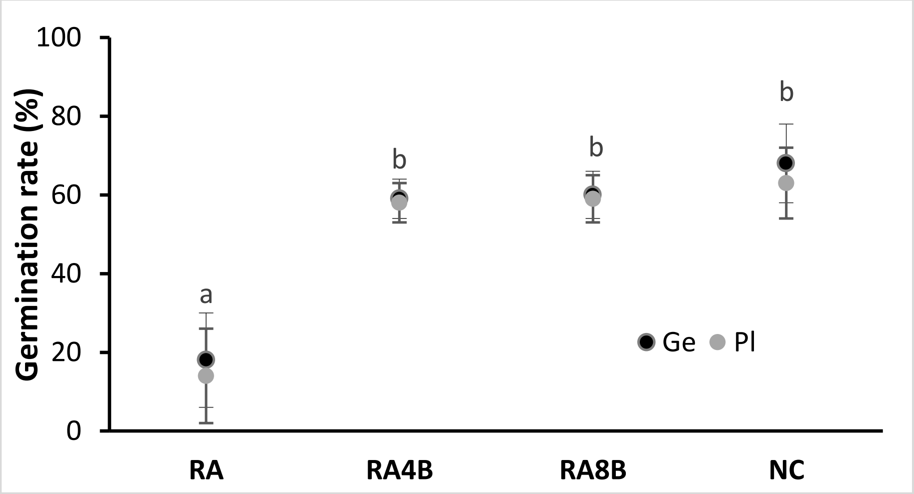

Figure 3. Germination rates (Ge) at two weeks and rate of number of plants present at the end of the experiment (Pl) in the uncontaminated soil (NC), in the unamended contaminated soil (RA) and in RA soil amended with biochar at 4% (RA4B) and 8% (RA8B). Error bars represent standard deviation. Different letters indicate significant differences ($p < 0.05$) between treatments by Tukey's post-hoc test.

The addition of biochar to the contaminated soil resulted in a significant increase in the germination rate in the RA4B and RA8B treatments with respect to RA, with no significant differences between the doses of amendment applied (Figure 3). Incubation time did not affect plant performance. In fact, the number of plants two months after sowing did not vary with respect to the initial germination in the soil amended with 4% (RA4B) and 8% (RA8B) biochar, suggesting that the treatments did not condition plant survival.

At the end of the experiment, no significant differences in the number of live plants were observed between the NC soil and the RA soil amended with biochar, irrespective of the amendment dose (Figure 3). Indeed, in NC, RA4B, and RA8B the survival rate of *Trifolium pratense* varied between 94 and 98% while in RA it was 70%.

3.3.2. Plant Biomass

The total biomass of the plants that grew in RA soil was significantly lower than the biomass in NC soil (Figure 4A).

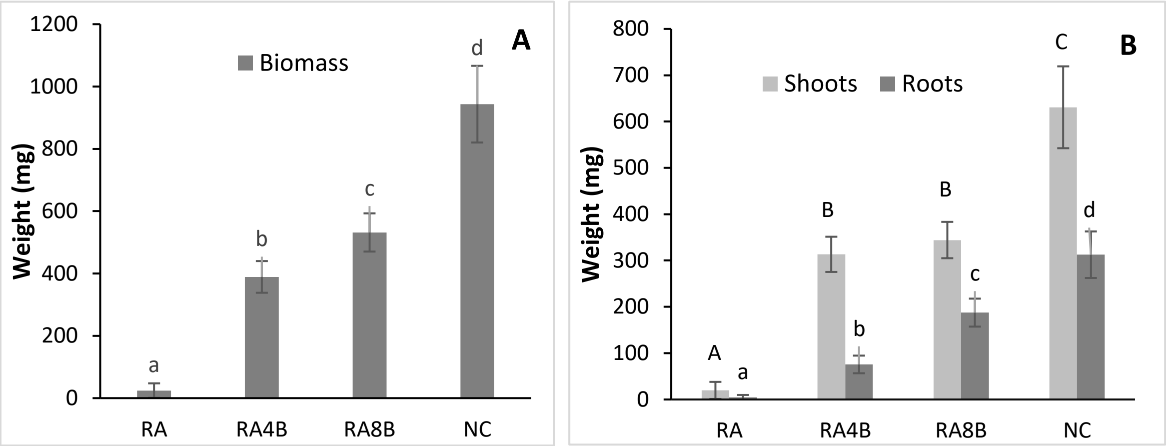

Figure 4. Values of total biomass (**A**) and biomass of the aerial part and roots (**B**) of *Trifolium pratense* in the tested treatments (RA, unamended contaminated soil; RA4B and RA8B, contaminated soil amended with 4 and 8% of biochar, respectively; and NC, non-contaminated soil). Error bars represent standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$) according to Tukey's post-hoc test.

The addition of biochar to contaminated soil significantly increased total plant biomass compared to unamended contaminated soil (Figure 4A). The plant biomass increased with the biochar dosage. Thus, the addition of 4 and 8% of biochar increased the total biomass 16 and 22-fold, respectively, both with respect to the unamended contaminated soil (Figure 4A), although this parameter remained below the values measured in NC (58.76% and 43.64% for RA4B and RA8B, respectively).

When comparing the aerial and root biomass of the amended soils, it can be observed that the increase in the amendment doses did not significantly modify the aerial biomass but it did increase the root biomass more than two times in the 8% treatment in relation to the 4% treatment (Figure 4B). However, shoot and root biomass in RA8B remains below (45% and 40%, respectively) the values in NC.

The values of the calculated growth indicators confirm the higher efficacy of the 8% biochar treatment compared to the 4% treatment (Table 3). Thus, the growth indicator values (Pt, Rt, and PR) for RA4B did not differ significantly from those for RA, unlike RA8B, which showed significant differences for the three coefficients analyzed in relation to RA and similar values to NC soil.

Table 3. Growth indicators (Pt, aerial/total biomass; Rt, root/total biomass; and PR, aerial/root ratio) of *Trifolium pratense* in the non-contaminated soil (NC), in the unamended contaminated soil (RA) and in RA soil amended with biochar at 4% (RA4B) and 8% (RA8B). Growth indicators were calculated two months after planting. Different letters indicate significant differences ($p < 0.05$) between treatments according to the post-hoc Tukey test.

	RA	RA4B	RA8B	NC
Pt	0.79 ± 0.07 b	0.81 ± 0.01 b	0.65 ± 0.01 a	0.67 ± 0.01 a
Rt	0.18 ± 0.02 a	0.19 ± 0.01 a	0.35 ± 0.01 b	0.33 ± 0.01 b
PR	3.86 ± 0.49 b	4.30 ± 0.25 b	1.86 ± 0.06 a	2.04 ± 0.08 a

3.4. Main Variables Affecting Soil Toxicity to Trifolium pratense

In order to visualize the relationship between the variables considered in this study, principal component analysis (PCA) was performed to determine which properties and elements control the toxicity in the tested soils. Total metal concentrations and growth indicators (Pt, Rt, and PR) were not considered to avoid creating noise in the analysis. The parameters considered explain 82.2% of the data.

The two main dimensions were used for the graph (Figure 5). The different soils studied (i.e., RA, RA4B, RA8B, and NC) are grouped in different parts of the diagram. The increase in Dim 1 and decrease in Dim 2 coefficients significantly differentiate RA soil from RA amended soils, by the increase in the dose of biochar applied. NC soil is significantly separated from RA soil by the increase in Dim 1 coefficient with no differences in Dim 2 coefficient. The large variability visible for the RA soil and NC soil is due to the presence of some outliers that influence the analysis.

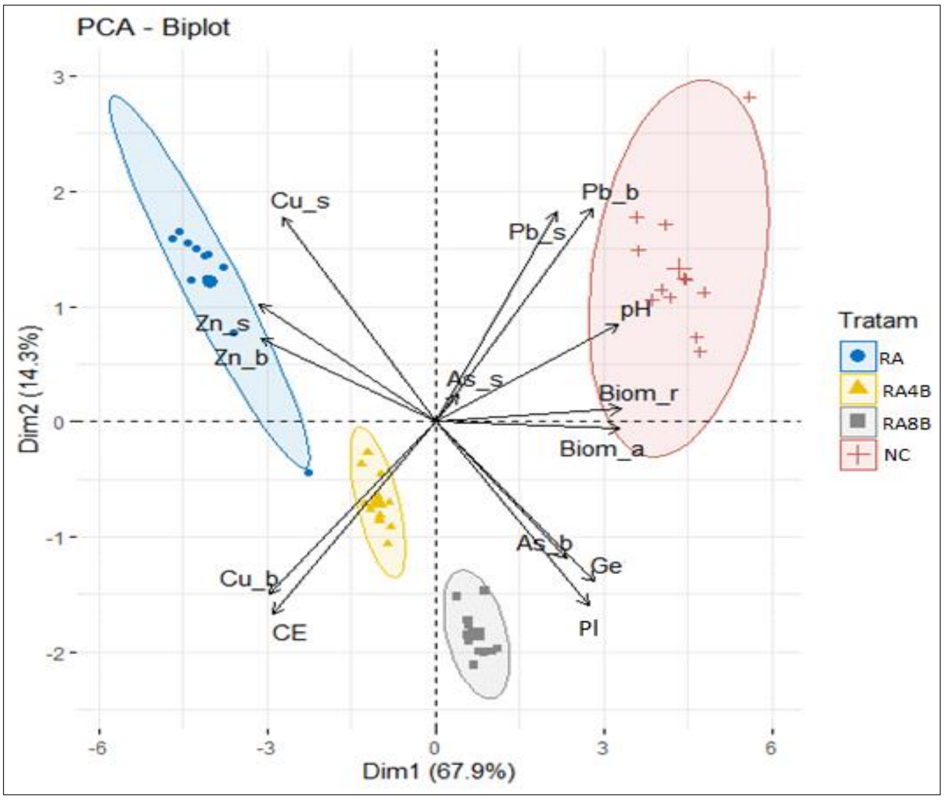

Figure 5. Principal component analysis (PCA) of the main variables in this study. The two components represented (Dim 1 and Dim 2) explain 82.2% of the data. The ellipses represent the variability of the treatments (RA, RA4B, RA8B, NC).

From the PCA results, it can be observed that the increase in biomass (both aerial and root) is directly related to the increase in pH and inversely related to the bioavailable forms of Cu and the EC. The location of the NC samples in relation to these variables indicate the high influence of the non-contaminated soil in the aerial and root biomass.

The germination rate (Ge) and the number of plants two months after planting (Pl) are directly related to the increase of As_b and inversely related to soluble and bioavailable Zn and soluble Cu. The location of the RA soil and RA amended soils in relation to these variables indicate the positive effect of the biochar amendment in relation to the increase of plant germination and plant growth and the decrease of soluble forms of Cu and Zn and bioavailable forms of Zn.

3.5. Metal(loid)s Uptake by Plant

3.5.1. Shoot and Root Uptake

Metal(loid)s uptake by plant was analysed at the end of the incubation time considering root and shoot uptake separately (Table 4). With regard to the aerial part (shoot) the same trend was observed, in general, for all of the metal(loid)s considered. Thus, the concentration of the different metal(loid)s in the shoot decreased following the order RA > RA4B > RA8B > NC, except for Cu, which did not show significant differences between RA4B and NC soils (Table 4).

Table 4. Plant uptake in shoot and root (mean ± SE) in the non-contaminated soil (NC), in the unamended contaminated soil (RA) and in RA soil amended with biochar at 4% (RA4B) and 8% (RA8B) at the end of the experiment. Different letters indicate significant difference ($p < 0.05$) between treatments according to Tukey's post-hoc test. SC/RC ratio was included.

Shoot	**RA**	**RA4B**	**RA8B**	**NC**
Pb (mg/kg)	35.79 ± 7.19 d	6.94 ± 1.00 c	4.53 ± 0.33 b	2.49 ± 0.55 a
As (mg/kg)	12.37 ± 1.92 d	3.92 ± 0.44 c	2.88 ± 0.14 b	0.87 ± 0.10 a
Zn (mg/kg)	1001.05 ± 155.24 d	334.52 ± 12.62 c	74.64 ± 2.59 b	44.77 ± 1.17 a
Cu (mg/kg)	54.19 ± 8.37 c	13.21 ± 0.75 b	7.87 ± 0.23 a	13.12 ± 0.71 b
Root				
Pb (mg/kg)	18.36 ± 2.84 a	129.79 ± 12.50 c	79.57 ± 3.36 b	23.82 ± 1.62 a
As (mg/kg)	4.45 ± 1.08 a	53.7 ± 4.57 c	36.95 ± 1.56 b	5.46 ± 0.27 a
Zn (mg/kg)	146.59 ± 20.57 b	431.76 ± 14.22 c	90.9 ± 2.41 a	75.93 ± 2.68 a
Cu (mg/kg)	35.03 ± 3.73 a	55.98 ± 2.09 b	28.26 ± 0.72 a	33.1 ± 1.41 a
SC/RC ratio				
Pb	1.95	0.05	0.06	0.10
As	2.78	0.07	0.08	0.16
Zn	6.83	0.77	0.82	0.59
Cu	1.55	0.24	0.28	0.40

The concentrations of metal(loid)s in root showed non-significant differences between RA and NC soils, expect for Zn, whose concentration in RA was around twice that in NC. In biochar amended soils different trends were observed depending on the biochar dosage. Thus, addition of biochar at 4% (RA4B) increased the root uptake of all of the metal(loid)s studied regarding to unamended contaminated RA soil. By contrast, with biochar treatment at 8% (RA8B) the root uptake increased for Pb and As, decreased for Zn and was not significative different for Cu, all with regard to RA. Non-significant differences were observed in the root uptake of Zn and Cu between RA8B and NC soils.

Generally, as evidenced by the SC/RC ratio, the metal(loid) concentration in the roots is considerably higher than in the aerial part, except for RA. The addition of biochar to RA soil decreased the SC/RC ratio to values below 1 (Table 4).

3.5.2. Bioaccumulation Factor

Bioaccumulation factor (BAF) using EDTA extraction of soils was calculated to evaluate the transfer of metal(loid)s into plants.

In general, BAF followed the same trend as described for shoot and root uptake. Thus, shoot BAFs decreased in the order RA > RA4B > RA8B > NC except for Cu, and higher root BAFs were observed in RA4B soil for all of the metal(loid)s studied, also for RA8B, although less pronounced (Table 5). Such high BAF values in contaminated soils, especially for Pb and As, depend on the higher concentration of metal(loid)s in the plant with respect to the bioavailable fraction present in the soil, indicating a further transfer of these elements from the soil to the plant. In contrast to amended soils, in RA the highest concentration of matal(loid)s is encountered in the BAF of the aerial part, probably due to the limited growth of the vegetative part of the plant, which for mathematical reasons increased the bioaccumulation factor.

Table 5. Bioaccumulation factor (BAF) in root and shoot, in the non-contaminated soil (NC), in the unamended contaminated soil (RA), and in soil amended with biochar at 4% (RA4B) and 8% (RA8B).

Shoot BAF	RA	RA4B	RA8B	NC
Pb (mg/kg)	1139.01	171.96	75.58	0.41
As (mg/kg)	215.27	68.82	30.47	10.36
Zn (mg/kg)	111.60	48.04	17.81	13.73
Cu (mg/kg)	9.02	2.51	1.40	4.97
Root BAF				
Pb (mg/kg)	584.27	3214.57	1326.78	3.93
As (mg/kg)	77.38	941.96	390.50	64.85
Zn (mg/kg)	16.34	62.01	21.69	23.28
Cu (mg/kg)	5.83	10.63	5.01	12.52

4. Discussion

4.1. Effect of Biochar on Soil Properties and Metal(oid)s Concentration

4.1.1. Soil Physico-Chemical Properties

Positive effects of the addition of biochar on the chemical characteristics of the contaminated soil were observed. The amendment addition raised the pH of the polluted soil depending on amendment dosage, with an increase by almost two units in the 8% treatment (Figure 3). Moreover, the addition of biochar slightly reduced the EC values in RA4B and RA8B with respect to RA. Previous studies have already shown the ability of biochar to modify soil characteristics when used as an amendment to restore contaminated soil [6,40]. Similar to our study, Beesley and Marmiroli [41] observed a strong increase in pH after adding biochar to contaminated soil samples. However, the soil response was not univocal. Considering that biochar properties vary considerably with feedstock and pyrolysis temperature, the results obtained will depend on the type of biochar and especially on the characteristics of the soil amended [11]. Thus, the greatest increase in pH was observed when alkaline biochar is added to a very acid soil [42]. Adding an alkaline biochar (pH between 7 and 9) to acidic soils results in a decrease in the mobility of cationic metal(loid)s due to reduced competition between the ions for cation exchange sites on the biochar surface [25]. However, it is possible that soil pH and soil solution pH may not always respond in the same way to biochar amendments [42]. The addition of biochar to neutral or alkaline soils may not result in a significant decrease in metal mobility [6], indicating that reduced values of this parameter are a consequence of biochar effect on acidic soils and not necessarily to biochar *per se* as an immobilizing agent.

4.1.2. Total Metal(loid)s

The high content of metal(loid)s in the unvegetated soils of the Guadiamar Green Corridor is a consequence of the continuous contamination process characterized by the oxidation of the remaining tailing in the soils [43]. The concentration of metal(loid)s in the contaminated soil treatments showed very high values compared to the values found in the non-contaminated soil, especially for As and Pb (Table 1). The only exception is represented by the Zn content. In fact, in NC soil high concentrations of this metal have been detected, even higher than those found in RA soil. These values of concentrations for Zn in NC soil were in the range of geochemical background values in the area [44], and the low values in RA may be related to the acid pH of this soils, which increased the leaching process of this element over time, lowering the total concentration in the polluted soil in relation to the non-polluted one [33]. However, in all treatments the total Zn content remained below the limits established by the Regional Government to declare a soil as contaminated (10,000 mg kg^{-1}) [45].

Regarding the total Cu content in RA, the values found do not differ much from the geochemical background values of the area [43], and in all treatment remained below the limits established in Decree 18/2015 of the Regional Government to declare a soil contaminated by this element (595 mg kg^{-1}). By contrast, total concentrations of As and Pb found in RA, RA4B, and RA8B exceed the regulatory levels established (275 mg kg^{-1} for Pb, 36 mg kg^{-1} for As) to declare a contaminated soil by the Regional Government [45], representing a threaten for the soil biota as evidenced in previous works [46].

The high concentrations of these two elements are related to their low mobility in soils, in the case of As, this element was strongly retained in the soils by iron oxides in the affected area [33,46]. In the case of Pb, the precipitation in the polluted soils is related to the retention by lead hydroxysulphates such as plumbojarosite [47].

4.1.3. Soluble and Bioavailable Forms

The increase in soluble forms of Cu and Zn is strongly related to soil properties, the acid pH being mainly responsible [43]. The opposite is observed for As, which tends to be more mobile at alkaline pH [48]. Indeed, unlike cationic metals, As is in the form of an oxyanion in solution, which make the remediation more difficult because its mobility in soil may increase with increasing pH when anion release occurs [49].

In our study, the addition of biochar significantly reduced the concentration of the mobile fractions of metals, especially for Zn and Cu. Thus, concentration of the mobile forms of Zn and Cu were inversely correlated with pH (r = 0.735–0.942, $p > 0.01$) and directly with EC (r = 0.507–0.834, $p < 0.01$) (Table A2). Previous studies have also reported a reduction of Zn mobility due to the application of biochar. Beesley and Marmiroli [41] observed that biochar reduced Zn concentration in the leachate through surface sorption. Novak et al. [50] also reported a decrease in Zn concentrations in the leachate after the addition of biochar, but it was related to an increase in pH. Zn bioavailability is drastically reduced in RA8B in response to the sharp increase in pH. This behavior was also observed by Fellet et al. [26], who found that the application of biochar to a mining-contaminated soil, increase pH and CEC, and reduced the bioavailable concentrations of metals. In the case of Cu, the bioavailability is reduced with the biochar addition, but this reduction was of a similar magnitude in the soil amended with biochar, regardless the biochar dosage applied. This result differs from those described by Jiang et al. [51], who observed a decrease in bioavailable forms related to the amount of biochar used, although these authors worked with much higher doses (15–25%) of biochar. The main mechanism related to the reduction of Cu mobility after biochar addition is cation exchange capacity [52], so a certain amount of Cu retained is in bioavailable forms.

For the mobile forms of Pb, a low correlation with soil properties was found, positive with pH (r = 0.539, $p < 0.01$ and r = 0.852, $p < 0.01$, for soluble and bioavailable forms, respectively) and negative with EC (r = −0.557, $p < 0.01$ and r = −0.732, $p < 0.01$, for soluble and bioavailable forms, respectively) (Table A2). In fact, Pb presented the highest solubility ratio in NC, with negligible fractions in RA. Lead is a very immobile element in soils, being clay minerals, pH and organic matter, the most important factors determining its fixation [53,54]. Probably in contaminated soils, Pb may have been immobilized by Fe hydroxysulphates [38,55], and Pb accumulation is also described in the most superficial part of soils, due to sorption by organic matter, by the formation of stable complexes, thus reducing phytotoxicity when the soil has an acidic pH [56]. However, in NC the presence of vegetation may have promoted the formation of complexes between Pb and organic matter contributing to the high solubility of Pb and consequently to the increase of its availability when the pH was raised above 6.5 [57].

Arsenic also showed a low mobility in the polluted soils. The anionic behavior of As favors adsorption under acidic conditions due to its binding to Fe/Mn oxides present in the soil [43,58]. However, when the pH approaches neutrality, an increase in bioaccessibility may also occur. According to correlation analyses, the variation of the soluble fraction of As with respect to pH value is not significant, while the bioavailable fraction is positively related to pH (r = 0.672, $p < 0.01$) and negatively related to EC (r = −0.311, $p = 0.032$) (Table A2). The addition of biochar did not lead to immobilization of this metal(loid), coinciding with the results obtained by Beesley and Marmiroli [41], who found that biochar had a negligible influence on mobile As concentrations in soil contaminated with multiple metals. In the current study, the 8% biochar dosage even caused an increase in the bioavailable fraction of As. This result agrees with previous studies that show some concerns about the application of biochar to manage soil contaminated with As since it may not lead to a reduction in As mobility, but to an increase [6]. To overcome such limitations associated with As mobilization, the use of solid amendments can be tested. For example, combining biochar with iron oxides may be a good option, since as mentioned above, iron oxides can reduce the mobility of As in the soil by anion exchange [59]. Aguilar et al. [46] confirmed the use of soils rich in free iron oxides as a suitable material in the remediation of the studied area. Thus, biochar in combination with iron-rich amendments can promote As retention capacity in soils and reduce the potential toxicity related to this element.

The mechanisms involved in the immobilization of metal(loid)s caused by biochar addition are different, depending mainly on the characteristics of the soil, the biochar used and the type of metal(loid)s present [26]. Although soil pH may explain the behavior of some of the metal(loid)s studied in relation to their mobility it is worthy to mention that immobilization of metals is also related to the ability of organic matter to bind these elements [60]. Thus, not only may biochar reduce the metal(loid)s mobility by an increase of the soil pH but also by the addition of organic matter to soil. However, soluble organic matter can generate ligand binding effects that increases the solubility of heavy metals [61,62]. Indeed, the toxicity of metals is greatly enhanced by their strong tendency to form organometallic complexes, which facilitates their availability and dispersion [63]. For example, in the case of Pb and As, bioavailability is controversial, and there could be a potential environmental risk in the long term if these areas are revegetated without adequate immobilization by additional amendments such as carbonate- and iron-rich materials. Consequently, the application of amendments to reduce the bioavailability of these elements requires monitoring over time.

Regarding to the potential toxicity of the soluble concentrations of metals analysed, for Zn and Cu, the 8% treatment manages to lower the soluble fraction to concentrations below the toxic values in soil solution (Cu = 0.7 mg kg^{-1}; Zn = 0.5 mg kg^{-1}) [64]; and in the case of Cu, also RA maintains a non-toxic concentration in soil solution. In all treatments, the soluble Pb and As content values are lower than the toxic values in soil solution (Pb = 1.0 mg kg^{-1}, As = 0.04 mg kg^{-1}) [64,65].

4.2. Effectiveness of Biochar Treatments to Modify Metal Toxicity to Plants

4.2.1. Germination

Germination and seed emergence are the first and crucial stages of the plant cycle [66]. Our results demonstrate the ability of biochar to promote vegetation emergence in soil with high metal(loid)s content.

The germination rate calculated for RA was very low (17.83 ± 3.54) respect to NC, suggesting the existence of phytotoxic concentrations of the metals in the soil that prevent plant emergence. In previous studies conducted in contaminated soils in the area, it was observed that the acidic pH value and the high concentration of metals resulted in a lack of available essential nutrients, a potential toxicity in soil solution, and an absence of vegetation [29,54]. Delgado-Caballero et al. [67] found that low pH together with high concentrations of Cd, Pb and Zn could have increased the solubility and toxicity of these elements, inhibiting seed germination in *Bouteloua dactyloides* and *Cynodon dactylon*. In fact, a significant correlation was found in our study between soil properties and germination rate, pH being the most explanatory variable with a positive coefficient r = 0.704, $p < 0.01$ (Table A3).

In soils amended with biochar, higher emergence rates were observed than in RA, regardless of the doses used. Probably due to the improvement of soil properties, mainly the increase in pH (with values above 4). In fact, according to García Carmona et al. [38]

and García Robles [68], the increase in pH and the decrease in EC represent favourable conditions for seed emergence and vegetation establishment. The increase of pH values would have promoted the reduction of metal toxicity by decreasing their bioavailability, being of particular importance the reduction of Cu and Zn bioavailable fractions, as evidenced by PCA (Figure 5) and correlation analyses. According to the correlation analysis, Ge would have risen by increasing the bioavailable fraction of Pb and As (r = 0.723, $p < 0.01$; r = 0.429, $p = 0.002$ respectively), and by decreasing the soluble Zn and Cu (r = −0.72, $p < 0.01$; r = −0.588, $p < 0.01$, respectively) and bioavailable Zn and Cu (r = −0.705, $p < 0.01$; r = −0.636, $p < 0.01$, respectively) (Table A3).

In addition, for RA, non-significant variations were observed in the rate of the number of plants present at the end of the experiment with respect to the initial germination. This variation determined the value of the survival percentage, which was lower in the contaminated soil than in the other treatments. Probably, the decrease in the number of plants observed in RA over the incubation time was due to the toxicity content of Zn and Cu.

In the 8% amended treatment the germination rate of *Trifolium pratense* is similar to that of the uncontaminated soil, although the biomass values differ greatly, which it could be related to the influence of the presence of a high content of metals on the normal growth and development of the plants. The existence of a positive correlation (r = 0.429, $p = 0.002$) between the bioavailable fraction of As and germination (Figure 5) indicate that the presence of this element in the soil could stimulate plant growth when it is present in low concentrations (µg/day). This is likely due to the fact that As, by interacting with both plant metabolism and nutrients, could act as a stimulator for the essential micronutrients uptake, such as phosphorus (P) [69]. However, controversial results are found in the literature regarding the role of As in plant growth.

4.2.2. Plant Biomass

The total biomass values inform about the effect of contamination on plant development, determining strong limitations for vegetation establishment. It is worth mentioning that the current experiments were conducted under controlled and fully favorable greenhouse conditions, which promoted the plant growth observed in contaminated soil. However, in real conditions, strong limitations prevent the establishment of vegetation in RA soil with no plant cover.

A significant increase in plant growth was observed in RA4B and RA8B with respect to RA, suggesting decline effect of metal phytotoxicity. As also observed in other studies [42,69], the use of biochar as an amendment improves plant performance. Root biomass values varied significantly among treatments depending on the dose of amendment used, but aerial biomass values did not differ significantly among the amended treatments. Probably the lower biomass of amended soil with respect to non-contaminated soil is due to the accumulation of metals in plant tissues, which may have caused limited growth [53,70]. Thus, it has been observed that the values of soluble and bioavailable fractions of Zn and Cu are negatively related to root (r = −0.937, $p < 0.01$; r = −0.77, $p < 0.01$) and aerial part biomass (r = −0.884, $p < 0.01$; r = −0.654, $p < 0.01$), (Figure 5 and Table A3).

The presence of large amounts of metal(loid)s can affect plant physiology by reducing respiration and growth. In addition, the accumulation of metal(loid)s in high concentrations can interfere with photosynthetic processes and inhibit fundamental enzymatic reactions [71]. When these metals are present in the soil at a low concentration, plants continue to grow uniformly despite accumulating these metals. The ability of plants to accumulate heavy metals in their tissues can therefore be used to control soil pollution and, in particular, the amount of heavy metals [71].

The bioavailable fractions of As and Pb showed positive correlation with the values of shoots (r = 0.895, $p < 0.01$; r = 0.679, $p < 0.01$) and roots (r = 0.875, $p < 0.01$; r = 0.505, $p < 0.01$) (Table A3) biomass. According to the biomass values and correlation data, it is observed that the presence in the soil of a high content of available Zn and Cu mainly affects the development of roots, limiting their growth, but does not influence too much the development of the aerial part. Otherwise, a small increase in the bioavailable fraction of Pb and As seems could promote plant growth, although it is not possible to give a complete answer since the mechanisms they implement are still unknown. (Figure 5).

The improved soil characteristics provided by the addition of biochar are related to increased plant growth. According to Atkinson et al. [72] and Sohi et al. [73], the increased water holding capacity and improved structure caused by the biochar addition represent material conditions conducive to plant growth. But the same properties that in some cases so effectively reduce metal mobility can also immobilize essential macronutrients [74], decreasing the fertility of an already compromised soil. Thus, if maintenance of soil quality and good conditions for plant growth is required, biochar alone may be ineffective for sustainable remediation and revegetation [75,76] and it should be complemented by the application of organic or inorganic fertilizer. For example, under nutrient-limited conditions, its combination with other organic amendments, such as compost, may be necessary [6]. In any case, the selection of a biochar that manages to maintain the balance between nutrient deficiency/retention and pollutant immobilization could be difficult. However, evidence of reductions in the mobility of different types of organic contaminants allows the same amendment to be implemented on multi-element contaminated sites, considering biochar as a cost-effective and versatile material for use on a wide variety of contaminated soils.

4.2.3. Plant Uptake

Biochar addition reduces the metal(loid)s concentration in the shoot as compared to plants grown in RA, but increase the concentration in the root, except for Zn and Cu in RA8B. The increase of As and Pb concentration in roots can be explained by increasing bioavailable fractions of these metal(oid)s in the amended soils. In addition, the presence of organic matter from biochar may increase the accumulation in vegetation of these metals due to the soluble organic ligands' formation for the root exudations that increase the uptake of the trace elements [77].

De Chen et al. [78] found that, in general, biochar consistently reduced the average concentrations of heavy metals in plant tissue. However, our results suggest that biochar may also reduce the translocation of metal(loid)s from root to shoot as indicated lower values of SC/RC ratio calculated in RA4B and RA8B than that in RA (Table 4).

Biochars from different feedstocks varied significantly in their capacity to reduce plant heavy metal concentrations. Thus, it is possible that nutrient enriched manure biochar enhanced plant growth, thereby diluting metal concentrations in the plant biomass [22]. Biochars produced at 450–500 °C are preferred for effectively reducing plant uptake of Cd, Pb, Cu, and Zn, since oxygen containing functional groups can effectively complex with heavy metals [52]. The biochar pH and the application rate of biochar significantly affected the percentage reductions in plant uptake of the heavy metals.

In the present study, bioaccumulation in *Trifolium pratense* seems not to be significant since the total metal content in the samples before the experiment (t0) did not differ significantly from the values found in the samples at the end of the experiment (tf) (Table A1). *Trifolium sp.* is herbaceous, with a limited biomass production. This plant is not considered as hyperaccumulator, since it does not remove significant amounts of metals in a highly contaminated soil by itself. Overall, *T. pratense* demonstrated its ability to survive, so it could represent a potential species to be used in phytostabilization both in the study area and in other similar contaminated areas. According to results in García Carmona et al. [38], *Trifoilium* sp., present in the studied area, showed a good capacity to cope with high levels of heavy metal contamination. These results agree with those of Bidar et al. [79], who reported that this species could grow in soils highly contaminated by Cd, Pb, and Zn, by accumulating them in its roots. It is likely that the bioaccumulation of metals could be an adaptive mechanism that allows plants to live under these stress conditions. In any case, the importance of using tolerant species for restoration trials of highly contaminated soils is highlighted [80]. More studies are needed to monitor the concentration of metals accumulated in the plant tissues to assess the potential transfer risk to the food chain under these polluted areas.

5. Conclusions

The results obtained show that the application of biochar modifies the properties of the treated soils, promoting in particular a rise in pH which leads to changes in the mobility of the metal(loid)s. In particular, the addition of biochar led to a reduction in the bioavailability of Zn and Cu, thus favoring the presence of vegetation in the contaminated soil. These results confirm the effectiveness of the amendment in terms of improving soil properties and its ability to stimulate revegetation in contaminated soils.

According to our study, this treatment could be advisable to ensure the positive evolution of unvegetated soils in the area and to promote vegetation growth. All of the results presented in this study provide valuable information on new remediation strategies that could be transferred to similar scenarios worldwide. However, before applying biochar in the field, it is important to understand its ability to immobilize contaminants in the long-term, which may change over time as its sorption sites may be occupied by native soil organic matter and compete with contaminants. In addition, it is important to consider the biogeochemistry of the soil, the characteristics of the site, and the nature of the contaminants before applying biochar for restoration purposes.

The species used as bioindicator, *Trifolium pratense*, has demonstrated a potential capacity to address severe soil contamination through the mechanism of phytostabilization. Although the bioaccumulation values encountered were low, bioaccumulation of metal(loid)s in the plant tissues must be considered. Indeed, plants with high concentrations of metals in shoot can represent a risk to the ecosystem and living organisms when introduced into the food chain (especially in the presence of livestock).

In light of the positive results obtained in the greenhouse experiment, the evaluation of the bioremediation potential of biochar under field conditions can be considered for the ecological restoration of highly contaminated sites. However, it is recommended that follow-up studies are conducted after each restoration program to avoid future mobilization of metal(loid)es, and continuous monitoring of the vegetation is carried out to ensure the safety of the ecosystem.

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Appendix A

Table A1. Soluble and bioavailable metals for the three treatments tested (RA, RA4B, RA8B) and the control soil (NC) at the beginning of the experiment. Different letters indicate statistically significant difference ($p < 0.05$) according to Kruskal-Wallis.

	RA	RA4B	RA8B	NC
Pb_s (μg/kg)	0.26 ± 0.26 a	0.63 ± 0.32 a	0.35 ± 0.35 a	20.35 ± 11.62 b
As_s (μg/kg)	3.55 ± 0.21 a	8.1 ± 4.15 a	5.2 ± 1.35 a	6.27 ± 0.87 a
Zn_s (mg/kg)	7.33 ± 0.08 d	4.12 ± 0.18 c	0.92 ± 0.01 b	0.031 ± 0.01 a
Cu_s (mg/kg)	0.45 ± 0.04 d	0.07 ± 0.01 c	0.02 ± 0.01 a	0.03 ± 0.01 b
Pb_b (μg/kg)	64.28 ± 3.22 a	49.11 ± 8.93 a	51.17 ± 11.04 a	6025.57 ± 54.47 b
As_b (μg/kg)	55.56 ± 1.77 a	53.33 ± 2.95 a	52.4 ± 4.19 a	92.43 ± 1.25 b
Zn_b (mg/kg)	10.39 ± 0.22 d	8.90 ± 0.35 c	7.39 ± 0.07 b	3.31 ± 0.09 a
Cu_b (mg/kg)	5.78 ± 0.06 d	4.66 ± 0.09 c	4.25 ± 0.06 b	2.84 ± 0.03 a

Table A2. Spearman correlation of soil properties pH and EC with mobile fractions of metals (_s = soluble; _b = bioavailable). NS = not significant; "**" = $p < 0.01$ and "*" = $p < 0.05$.

	pH	Sig.	CE	Sig.
Pb_s	0.539	**	−0.557	**
As_s	NS	NS	NS	NS
Zn_s	−0.942	**	0.804	**
Cu_s	−0.799	**	0.507	**
Pb_b	0.852	**	−0.732	**
As_b	0.672	**	−0.311	*
Zn_b	−0.924	**	0.739	**
Cu_b	−0.735	**	0.834	**

Table A3. Spearman correlation of germination, aboveground biomass (Biom_a) and root biomass (Biom_r) with soil properties and metal(loid)s mobility. NS = not significant; "**" = $p < 0.01$ and "*" = $p < 0.05$.

	Ge	Sig.	Biom_a	Sig.	Biom_r	Sig.
pH	0.704	**	0.862	**	0.921	**
CE	−0.715	**	−0.831	**	−0.773	**
Pb_s	0.311	*	0.492	**	0.486	**
As_s	NS	NS	NS	NS	NS	NS
Zn_s	−0.720	**	−0.884	**	−0.937	**
Cu_s	−0.588	**	−0.654	**	−0.77	**
Pb_b	0.723	**	0.875	**	0.895	**
As_b	0.429	**	0.505	**	0.679	**
Zn_b	−0.705	**	−0.877	**	−0.945	**
Cu_b	−0.636	**	−0.782	**	−0.712	**

References

1. FAO and ITPS. *Status of the World's Soil Resources (SWSR) Main Report*; Food and Agriculture Organization of the United Nations and Intergovernmental Technical Panel on Soils: Rome, Italy, 2015.
2. Muyessar, T.; Linsheng, Y. Trace Elements Contamination and Human Health Risk Assessment in Drinking Water from the Agricultural and Pastoral Areas of Bay County, Xinjiang, China. *Int. J. Environ. Res. Public Health* **2016**, *13*, 938.
3. Smolders, E.; Oorts, K.; Van Sprang, P.; Schoeters, I.; Janssen, C.R.; McGrath, S.P.; McLaughlin, M.J. Toxicity of trace metals in soil as affected by soil type and aging after contamination: Using calibrated bioavailability models to set ecological soil standards. *Environ. Toxicol. Chem.* **2009**, *28*, 1633–1642. [CrossRef] [PubMed]
4. Jiménez Ballesta, R. Contaminación de Suelos por Metales Pesados. In *Introducción a la Contaminación de Suelos*; Mundi Prensa Ed.: Madrid, Spain, 2017; pp. 42–43.

5. Kumpiene, J.; Antelo, J.; Brännvall, E.; Carabante, I.; Ek, K.; Komárek, M.; Söderberg, C.; Wårell, L. In situ chemical stabilization of trace element-contaminated soil—Field demonstrations and barriers to transition from laboratory to the field—A review. *J. Appl. Geochem.* **2019**, *100*, 335–351. [CrossRef]
6. Beesley, L.; Moreno Jiménez, E.; Gomez Eyles, J.L.; Harris, E.; Robisnon, B.; Sizmur, T. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environ. Pollut.* **2011**, *159*, 3269–3282. [CrossRef]
7. Zeng, G.; Wu, H.; Liang, J.; Guo, S.; Huang, L.; Xu, P.; Liu, Y.; Yuan, Y.; He, X.; He, Y. Efficiency of biochar and compost (or composting) combined amendments for reducing Cd, Cu, Zn and Pb bioavailability, mobility and ecological risk in wetland soil. *RSC Adv.* **2015**, *5*, 34541–34548. [CrossRef]
8. *IBI-STD-2.1*; Standardized Product Definition and Product Testing Guidelines for Biochar That is Used in Soil 2.1. International Biochar Initiative (IBI): Canandaigua, NY, USA, 2015.
9. Lehmann, J.; Joseph, S. Biochar for environmental management: An introduction. In *Biochar for Environmental Management: Science, Technology and Implementation*; Lehmann, J., Joseph, S., Eds.; Taylor and Francis: London, UK, 2015; pp. 1–13.
10. Cueto García, M.J. Potencial de Producción de Biochar en España a Partir de Residuos de la Industria Papelera, de Lodos de E.D.A.R., de Residuos Sólidos Urbanos y de Residuos Ganaderos: Estudio de la Fijación de Carbono. Ph.D. Thesis, Universidad Politéecnica de Madrid, Madrid, Spain, 2016.
11. Chen, T.; Zhang, Y.X.; Wang, H.T.; Lu, W.J.; Zhou, Z.Y.; Zhang, Y.C.; Ren, L.L. Influence of pyrolysis temperature on characteristics and heavy metal adsorptive performance of biochar derived from municipal sewage sludge. *Bioresour. Technol.* **2014**, *164*, 47–54. [CrossRef]
12. Jin, J.W.; Li, Y.A.; Zhang, J.Y.; Wu, S.C.; Cao, Y.C.; Liang, P.; Zhang, J.; Wong, M.H.; Wang, M.Y.; Shan, S.D.; et al. Influence of pyrolysis temperature on properties and environmental safety of heavy metals in biochars derived from municipal sewage sludge. *J. Hazard. Mater.* **2016**, *320*, 417–426. [CrossRef]
13. Kavitha, B.; Venkata Laxma Reddy, P.; Kim, B.; Soo Lee, S.; Kumar Pandey, S.; Kim, K. Benefits and limitations of biochar amendment in agricultural soils: A review. *J. Environ. Manag.* **2018**, *227*, 146–154. [CrossRef]
14. Jin, Z.; Chen, C.; Chen, X.; Jiang, F.; Hopkins, I.; Zhang, X. Soil acidity, available phosphorus content, and optimal biochar and nitrogen fertilizer application rates: A five-year field trial in upland red soil, China. *Field Crops Res.* **2019**, *232*, 77–87. [CrossRef]
15. Langeroodi, A.R.S.; Campiglia, E.; Mancinelli, R.; Radicetti, E. Can biochar improve pumpkin productivity and its physiological characteristics under reduced irrigation regimes? *Sci. Hortic.* **2019**, *247*, 195–204. [CrossRef]
16. Joseph, S.D.; Downie, A.; Crosky, A.; Lehmann, J.; Munroe, P. Biochar for carbon sequestration, reduction of greenhouse gas emissions and enhancement of soil fertility; a review of the materials science. *Rend. Circ. Mat. Palermo* **2007**, *48*, 101–106.
17. Nguyen, B.; Lehmann, J.; Kinyangi, J.; Smernik, R.; Riha, S.; Engelhard, M. Long-term black carbon dynamics in cultivated soil. *Biogeochemistry* **2009**, *92*, 163–176. [CrossRef]
18. Farrell, M.; Macdonald, L.M.; Butler, G.; Chirino-Valle, I.; Condrom, L.M. Biochar and fertiliser applications influence phosphorus fractionation and wheat yield. *Biol. Fertil. Soils* **2014**, *50*, 169–178. [CrossRef]
19. Gao, S.; De Luca, T.H.; Cleveland, C.C. Biochar additions alter phosphorus and nitrogen availability in agricultural ecosystems: A meta-analysis. *Sci. Total Environ.* **2019**, *654*, 463–472. [CrossRef] [PubMed]
20. Meng, L.; Sun, T.; Li, M.; Saleem, M.; Zhang, Q.; Wang, C. Soil-applied biochar increases microbial diversity and wheat plant performance under herbicide fomesafen stress. *Ecotoxicol. Environ. Saf.* **2019**, *171*, 75–83. [CrossRef]
21. Palansooriya, K.N.; Ok, Y.; Awad, Y.; Lee, S.; Sung, J.; Koutsospyros, A.; Moon, D. Impacts of biochar application on upland agriculture: A review. *J. Environ. Manag.* **2019**, *234*, 52–64. [CrossRef]
22. Park, J.H.; Lamb, D.; Paneerselvam, P.; Choppala, G.; Bolan, N.; Chung, J.W. Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils. *J. Hazard. Mater.* **2011**, *185*, 549–574. [CrossRef]
23. Yuan, J.H.; Xu, R.K.; Zhang, H. The forms of alkalis in the biochar produced from crop residues at different temperatures. *Bioresour. Technol.* **2011**, *102*, 3488–3497. [CrossRef]
24. Liang, B.; Lehmann, J.; Solomon, D.; Kinyang, J.; Grossman, J.; O'Neill, O.; Skjemstad, J.O.; Thies, J.; Luizao, F.J.; Petersen, J.; et al. Black carbon increases cation exchange capacity in soils. *Soil Sci. Soc. Am. J.* **2006**, *70*, 1719–1730. [CrossRef]
25. Xie, T.; Reddy, K.; Wang, C.W.; Yargicoglu, E.; Spokas, K. Characteristics and applications of biochar for environmental remediation: A review. *Crit. Rev. Environ. Sci. Technol.* **2015**, *45*, 939–969. [CrossRef]
26. Fellet, G.; Marchiol, L.; Delle Vedove, G.; Peressotti, A. Application of biochar on mine tailings: Effects and perspectives for land reclamation. *Chemosphere* **2011**, *83*, 1262–1267. [CrossRef] [PubMed]
27. Shen, Z.; Som, A.M.; Wang, F.; Jin, F.; McMillan, O.; Al-Tabbaa, A. Long-term impact of biochar on the immobilisation of nickel (II) and zinc (II) and the revegetation of a contaminated site. *Sci. Total Environ.* **2016**, *542*, 771–776. [CrossRef] [PubMed]
28. Yang, X.; Liu, J.; McGrouther, K.; Huang, H.; Lu, K.; Guo, X.; He, L.; Lin, X.; Che, L.; Ye, Z.; et al. Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in soil. *Environ. Sci. Pollut. Res.* **2016**, *23*, 974–984. [CrossRef]
29. Pastor-Jáuregui, R.; Paniagua-López, M.; Aguilar-Garrido, A.; Martín Peinado, F.; Sierra-Aragón, M. Long-term assessment of remediation treatments applied to an area affected by a mining spill. *Land Degrad. Dev.* **2021**, *32*, 2481–2492. [CrossRef]
30. Cabrera, F.; Clemente, L.; Díaz Barrientos, E.; López, R.; Murillo, J.M. Heavy metal pollution of soils affected by the Guadiamar toxic flood. *Sci. Total Environ.* **1999**, *242*, 117–129. [CrossRef]

31. IUSS (International Union of Soil Sciences). Working Group WRB. World Reference Base for Soil Resources. In *International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*; World Soil Resources Reports N 106; FAO: Rome, Italy, 2015.
32. Martín-Peinado, F.J.; Romero-Freire, A.; García-Fernández, I.; Sierra-Aragón, M.; Ortiz-Bernad, I.; Simón-Torres, M. Long-term contamination ina recovered area affected by a mining spill. *Sci. Total Environ.* **2015**, *514*, 219–223. [CrossRef]
33. Aguilar, J.; Dorronsoro, C.; Fernández, E.; Fernández, J.; García, I.; Martín, F.; Simón, M. Soil pollution by a pyrite mine spill in Spain: Evolution in time. *Environ. Pollut.* **2004**, *132*, 395–401. [CrossRef]
34. MAPA (Spanish Ministry of Agricultural, Fishing and Food) Ministerio de Agricultura, Pesca y Alimentación). Official Methods of Analysis. Métodos Oficiales de Análisis. In *Tomo III. Spanish Ministry of Agricultural, Fishing and Food Secretaría General Técnica del Ministerio de Agricultura*; Pesca y Alimentación: Madrid, Spain, 1994; Volume III, p. 195.
35. Sposito, G.; Lund, L.J.; Chang, A.C. Trace metal chemistry in arid-zone field soils amended with sewage sludge. I: Fractionation of Ni, Cu, Zn, Cd and Pb in solid phases. *Soil Sci. Soc. Am. J.* **1982**, *46*, 260–264. [CrossRef]
36. Quevauviller, P.; Lachica, M.; Barahona, E.; Gomez, A.; Rauret, G.; Ure, A.; Muntau, H. Certified reference material for the quality control of EDTA-and DTPA-extractable trace metal contents in calcareous soil (CRM 600). *Fresenius J. Anal. Chem.* **1998**, *360*, 505–511. [CrossRef]
37. U.S. EPA. *Method 3050B: Acid Digestion of Sediments, Sludges, and Soils*; Revision 2; EPA: Washington, DC, USA, 1996; p. 2.
38. García-Carmona, M.; García-Robles, H.; Turpín Torranoa, C.; Fernández Ondoño, E.; Lorite Moreno, J.; Sierra Aragón, M.; Martín Peinado, F.J. Residual pollution and vegetation distribution in amended soils 20 years after a pyrite mine tailings spill (Aznalcóllar, Spain). *Sci. Total Environ.* **2019**, *650*, 933–940. [CrossRef]
39. Zuur, A.; Ieno, E.; Elphick, C. A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* **2010**, *1*, 3–14. [CrossRef]
40. Tang, J.; Zhu, W.; Kookana, R.; Katayama, A. Characteristics of biochar and its application in remediation of contaminated soil. *J. Biosci. Bioeng.* **2013**, *116*, 653–659. [CrossRef] [PubMed]
41. Beesley, L.; Marmiroli, M. The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. *Environ. Pollut.* **2011**, *159*, 474–480. [CrossRef] [PubMed]
42. Karami, N.; Clemente, R.; Moreno-Jiménez, E.; Lepp, N.W.; Beesley, L. Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. *J. Hazard. Mater.* **2011**, *191*, 41–48. [CrossRef]
43. Simón, M.; García, I.; Martín, F.; Díez, M.; del Moral, F.; Sánchez, J.A. Remediation measures and displacement of pollutants in soils affected by the spill of a pyrite mine. *Sci. Total Environ.* **2008**, *407*, 23–39. [CrossRef]
44. Simón, M.; Ortiz, I.; García, I.; Fernández, E.; Fernández, J.; Dorronsoro, D.; Aguilar, J. Pollution of soils by the toxic spill of a pyrite mine (Aznalcollar, Spain). *Sci. Total Environ.* **1999**, *242*, 105–115. [CrossRef]
45. Decreteo 18/2015. *Official del 27 de Enero, por el que se Aprueba el Reglamento que Regula el Régimen Aplicable a los Suelos Contaminados*; Bulletiín Oficial de laof Andalusian Goverment, Junta de Andalucía: Andalusia, Spain, 2015; Volume 38, pp. 28–64.
46. Aguilar, J.; Dorronsoro, C.; Fernández, E.; Fernández, J.; García, I.; Martín, F.; Sierra, M.; Simón, M. Remediation of As-contaminated soils in the Guadiamar river basin (SW, Spain). *Water Air Soil Pollut.* **2007**, *180*, 109–118. [CrossRef]
47. Martín, F.; García, I.; Díez, M.; Sierra, M.; Simon, M.; Dorronsoro, C. Soil alteration by continued oxidation of pyrite tailings. *J. Appl. Geochem.* **2008**, *23*, 1152–1165. [CrossRef]
48. Galán Huertos, E.; Romero, A. Contaminación de Suelos por metales pesados. *Macla Rev. Soc. Esp. Mineral.* **2008**, *10*, 48–60.
49. Romero-Freire, A.; Sierra, M.; Ortiz, I.; Martín, F. Toxicity of arsenic in relation to soil properties: Implications to regulatory purposes. *J. Soils Sediments* **2014**, *14*, 968–979. [CrossRef]
50. Novak, J.M.; Busscher, W.J.; Laird, D.L.; Ahmedna, M.; Watts, D.W.; Niandou, M.A.S. Impact of Biochar Amendment on Fertility of a Southeastern Coastal Plain Soil. *Soil Sci.* **2009**, *174*, 105–112. [CrossRef]
51. Jiang, J.; Xu, R.K.; Jiang, T.Y.; Li, Z. Immobilization of Cu(II), Pb(II) and Cd(II) by the addition of rice straw derived biochar to a simulated polluted Ultisol. *J. Hazard. Mater.* **2012**, *229–230*, 145–150. [CrossRef] [PubMed]
52. Uchimiya, M.; Klasson, K.; Wartelle, L.; Lima, I. Influence of soil properties on heavy metal sequestration by biochar amendment: 1. Copper sorption isotherms and the release of cations. *Chemosphere* **2011**, *82*, 1431–1437. [CrossRef] [PubMed]
53. Kabata-Pendias, A. Trace Elements in Soils and Plants, 4th ed.CRC Press: Boca Raton, FL, USA; London, UK; New York, NY, USA, 2011; pp. 125–140.
54. Romero-Freire, A.; Martín, F.; van Gestel, C.A.M. Effect of soil properties on the toxicity of Pb: Assessment of the appropriateness of guideline values. *J. Hazard. Mater.* **2015**, *289*, 46–53. [CrossRef] [PubMed]
55. Simón, M.; Diez, M.; González, V.; García, I.; Martín, F.; de Haro, S. Use of liming in the remediation of soils polluted by sulphide oxidation: A leaching-column study. *J. Hazard. Mater.* **2010**, *180*, 241–246. [CrossRef]
56. Udom, B.E.; Mbagwu, J.S.C.; Adesodun, K.; Agbim, N.N. Distributions of zinc, copper, cadmium and lead in a tropical ultisol after long-term disposal of sewage sludge. *Environ. Int.* **2004**, *30*, 467–470. [CrossRef]
57. Sauvé, S.; McBride, M.; Hendershot, W. Soil Solution Speciation of Lead (II): Effects of Organic Matter and pH. *Soil Sci. Soc. Am. J.* **1998**, *62*, 618–621. [CrossRef]
58. Acosta, A.J.; Arocena, J.M.; Faz, A. Speciation of arsenic in bulk and rhizosphere soils from artisanal cooperative mines in Bolivia. *Chemosphere* **2015**, *138*, 1014–1020. [CrossRef]
59. Warren, G.P.; Alloway, B.J.; Lepp, N.W.; Singh, B.; Bochereau, F.J.; Penny, C. Field trials to assess the uptake of arsenic by vegetables from contaminated soils and soil remediation with iron oxides. *Sci. Total Environ.* **2003**, *311*, 19–33. [CrossRef]

60. Sierra-Aragón, M.; Mitsui-Nakamaru, Y.; García-Carmona, M.; Martínez-Garzón, F.J.; Martín-Peinado, F.J. The role of organic amendment in soils affected by residual pollution of potentially harmful elements. *Chemosphere* **2019**, *237*, 124–549. [CrossRef]
61. Tang, W.W.; Zeng, G.M.; Gong, J.L.; Liang, J.; Xu, P.; Zhang, C.; Huang, B.B. Impact of humic/fulvic acid on the removal of heavy metals from aqueous solutions using nanomaterials: A review. *Sci. Total Environ.* **2014**, 468–469. [CrossRef] [PubMed]
62. Kohler, J.; Caravaca, F.; Azcón, R.; Díaz, G.; Roldán, A. The combination of compost addition and arbuscular mycorrhizal inoculation produced positive and synergistic effects on the phytomanagement of a semiarid mine tailing. *Sci. Total Environ.* **2015**, *514*, 42–48. [CrossRef] [PubMed]
63. Aguilar, J.; Gómez, J.L.; Galán, E. Criterios y estándares para declarar un suelo contaminado en Andalucía y la metodología y técnicas de toma de muestra y análisis para su investigación. In *Investigación y desarrollo Medioambiental en Andalucia*; Universidad de Sevilla, Consejería de Medio Ambiente de la Junta de Andalucía: Andalucía, Spain, 1999; p. 257.
64. Ewers, W. Metals and Their Compounds in the Environment. In *Standards, Guidelines and Legislative Regulatory Concerning Metals and Their Compounds*; Merian, E., Ed.; VCH Publishers: Weinheim, Germany, 1991; pp. 707–711.
65. Bohn, H.L.; McNeal, B.L.; O'Connor, G.A. *Soil Chemistry*, 2nd ed.; Wiley & Sons Ed.: New York, NY, USA, 1985; pp. 257–260.
66. Fenner and Thompson. Germination. In *The Ecology of Seeds*; Cambridge University Press: Cambridge, UK, 2005; pp. 110–131.
67. Delgado-Caballero, M.; Alarcón-Herrera, M.; Valles-Aragón, M.; Melgoza, A.; Ojeda-Barrios, D.; Leyva-Chávez, A. Germination of Bouteloua dactyloides and Cynodon dactylon in a Multi-Polluted Soil. *Sustainability* **2017**, *9*, 81. [CrossRef]
68. García Robles, H. The Role of Gypsum in Bioremediation; Recovery of Ecosystem Functions and Services of Pollutes Soils. Ph.D. Dissertation, University of Granada, Granada, Spain, 2020; p. 278.
69. Beesley, L.; Inneh, O.S.; Norton, G.J.; Moreno-Jimenez, E.; Pardo, T.; Clemente, R.; Dawson, J.J. Assessing the influence of compost and biochar amendments on the mobility and toxicity of metals and arsenic in a naturally contaminated mine soil. *Environ. Pollut.* **2014**, *186*, 195–202. [CrossRef]
70. Chibuike, G.U.; Obiora, S. Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods. *Appl. Environ. Soil Sci.* **2014**, *2014*, 752708. [CrossRef]
71. Malizia, D.; Giuliano, A.; Ortaggi, G.; Masotti, A. Common plants as alternative analytical tools to monitor heavy metals in soil. *Chem. Cent. J.* **2012**, *6*, S6. [CrossRef]
72. Atkinson, C.; Fitzgerald, J.; Hipps, N. Potential Mechanisms for Achieving Agricultural Benefits from Biochar Application to Temperate Soils: A Review. *Plant Soil* **2010**, *337*, 1–18. [CrossRef]
73. Sohi, S.; Krull, E.; Lopez-Capel, E.; Bol, R. A Review of Biochar and Its Use and Function in Soil. *Adv. Agron.* **2010**, *105*, 47–82.
74. Rondon, M.; Lehmann, J.; Ramirez, O.J.; Hurtado, M.D. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. *Biol. Fertil. Soils* **2007**, *43*, 699–708. [CrossRef]
75. Chan, K.Y.; Van Zwieten, L.; Meszaros, I.; Downie, A.; Joseph, S. Agronomic Values of Green Waste Biochar as a Soil Amendment. *Aust. J. Soil Res.* **2007**, *45*, 629–634. [CrossRef]
76. Van Zwieten, L.; Kimber, S.; Morris, S.; Chan, K.Y.; Downie, A.; Rust, J.; Joseph, S.; Cowie, A. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant Soil* **2010**, *327*, 235–246. [CrossRef]
77. Kabas, S.; Faz, A.; Acosta, J.; Zornoza, R.; Martínez-Martínez, S.; Carmona, D.M.; Bech, J. Effect of marble waste and pig slurry on the growth of native vegetation and heavy metal mobility in a mine tailing pond. *J. Geochem. Explor.* **2012**, *123*, 69–76. [CrossRef]
78. De Chen, X.L.; Rongjun Bian, K.C.; Xuhui Zhang, J.Z.; Stephen Joseph, D.C.; Genxing Pan, L.L. Effects of biochar on availability and plant uptake of heavy metals—A meta-analysis. *J. Environ. Manag.* **2018**, *222*, 76–85. [CrossRef] [PubMed]
79. Bidar, G.; Garçon, G.; Pruvot, C.; Dewaele, D.; Cazier, F.; Douay, F.; Shirali, P. Behavior of Trifolium repens and Lolium perenne growing in a heavy metal contaminated field: Plant metal concentration and phytotoxicity. *Environ. Pollut.* **2007**, *147*, 546–553. [CrossRef] [PubMed]
80. Nirola, R.; Megharaj, M.; Aryal, R.; Naidu, R. Screening of metal uptake by plant colonizers growing on abandoned copper mine in Kapunda, South Australia. *Int. J. Phytoremediation* **2016**, *18*, 399–405. [CrossRef]