environments

Article

Biochar, Ochre, and Manure Maturation in an Acidic Technosol Helps Stabilize As and Pb in Soil and Allows Its Vegetation by *Salix triandra*

Melissa Simiele [1], Manhattan Lebrun [2], Sylvain Bourgerie [2], Dalila Trupiano [1], Gabriella Stefania Scippa [1] and Domenico Morabito [2,*]

[1] Department of Biosciences and Territory, University of Molise, 86090 Pesche, Italy; melissa.simiele@unimol.it (M.S.); dalila.trupiano@unimol.it (D.T.); scippa@unimol.it (G.S.S.)
[2] LBLGC EA 1207, INRAE USC1328, Orléans University, BP 6759, CEDEX 2, 45067 Orléans, France; manhattan.lebrun@univ-orleans.fr (M.L.); sylvain.bourgerie@univ-orleans.fr (S.B.)
* Correspondence: domenico.morabito@univ-orleans.fr

Abstract: Past mining extraction activities still have a negative impact in the present time, the resulting metal(loid) contaminated soils affecting both the environment and human health. Assisted phytostabilization technology, combining soil conditioner application to immobilize metal(loid)s and plant growth to reduce erosion and leaching risks, is a useful strategy in the restoration of metal(loid) contaminated lands. However, contaminants will respond differently to a particular amendment, having their own specific characteristics. Therefore, in multi-contaminated soils, soil conditioner combination has been suggested as a good strategy for metal(loid) immobilization. In the present study, in a mesocosm experiment, organic (biochar and manure) and inorganic (ochre) amendments were evaluated in single and combined applications for their effect on metal(loid) stabilization and *Salix triandra* growth improvement, in an arsenic and lead highly contaminated soil. Specifically, the effects of these amendments on soil properties, metal(loid) behavior, and plant growth were evaluated after they aged in the soil for 6 months. Results showed that all amendments, except biochar alone, could reduce soil acidity, with the best outcomes obtained with the three amendments combined. The combination of the three soil conditioners has also led to reducing soil lead availability. However, only ochre, alone or combined with the other soil fertilizers, was capable of immobilizing arsenic. Moreover, amendment application enhanced plant growth, without affecting arsenic accumulation. On the contrary, plants grown on all the amended soils, except plants grown on soil added with manure alone, showed higher lead concentration in leaves, which poses a risk of return of lead into the soil when leaves will shed in autumn. Considering that the best plant growth improvement, together with the lowest increase in lead aerial accumulation, was observed in manure-treated soil, the addition of manure seems to have potential in the restoration of arsenic and lead contaminated soil.

Keywords: aging; amendment; metal(loid); phytostabilization; *Salicaceae*

check for updates

Citation: Simiele, M.; Lebrun, M.; Bourgerie, S.; Trupiano, D.; Scippa, G.S.; Morabito, D. Biochar, Ochre, and Manure Maturation in an Acidic Technosol Helps Stabilize As and Pb in Soil and Allows Its Vegetation by *Salix triandra*. *Environments* **2022**, *9*, 87. https://doi.org/10.3390/environments9070087

Academic Editor: Yu-Pin Lin

Received: 8 June 2022
Accepted: 4 July 2022
Published: 8 July 2022

1. Introduction

The intensification of various human activities, including mining extraction, fossil fuel combustion, agricultural use of fertilizers and pesticides, and landfill leaching have led to soil contamination by metal(loid)s [1]. Metal(loid)s, such as arsenic, cadmium, chromium, lead, and mercury, are considered some of the most toxic elements in the environment [2]. Metal(loid) soil contamination is a serious and global environmental issue because, unlike organic contaminants, metal(loid)s cannot be degraded into harmless forms; therefore, they persist in soils for a long time [3]. Due to the absence of vegetation in contaminated soils, metal(loid)s may also leach into groundwater, and be transported to the surrounding environment via erosion, which could contaminate nearby agricultural soils, reducing yield and leading to entry into the food chain [4]. Consequently, there is an urgent need for the treatment and remediation of metal(loid)-contaminated soils.

Phytoremediation technology, which consists of the use of plants to reduce the toxicity and risks associated with the presence of contaminants in soils, has attracted increasing attention over the years [5]. Among different phytoremediation strategies, phytostabilization—the use of plants to reduce bioavailability and mobility of contaminants in soils and limit exposure to them [6]—is usually chosen over others. In phytostabilization methods, metal(loid) immobilization in the soil can be significantly increased by selecting appropriate plant species [7].

Willows are promising in both phytoextraction and phytostabilization strategies [8–10]. They can thrive on contaminated soils, and they have a high biomass production, and a deep and wide root system that is able to stabilize metal(loid)s into the soil [11]. Although willows can grow on metal(loid)-contaminated areas, high metal(loid) concentrations, associated with the poor physicochemical properties of these soils (extreme pH, low nutrient and organic matter content), prevent soil vegetation [12].

It follows that phytoremediation efficiency can be enhanced by overcoming two main challenges: (i) reducing metal(loid) toxicity by enhancing their stabilization in soil, and (ii) increasing the low fertility of contaminated lands. To overcome these issues, assisted phytostabilization, which consists of applying amendments and/or fertilizers to increase soil metal(loid) stabilization and ameliorate soil conditions, is often required [7].

Numerous amendments are used to immobilize metal(loid)s and reduce their bioavailability to facilitate the establishment of plants in metal(loid) contaminated soils, and they include both organic and inorganic soil conditioners [13]. Among different organic amendments, biochar—charcoal produced by the pyrolysis of various organic feedstocks—has received attention [14,15]. On the one hand, amending soils with biochar can reduce leaching and phytoavailability of metal(loid) cations by direct and indirect mechanisms [16]. Biochar interacts with metal(loid)s directly through negatively charged functional groups present on its surface (e.g., carboxyl, hydroxyl, and phenolic groups) [17]. Indirect mechanisms through which biochar immobilizes metal(loid)s in soil are related to its pH modification ability and high cation exchange enhancement capacity [16]. On the other hand, biochar can improve soil physicochemical and biological properties by ameliorating soil texture and structure, by enhancing nutrient availability and water retention [18], by modifying soil biological community composition and abundance [19].

Immobilization of metal(loid)s may also be performed by inorganic soil conditioners such as ochre [20]. Ochre is a by-product found in the outflows of some mining systems [21]. It has high levels of iron oxides, and for this reason, it is able to adsorb metal(loid)s (e.g., arsenic, lead, and zinc) reducing their availability [22], leaching, and toxicity [23].

Manure is commonly used as a fertilizer that improves soil physicochemical characteristics, and supplies organic matter, microorganisms, and essential nutrients (e.g., nitrogen, phosphorus, and potassium) that support plant growth [24]. Moreover, previous studies have demonstrated its ability to immobilize metal(loid)s such as cadmium, copper, lead, and zinc [24–26]. Nevertheless, manure organic matter content may be responsible for increased mobilization of certain metal(loid) ions, such as arsenic anions [27].

From the above, it is clear that a specific soil conditioner may be effective in immobilizing one metal(loid) but ineffective for another, or it may even increase the mobility of other metal(loid)s [28,29]. In addition, some soil conditioners, while effective in immobilizing metal(loid)s, and reducing their toxicity, are not able to provide nutrients and organic matter required for plant growth [23]. Since the ideal amendment should not only reduce the availability of toxic metal(loid)s in soil, but also improve soil characteristics and plant growth [30], it follows that the combined use of amendments and fertilizers could be a promising choice to assist phytostabilization of multi-metal(loid) contaminated areas [16]. For example, Beesley et al. [31] found that the low vegetation growth caused by nutrient deficiency in a biochar-amended substrate could be avoided by adding fertilizers, such as compost and manure, to the biochar medium, while in order to overcome the problem related to the mobilization of metal(loid) anions (such as arsenic anions), induced by organic matter content of some amendments (e.g., biochar, manure), iron-based soil conditioners, such as ochre, could be combined with organic amendments [32,33].

Impacts of amendments on soil properties and plant productivity in metal(loid)-contaminated lands vary not only with soil characteristics, plant species, amendment properties, and application rates, but also with aging time [34–36]. Among all, the soil conditioner aging time is one of the factors that influence the efficacy of phytoremediation and on which much clarity still needs to be given [37].

In this context, the present study is a follow-up of the study of Lebrun et al. [38], in which the authors showed that biochar, ochre, and manure, alone or combined, improved characteristics of a contaminated soil, which in turn allowed a better *Agrostis capillaris* plant growth, especially with manure, due to its high nutrient supply.

Based on these results and using the same specific soil—a former mine technosol being highly contaminated by arsenic and lead, and having extreme conditions (acidic pH, low organic matter and nutrient contents)—the goals of this study were to evaluate the effects of single and combined application of biochar, ochre, and manure, after 6 months of aging in soil, on (i) soil physicochemical properties, (ii) arsenic and lead immobilization, and (iii) *Salix triandra* growth.

2. Materials and Methods

2.1. Soil and Amendments

The studied soil was a technosol which is a type of soil that has been heavily modified due to long-term anthropic activities [39]. The technosol was sampled on the former silver-lead extraction mine located in the Pontgibaud district (Auvergne-Rhône-Alpes, France). The latest mining activity goes back to 1897 and led to a high contamination by arsenic (1068 mg·kg^{-1}) and lead (23,387 mg·kg^{-1}) [40]. The technosol was sampled at the second settling pond (GPS coordinates 45°47′27″ N and 2°49′38″ E), between 0 and 20 cm depth.

The biochar (B) was a commercial product (La Carbonerie, Crissey, France) derived from hardwood biomass (oak, beech and charm wafers and chips), slow pyrolyzed at a temperature of 500 °C with a heating rate of 2.5 °C·min^{-1}, and a 3 h residence time. It was crushed and sieved between 0.5 and 1 mm.

The ochre (I) was collected at a former charcoal mine (Alès) in France, as described in Thouin et al. [22], and it was dried and crushed to obtain a fine powder.

The cow manure (M) was provided by a local farmer located in Pontgibaud (GPS coordinates 45°47′26″ N and 2°49′44″ E).

The main physicochemical properties of the Pontgibaud technosol (P0%) and the three amendments used were determined in previous studies [38–43] and are presented in Table 1.

Table 1. Main physicochemical properties of Pontgibaud technosol (P0%) and the three used amendments: biochar (B), ochre (I), and manure (M). (n = 3; nd = non determined).

	P0% *	B *	I **	M *
pH	4.82 ± 0.01	8.46 ± 0.01	8.29 ± 0.03	9.5 ± 0.0
Electrical conductivity (µS·cm^{-1})	nd	302 ± 1	7765 ± 14	9476 ± 138
Redox potential (mV)	nd	nd	217 ± 9	88.2 ± 0.9
Water holding capacity (%)	29.8 ± 1	212 ± 4	nd	nd
Cation exchange capacity (cmol(+)·kg^{-1})	0.65 ± 0.02	<1.5	nd	nd
Specific surface area (m^2·g^{-1})	nd	4.38	nd	nd
Total pore volume (cm^3·g^{-1})	nd	0.01	nd	nd

Table 1. *Cont.*

	P0% *	B *	I **	M *
Mean pore diameter (nm)	nd	9.13	nd	nd
Content of carbon (%)	0.14 ± 0.03	79 ± 1	nd	nd
Content of hydrogen (%)	0.22 ± 0.00	1.74 ± 0.07	nd	nd
Content of nitrogen (%)	0.09 ± 0.02	2.4 ± 0.8	nd	nd
[As] ($mg \cdot kg^{-1}$) ***	1501 ± 326	0.9 ± 0.1	0.24 ± 0.09	0.8 ± 0.3
[Fe] ($mg \cdot kg^{-1}$) ***	6518 ± 1639	18 ± 5	1.04 ± 0.53	2.4 ± 0.2
[K] ($mg \cdot kg^{-1}$) ***	nd	752 ± 30	356 ± 0.5	16,096 ± 65
[P] ($mg \cdot kg^{-1}$) ***	nd	8 ± 1	0.63 ± 0.07	250 ± 3
[Pb] ($mg \cdot kg^{-1}$) ***	19,228 ± 1531	1.6 ± 5.1	0.31 ± 0.05	0.57 ± 0.06

* data from Nandillon et al. [40], Lebrun et al. [41], Lebrun et al. [42], and Lebrun et al. [43]. ** data from Lebrun et al. [38]. *** For Pontgibaud technosol, total concentrations of arsenic [As], iron [Fe], potassium [K], phosphorus [P], and lead [Pb] were previously determined in Lebrun et al. [43]. For the three amendments, phytoavailable [As], [Fe], [K], [P], and [Pb] were determined by a method of NH_4NO_3 extraction as described in Pueyo et al. [44].

2.2. Experimental Setup

A pot experiment was carried out using the contaminated soil manually mixed with the biochar, ochre, and cow manure, which were added both alone and in combination. All the three amendments were applied at the rate of 1% (*w*/*w*). The 1% application rate was used both to test a low dose of amendment (easier to apply in field studies) and to utilize the same rates reported in Lebrun et al. [38] because this study, as reported in the Introduction section, is based on the previous one [38].

Thus, the experiment included eight treatments (Table 2):

1. Untreated Pontgibaud soil (P0%);
2. P0% soil + 1% biochar (PB);
3. P0% soil + 1% ochre (PI);
4. P0% soil + 1% manure (PM);
5. P0% soil + 1% biochar + 1% ochre (PBI);
6. P0% soil + 1% biochar + 1% manure (PBM);
7. P0% soil + 1% ochre + 1% manure (PIM);
8. P0% soil + 1% biochar + 1% ochre + 1% manure (PBIM).

Table 2. Components and their percentage (%) of the eight used treatments (P0%, PB, PI, PM, PBI, PBM, PIM, and PBIM).

Treatment	Components	Percentage (%)
P0%	Pontgibaud technosol (P0%)	-
PB	Pontgibaud technosol (P0%)	-
	Biochar (B)	1%
PI	Pontgibaud technosol (P0%)	-
	Ochre (I)	1%
PM	Pontgibaud technosol (P0%)	-
	Manure (M)	1%
PBI	Pontgibaud technosol (P0%)	-
	Biochar (B)	1%
	Ochre (I)	1%
PBM	Pontgibaud technosol (P0%)	-
	Biochar (B)	1%
	Manure (M)	1%

Table 2. *Cont.*

Treatment	Components	Percentage (%)
PIM	Pontgibaud technosol (P0%)	-
	Ochre (I)	1%
	Manure (M)	1%
PBIM	Pontgibaud technosol (P0%)	-
	Biochar (B)	1%
	Ochre (I)	1%
	Manure (M)	1%

Five replicates of each treatment were potted in plastic pots. Pots were then watered every 2 days for 6 months to maintain moisture and let the mixtures mature, in order to evaluate the effect of soil conditioners after medium-time aging.

After this 6-month maturation, one non-rooted cutting of *Salix triandra* was placed in each pot, this was considered the beginning of the experiment (T0). Plants were grown for 60 days in a growth chamber under the following controlled conditions: day/night temperatures (22 °C/16 °C), 16 h of light/8 h of darkness, and with a light intensity of 400 W, and daily watered with tap water.

Thus, the current study had two novel aspects compared to Lebrun et al. [38]: (i) the assessment of the aging time of the soil conditioners; and (ii) the use of *Salix triandra* versus *Agrostis capillaris* plants.

The choice of the soil conditioner 6-month aging was based on the fact that the interactions among soil, soil improvers, contaminants, plants, and soil microbiome change over time. The short-term period (1–3 weeks) is dominated by the dissolution of soil improver compounds [45]. Therefore, changes in the physicochemical properties of the soil and soil conditioners can affect the initial phase of plant growth. In contrast, medium-term reactions (1–6 months) are characterized by plant root interception and interactions with soil conditioners [46]. Thus, because of the aims of the present study in studying how interactions between soil, soil conditioners, contaminant, and plants influence *Salix triandra* growth, health, and ability in phytoremediation, we decided to combine two medium-term periods: the 6-month amendment aging period with 60-day plant growth in the 8 amendment-treated soils. Moreover, we decide to use a medium-term study because, even though long-term reactions (1 or more years) are also very useful in understanding all the complex interactions occurring in soil, these studies are usually conducted in the field [45].

In the study by Lebrun et al. [38], the choice of *Agrostis capillaris* species was made based on the species that naturally colonize the Pontgibaud site. In our study, the choice was made on the basis that *Salix triandra* is a species that produces high biomass and, compared to *A. capillaris*, therefore might have a greater ability to stabilize or accumulate contaminants in phytoremediation strategies.

2.3. Soil Pore Water, Soil, and Plant Analysis

Soil pore water (SPW) was collected at the beginning (T0) and the end (T60) of the experiment in all potted soils using soil moisture samplers (Rhizon®, Rhizosphere Research Product, Wageningen, The Netherlands). The pH, electrical conductivity (EC), and redox potential (Eh) of SPW samples were directly measured using a multimeter (Mettler-Toledo, Serveur Excellence, Columbus, OH, USA). SPWs were then acidified (by HNO_3), as described in Bart et al. [47], and arsenic (As), and lead (Pb) concentrations were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (ULTIMA 2, HORIBA, Labcompare, San Francisco, CA, USA), using standard solutions prepared from mother solutions of As and Pb provided by Alfa Aesar-Fisher (Karlsruhe, Germany), and five-point calibration curves. The detection limits of the apparatus were 0.005 mg·L^{-1} for As and 0.001 mg·L^{-1} for Pb.

At T60, soils were sampled in each pot, and the phytoavailable fractions of As and Pb for the eight treatments were obtained using 1 M NH_4NO_3 extractant (solid/liquid ratio 1:5 w/v) as described by Qasim et al. [48]. As and Pb concentrations were measured using ICP-AES, as reported above.

At T60, plant leaves, stems, and roots were harvested, washed with tap water, rinsed with distilled water, and oven-dried at 60 °C for 72 h until constant weight, and then weighed for determining the leaf, stem, and root dry weight (DW). Dried leaves, stems, and roots were ground (propeller mill, IKA, Staufen, Germany) and, according to the protocol of Bart et al. [47], 200 mg of plant material were treated using an acid mixture (3 mL of HCl 37% and 6 mL of HNO 65%) and mineralized using a microwave (15 min increase up to 180 °C, 15 min hold at 180 °C, cooling) (Multiwave 5000, Anton Paar, Courtaboeuf, France). The digested samples were adjusted to 50 mL with deionized water and filtered by a HAWP Millipore membrane 0.45 µm [47], and As and Pb concentrations were measured by ICP-AES.

To evaluate the As and Pb uptake in *Salix triandra* plants, the translocation factor (TF) and the organ-specific (leaf, stem, and root) bioconcentration factor (BCF) were then calculated. The TF was calculated as the ratio of As or Pb concentration in aboveground (leaves and stems) to the concentrations in the belowground (roots) plant compartments. The BCF was calculated as the ratio of As or Pb concentrations in leaves, stems, and roots to the concentrations in the soil.

2.4. Aim of Analyses

This section reports the reasons why the specific analyses described above were conducted at the two different sampling times in the present study.

On SPW, analyses were conducted at both T0 and T60. Measurements made on SPW, at the T0 sampling time, allowed the determination of the effects of the three soil conditioners, subjected to 6 months of aging, on soil parameters (such as pH, EC, and Eh) and on the immobilization/mobilization and availability of As and Pb.

The determination of the same parameters on SPW (pH, EC, Eh, As and Pb concentrations), at T60, was aimed at assessing how the soil conditioner–plant interaction affected soil characteristics and the stabilization or accumulation of metal(loid)s.

Analyses conducted exclusively at the T60 sampling time, included measurements of (i) As and Pb phytoavailable concentration in untreated and soil conditioner-treated substrates; (ii) plant dry weight; (iii) and metal(loid) concentrations in *Salix triandra* organs (roots, stem, and leaves).

Measurements of metal(loid) phytoavailable concentration in the soil, such as those made in SPW, indicated how the soil conditioner-plant synergy had affected the availability of As and Pb.

Results obtained on plant dry weight could show the effects of the soil conditioners on the *Salix triandra* growth.

Finally, determinations of As and Pb concentrations in plants provided information about the stabilization and accumulation abilities of *Salix triandra* in soils treated with the three amendments.

2.5. Statistical Analysis

All statistical analyses were performed using R software version 3.4.3 (R Development Core Team, Vienna, Austria, 2017). Normality and homoscedasticity of the data were evaluated using Shapiro and Bartlett tests, respectively. Next, ANOVA and Kruskal–Wallis tests were performed for normal and non-normal data, respectively, followed by a post hoc test (Tukey HSD or pairwise Wilcox tests) to assess two-by-two differences. Differences were considered statistically significant at $p < 0.05$. Moreover, for soil pore water, a comparison between T0 and T60 was evaluated in each treatment, using the same procedure, but using the parametric Paired Student test for mean comparison, to assess "time effect".

3. Results and Discussion

3.1. Soil Pore Water pH

Table 3 shows the physicochemical properties of the soil pore water (SPW) collected, both at the beginning (T0) and the end (T60) of the experiment, from the control soil and the different soils added with the various combinations of the biochar (B), ochre (I), and cow manure (M).

Table 3. Soil pore water (SPW) pH, electrical conductivity (EC) (μS·cm^{-1}) and redox potential (Eh) (mV) determined in the eight studied treatments. SPWs were sampled at the beginning of the experiment (T0) and after 60 days of *Salix triandra* plant growth (T60). The "treatment effect" for each sampling time is represented by lowercase letters for T0 and capital letters for T60 ($p < 0.05$) ($n = 5 \pm$ SE). In detail, for each sampling time, different letters indicate a significant difference among the treatments. The letters "a" and "A" are used for the highest value found. Different treatments with at least one letter in common show no significant difference. Thus, different treatments not having any letter in common are significantly different from each other. "Time effect" for each parameter and treatment is shown by: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), ns (non-significant). In detail, the "time effect" concerns any differences found between the two sampling times.

Treatment	Sampling Time	pH	pH Time Effect	EC (μS·cm^{-1})	EC Time Effect	Eh (mV)	Eh Time Effect
P0%	T0	3.9 ± 0.0 c	ns	609 ± 63 c	**	455 ± 11 a	**
	T60	4.6 ± 0.3 C		1122 ± 126 B		399 ± 13 A	
PB	T0	4.9 ± 0.3 c	ns	664 ± 49 c	***	399 ± 14 b	ns
	T60	5.1 ± 0.2 C		1433 ± 113 AB		377 ± 11 A	
PI	T0	6.5 ± 0.1 ab	ns	1072 ± 70 b	*	307 ± 5 cd	**
	T60	6.2 ± 0.1 B		2088 ± 263 AB		339 ± 6 B	
PM	T0	5.9 ± 0.4 b	ns	1457 ± 131 ab	ns	341 ± 21 c	ns
	T60	6.5 ± 0.1 AB		1896 ± 503 AB		323 ± 5 BC	
PBI	T0	6.6 ± 0.1 ab	ns	1076 ± 28 b	ns	338 ± 9 cd	ns
	T60	6.4 ± 0.1 AB		1525 ± 186 AB		322 ± 5 BC	
PBM	T0	6.4 ± 0.1 b	ns	1722 ± 45 a	ns	313 ± 7 cd	ns
	T60	6.7 ± 0.0 AB		2089 ± 282 AB		322 ± 2 BC	
PIM	T0	6.6 ± 0.1 ab	ns	1782 ± 62 a	**	277 ± 2 d	***
	T60	6.7 ± 0.0 AB		2508 ± 201 A		308 ± 4 C	
PBIM	T0	7.2 ± 0.0 a	ns	1779 ± 190 a	ns	313 ± 9 cd	ns
	T60	6.9 ± 0.1 A		2247 ± 450 AB		300 ± 5 C	

P0%—Pontgibaud technosol; PB—Pontgibaud amended with 1% biochar; PI—Pontgibaud amended with 1% ochre; PM—Pontgibaud amended with 1% manure; PBI—Pontgibaud amended with 1% biochar and 1% ochre; PBM—Pontgibaud amended with 1% biochar and 1% manure; PIM—Pontgibaud amended with 1% ochre and 1% manure; PBIM—Pontgibaud amended with 1% biochar, 1% ochre and 1% manure.

At time T0, the SPW sampled from the non-treated Pontgibaud soil (P0%) had an acidic pH of 3.9. All the treatments, except the soil added with the biochar alone (PB), increased SPW pH (Table 3). In detail, SPW pH rose by 2.6 units in the treatment with the ochre (PI), by 2.0 units in the soil added with the cow manure (PM), and by 2.7 in the soil with the biochar–ochre combination (PBI). The combination of the manure with the biochar (PBM) and the ochre (PIM), and the combination of all the three soil conditioners (PBIM) increased SPW pH by 2.5, 2.7, and 3.3 units, respectively (Table 3).

Likely, the SPW pH rises were primarily due to the amendment alkalinity, although this was found to be true only for the ochre and manure but not for the biochar. As shown in Table 1, the ochre and cow manure were alkaline with a pH of 8.29 and 9.5, respectively.

Soil pH increases due to ochre addition have been observed previously [49] and attributed to the dissolution of calcite [50]. Indeed, calcite may be present as an impurity in ochre amendments, giving the material's highly alkaline nature [50].

Besides the alkalinity of the manure, which contributed to increasing SPW pH, the beneficial action of manure on raising pH may have been due to its ash, bicarbonate, and carbonate content, and to the presence of carboxyl, hydroxyl, and phenolic groups which can bind organic acids, and consequently buffer soil acidity [51]. Indeed, in general, the alkalinity of manure following the decarboxylation of organic anions and the ammonification of organic nitrogen (N) are the major causes of increases in soil pH [52].

The positive effects of biochar on soil acidity by increasing substrate pH are well known and documented in many different studies [53,54]. However, our results reported a negligible effect on SPW pH when the biochar was added alone to the Pontgibaud soil (PB), which is consistent with other findings [55,56], and could be attributed to the biochar aging. Indeed, Lebrun et al. [38], evaluating the effects of the soil improvers at T0, showed that in all the treated soils (including biochar-treated substrate) there was a SPW pH increase. Thus, in our research, biochar aging may induce changes in biochar physicochemical properties, and thus biochar effectiveness may fade [57]. The alkalinity of biochar may decrease due to the leaching of alkaline minerals and slow surface oxidation [55]. Moreover, the degradation of biochar properties (e.g., surface area, adsorption properties) was found to affect the potential capacity of biochar to amend soil acidity [58].

The reason for the fact that the aging of the amendments, in the other treatments in this experiment, did not lead to a decrease in pH but rather to an increase in it could be explained as follows. Usually, it is true that, during the aging process of soil organic fertilizers, microbial decomposition and mineralization of manure, resulting in the release of organic acids, also leads to decreases in soil pH [59]. However, over time, pH could return to higher values due to the buffering capacity of soil conditioners [60]. In our study, the increase of SPW pH values recorded in all manure treatments can be explained by the fact that manure was the amendment characterized by the highest pH value (Table 1). Although, the biochar had a higher pH value than ochre (Table 1), in ochre treatments, the pH still increased and was not negatively affected by aging; this is probably because the ochre, being an inorganic amendment, did not undergo the decomposition and mineralization processes that instead characterized soil conditioners of an organic nature [59].

Our results showed that the synergy amendment-plant did not affect the SPW pH because no difference was observed between the beginning (T0) and the end (T60) of the experiment (Table 3).

3.2. Soil Pore Water Electrical Conductivity and Redox Potential

At the beginning of the experiment (T0), SPW electrical conductivity (EC) was low in the P0% treatment (609 $\mu S \cdot cm^{-1}$) and increased by twofold on average in the six treated soils (PI, PM, PBI, PBM, PIM, and PBIM) with the exception of the PB condition (Table 3).

The increase in SPW EC as impacted by addition of the amendments might be due to the amount of dissolved salts in these soil conditioners [61–64]. Furthermore, the increase in SPW pH that occurred with the amendment addition (Table 3) probably raised salt dissolution and nutrient leaching [63,64]. The ochre and manure could release soluble alkaline elements (especially K^+, Table 1), enhancing EC values [63,65]. Although the biochar had a higher K^+ cation content than the ochre, it was characterized by a low electrical conductivity (Table 1). Indeed, the EC rise observed at time T0, in the PI, PM, PBI, PBM, PIM, and PBIM soils, might be due to the high EC of ochre and manure, 7765 $\mu S \cdot cm^{-1}$ and 9476 $\mu S \cdot cm^{-1}$ (Table 1), much higher than soil EC, while biochar had a lower EC (302 $\mu S \cdot cm^{-1}$) (Table 1). Likewise, Lebrun et al. [38] observed that the biochar alone did not have an effect on SPW EC because of its low EC. Manure is also known to have many functional groups, such as hydroxyls, carboxyl, and phenolic, on its surface that can be dissolved, dissociated, or protonated once the amendment is added to soil [16,51], resulting in higher SPW EC values.

At the end of the experiment (T60), a significant SPW EC rise was only found when the ochre and manure were added in combination to the soil (PIM) (2508 $\mu S \cdot cm^{-1}$) compared to the value in the P0% soil (1122 $\mu S \cdot cm^{-1}$). This increase could be related to the fact that the ochre and manure had the highest EC values and likely just their combination led to the EC rise at the end of our experiment.

Besides, SPW EC increased significantly between the beginning (T0) and the end (T60) of the experiment in the P0%, PB and PI treatments with a two-fold increase, and in PIM with a one-fold increase (Table 3). The differences observed between the two times of the experiment could be explained by different factors, such as plant growth, release of root exudates, and plant–amendment interaction [66]. Biochar and manure adsorption of root exudates may cause acidification and mineral dissolution that contribute to increase soil EC [67–69].

At time T0, SPW redox potential (Eh) was 455 mV in the untreated soil (P0%) and it decreased with the amendment application (Table 3), as observed in Lebrun et al. [38]. Indeed, an SPW Eh decrease was found in all the amended soils: PB (399 mV), PM (341 mV), PBI (338 mV), PBM (313 mV), PBIM (313 mV), PI (307 mV), and PIM (277 mV).

At time T60, except for the soil amended with biochar alone (PB), in the amended soils an SPW Eh decrease was observed with respect to the P0% condition (399 mV) (Table 3). SPW Eh decreased by 15% for PI, 19% for PM, PBI and PBM, 23% for PIM, and 25% for the PBIM treatment.

In addition, between the two times of sampling, SPW Eh decreased in the P0%, PI and PIM soils (Table 3). In this study, it was found that, on one hand, the addition of biochar, ochre, and manure to P0% soil increased SPW pH while, on the other hand, it reduced SPW Eh.

Showing that the Eh values followed an opposite trend to that of the pH values is in accordance with the fact that the two parameters are negatively correlated in soils [70].

Comparing the results of this study with those of Lebrun et al. [38] it would appear that, except for what was observed for the biochar, the 6-month aging of the soil improvers did not adversely affect their ability to improve the soil's physicochemical characteristics.

3.3. Phytoavailable Metal(Loid) Concentrations

3.3.1. Soil Pore Water Arsenic Concentration

The total concentration of metal(loid)s in soils does not necessarily provide information about their toxicity or environmental impact, whereas an estimation of the phytoavailable fraction may be very informative to predict the hazard posed by metal(loid)s towards plants and ecosystem functioning [71]. For these reasons, in the present study, the SPW and soil phytoavailable concentrations of As and Pb, in the eight studied treatments, were determined. Table 4 reports SPW metal(loid) concentrations, both at time T0 and T60 of the experiment.

Table 4. Soil pore water (SPW) arsenic [As] and lead [Pb] concentration ($mg \cdot L^{-1}$) determined in the eight studied treatments. SPWs were sampled at the beginning of the experiment (T0) and after 60 days of *Salix triandra* plant growth (T60). The "treatment effect" for each sampling time is represented by lowercase letters for T0 and capital letters for T60 ($p < 0.05$) ($n = 5 \pm$ SE). In detail, for each sampling time, different letters indicate a significant difference among the treatments. The letters "a" and "A" are used for the highest value found. Different treatments with at least one letter in common show no significant difference. Thus, different treatments not having any letter in common are significantly different from each other. "Time effect" for each parameter and treatment is shown by: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), ns (non-significant). In detail, the "time effect" concerns any differences found between the two sampling times.

Treatment	Sampling Time	[As] ($mg \cdot L^{-1}$)	[As] Time Effect	[Pb] ($mg \cdot L^{-1}$)	[Pb] Time Effect
P0%	T0	0.18 ± 0.01 a	***	7.5 ± 0.8 ab	ns
	T60	0.08 ± 0.01 ABC		6.1 ± 0.6 A	
PB	T0	0.15 ± 0.01 ab	***	6.7 ± 0.7 abc	**
	T60	0.05 ± 0.01 C		3.9 ± 0.2 BC	

Table 4. *Cont.*

Treatment	Sampling Time	[As] (mg·L^{-1})	[As] Time Effect	[Pb] (mg·L^{-1})	[Pb] Time Effect
PI	T0 T60	0.15 ± 0.00 b 0.07 ± 0.01 BC	***	4.4 ± 0.6 bc 4.1 ± 0.4 B	**ns**
PM	T0 T60	0.15 ± 0.02 ab 0.06 ± 0.01 BC	***	10.4 ± 2.3 a 2.7 ± 0.6 BCDE	**
PBI	T0 T60	0.13 ± 0.00 b 0.06 ± 0.01 BC	***	5.1 ± 0.1 bc 3.7 ± 0.4 BCD	*
PBM	T0 T60	0.15 ± 0.01 ab 0.12 ± 0.01 A	**ns**	7.9 ± 1.1 ab 1.8 ± 0.4 E	**
PIM	T0 T60	0.14 ± 0.01 b 0.10 ± 0.00 AB	*	5.2 ± 0.8 bc 2.6 ± 0.1 BCDE	*
PBIM	T0 T60	0.14 ± 0.00 b 0.10 ± 0.01 AB	*	3.4 ± 0.2 c 2.1 ± 0.5 E	*

P0%—Pontgibaud technosol; PB—Pontgibaud amended with 1% biochar; PI—Pontgibaud amended with 1% ochre; PM—Pontgibaud amended with 1% manure; PBI—Pontgibaud amended with 1% biochar and 1% ochre; PBM—Pontgibaud amended with 1% biochar and 1% manure; PIM—Pontgibaud amended with 1% ochre and 1% manure; PBIM—Pontgibaud amended with 1% biochar, 1% ochre and 1% manure.

As regards the As concentration in SPW, at time T0, all the treatments in which there was the addition of ochre to the soil (PI, PBI, PIM, and PBIM) led to a decrease of As concentration in SPW compared to the untreated P0% soil (Table 4). In detail, SPW As concentration decreased by 21% in PI, 28% in PBI soil, 25% in PIM, and 26% in PBIM treatment with respect to the P0% condition (0.18 mg·L^{-1}); whereas, treatments with only biochar and/or manure (PB, PM and PBM) had no effect on SPW As concentration (Table 4). On the contrary, Lebrun et al. [38] reported that there was no immediate effect from amendment application, either alone or combined, on SPW As concentration. However, the authors observed that at the beginning of the experiment, the biochar alone (PB) or in combination with the manure (PBM) increased soil phytoavailable As concentration [38]. In the present study, as reported above, SPW pH significantly increased after the application of soil amendments (Table 3), and many studies have reported a positive correlation between soil pH rise and soil desorption of As anions [72–75].

Owing to the SPW pH increase, in the PM, and PBM treatments no effect on As concentration was found in SPW. Although soil pH would appear to be the most important factor in influencing changes in soil metal(loid) bioavailability, amendment aging time also plays an important role [57]. During the biochar and manure aging processes, immobilized metal(loid)s in soils are at risk of release because their stability is affected by many factors, such as amendment pore structure, soluble organic carbon content and mineralization degree [57]. For example, aged biochar has less oxygen-containing functionality and mineral coating than fresh biochar [56], and this reduces its ability to immobilize metal(loid)s in soil.

Otherwise, if on one hand the ochre caused an SPW pH increase due to its own alkaline nature (Table 3), on the other hand the ochre addition in the PI, PBI, PIM, and PBIM treatments led to a decrease of SPW As concentration probably due to its characteristic composition with high content of iron (Fe) oxides which play an important role in As immobilization. Indeed, Olimah et al. [21] explained that the response of As in contaminated soils amended with ochre may appear as either increased As mobility due to a high pH or immobilization through the Fe oxide and oxyhydroxide phases (e.g., goethite and hematite) of ochre. Garau et al. [71] evaluated the efficiency of red mud to immobilize the arsenic present in a contaminated acidic soil and found that its addition caused a pH increase, while reduced the As mobility decreasing the water-soluble As concentration by two-fold compared to the untreated soil.

Between the beginning (T0) and the end (T60) of the experiment, in all the different treatments, with except the PMB, a decrease of As concentration in SPW was recorded (Table 4). These results may be explained by the complex interactions that are established in the soil among amendment, metal(loid), plant root system, and rhizosphere area [76]. Indeed, in the present study, such SPW As decreases might be attributed to the metalloid uptake in plant roots and, consequently, in the aboveground part.

3.3.2. Soil Pore Water Lead Concentration

Regarding the concentration of lead (Pb) in soil pore water (Table 4), at time T0, it decreased significantly only in the PBIM treatment (by 55%), with respect to the P0% control condition (7.5 mg·L^{-1}). In contrast, Lebrun et al. [38] found that in all the treated soils, there was a decrease of Pb in SPW compared to the untreated soil. As reported above, probably, the 6-month aging processes of the soil improvers reduced their ability to immobilize metal(loid)s in the soil.

The biochar, ochre, and cow manure may have changed soil physicochemical properties, enzyme activities, and microbial community structure, and thereby changed the bioavailability of Pb into the different amended soils. Indeed, several factors might have controlled the effectiveness of the three amendments for Pb immobilization in the contaminated soils. However, above all, it is soil pH which plays a significant role in controlling Pb mobility [77], and the SPW pH rises, observed in this study, after the addition of the three used amendments, were likely related to the Pb concentration decreases. The biochar and manure also contain several functional groups (e.g., carboxyl, hydroxyl, and phenolic groups) [16] on which Pb may be directly adsorbed. In addition, these groups may have increased negative surface charge of the soil able to chelate Pb cations and, consequently, decrease their concentration in SPW. Moreover, specific components in the biochar, ochre, and manure could contribute to reduce the mobility/availability of lead. Many metal(loid)s, among which lead, may co-precipitate with carbonates, phosphates, oxides, and hydroxides of biochar, ochre, and manure [57,78] to form compounds with an extremely low solubility, reducing, in turn, their own mobility and potential toxicity [52,79,80]. All these characteristics suggest that the biochar, ochre, and manure could have adsorbed or chelated Pb ions from the contaminated soils, thereby decreasing the metal concentration in the soil pore water. Moreover, all these different mechanisms related to each soil amendment might have a synergistic effect if amendments are used in combination with each other. Indeed, the soils added with the combination of all three soil conditioners (biochar, ochre, and manure) resulted in a greater decrease in the SPW Pb concentration (Table 4).

At the end of the experiment (T60), a SPW Pb concentration reduction was observed in all the amended soils compared to the P0% treatment (Table 4). In detail, Pb concentration decreased by 70% in PBM, 66% in PBIM, 58% in the PM treatment, 57% in PIM, 39% in the PBI soil, 36% in the PB treatment, and 33% in the PI condition with respect to the non-amended P0% soil (Table 4).

Moreover, as shown in Table 4, between the two times of sampling, a significant decrease of SPW Pb concentration was found in all the treatments except in the non-amended Pontgibaud soil (P0%), and the soil added with the ochre alone (PI). As explained above for arsenic, such SPW Pb decreases might be attributed to the uptake by *Salix triandra* plants.

3.3.3. Soil

Figure 1 shows As and Pb phytoavailable concentrations in soil measured after the 60 days of *Salix triandra* growth.

The results indicated that only the ochre addition to the Pontgibaud technosol (PI) led to a soil phytoavailable As concentration decrease by 26% with respect to the control condition (0.98 mg·kg^{-1}) (Figure 1a).

The low soil phytoavailable concentrations of Pb in the substrates added with the three soil improvers (Figure 1b), reflected its SPW concentrations which were registered at the end of the experiment (T60) (Table 4). Unlike what was observed for arsenic (Figure 1a), in all the amended soils there was a phytoavailable Pb concentration decrease with respect to the untreated Pontgibaud technosol (Figure 1b). Indeed, the phytoavailable concentration of Pb decreased from 539 mg·kg^{-1} in the P0% condition to 353 mg·kg^{-1} in the PB soil, 230 mg·kg^{-1} in the PI treatment, 154 mg·kg^{-1} in PM, 280 mg·kg^{-1} in the PBI soil, 147 mg·kg^{-1} in the PBM condition, 167 mg·kg^{-1} in the PIM treatment and 123 mg·kg^{-1} in PBIM (Figure 1b).

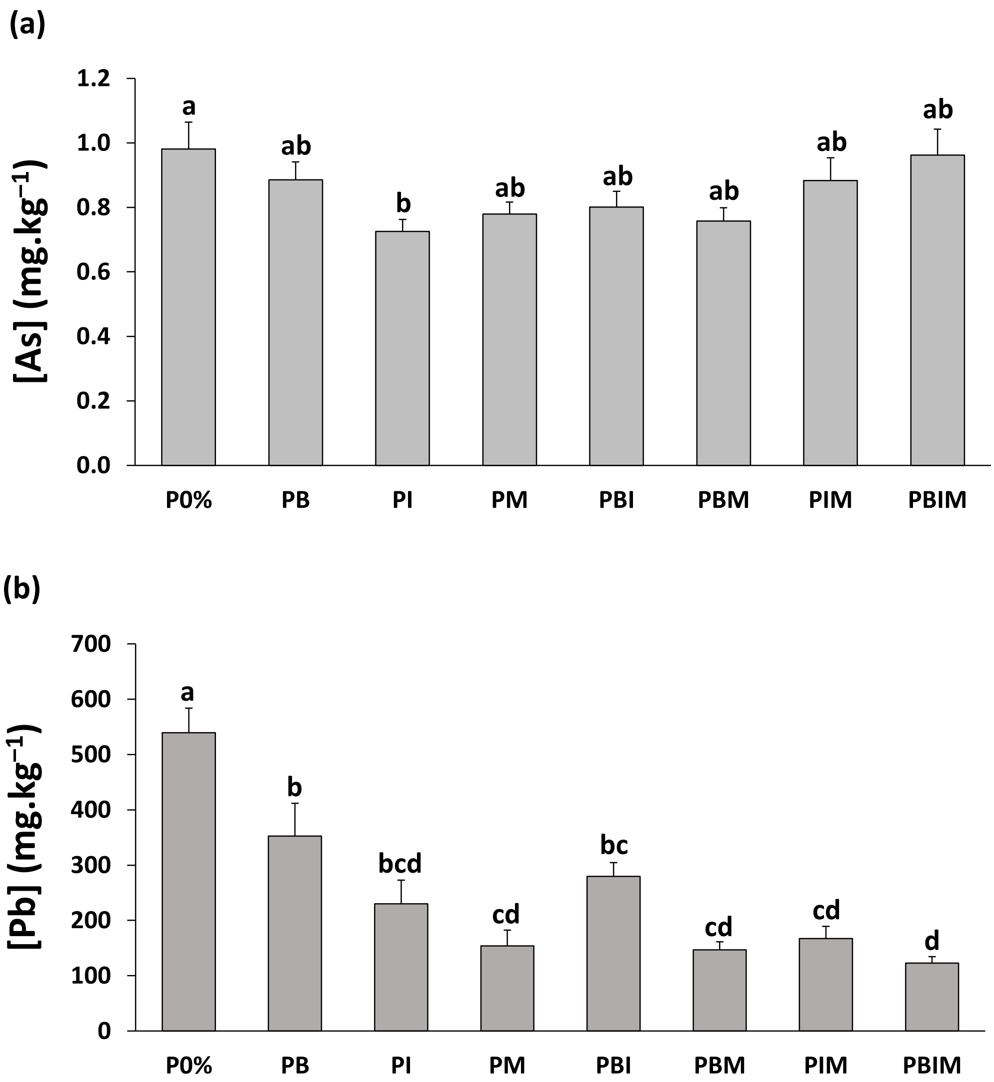

Figure 1. Soil phytoavailable arsenic (**a**) and lead (**b**) concentration (mg·kg^{-1}) determined after 60 days of *Salix triandra* growth (T60) in the eight studied treatments. Different letters indicate significant difference among the treatments ($p < 0.05$) ($n = 5$). The letter "a" is used for the highest value found. Different treatments with at least one letter in common show no significant difference. Thus, different treatments not having any letter in common are significantly different from each other. P0%—Pontgibaud technosol; PB—Pontgibaud amended with 1% biochar; PI—Pontgibaud amended with 1% ochre; PM—Pontgibaud amended with 1% manure; PBI—Pontgibaud amended with 1% biochar and 1% ochre; PBM—Pontgibaud amended with 1% biochar and 1% manure; PIM—Pontgibaud amended with 1% ochre and 1% manure; PBIM—Pontgibaud amended with 1% biochar, 1% ochre and 1% manure.

As reported previously in the present study, numerous complex interactions occur in the soil among contaminants, soil conditioners, and the rhizosphere [76]. What was found about phytoavailable arsenic concentrations can therefore be explained by the soil conditioner–*Salix triandra* plant interactions. Indeed, we found that among the three soil conditioners used in our experiment, only the ochre appeared to have an immobilizing effect on arsenic (see Table 4, at the T0 sampling time). Consequently, in the different treatments, the plants, as they grew, had similar amounts of arsenic available for uptake.

Additionally, indeed, as will be explained later in this paper (Section 3.4.2), in all the amended soils, arsenic concentrations measured at the level of the different plant organs did not show significant differences from those found in plants grown on the untreated Pontgibaud technosol. This could explain the fact that, at time T60, the concentrations found in the soil were almost the same in all treatments, except the PI treatment, compared to the P0% condition (Figure 1).

Moreover, our results showed that the As phytoavailable concentrations were extremely low (<1 mg·kg^{-1}) with respect to the total As concentration in the Pontgibaud soil (1501 mg·kg^{-1}, Table 1), testifying to a general scarce mobility of As.

Regarding Pb, as seen above, the soil improvers appeared to have no effect on soil Pb immobilization except when combined all three together (see Table 4, at the T0 sampling time). However, Pb availability in the soil decreased in all the treatments compared to the untreated soil (Figure 1b), and this, again, could be related to Pb uptake by plants.

In fact, *Salix traindra* plants, growing better on some of the soil treated with the soil conditioners (Figure 2), had greater biomass in which to assimilate greater amounts of the metal (Figures 2 and 3; Section 3.4).

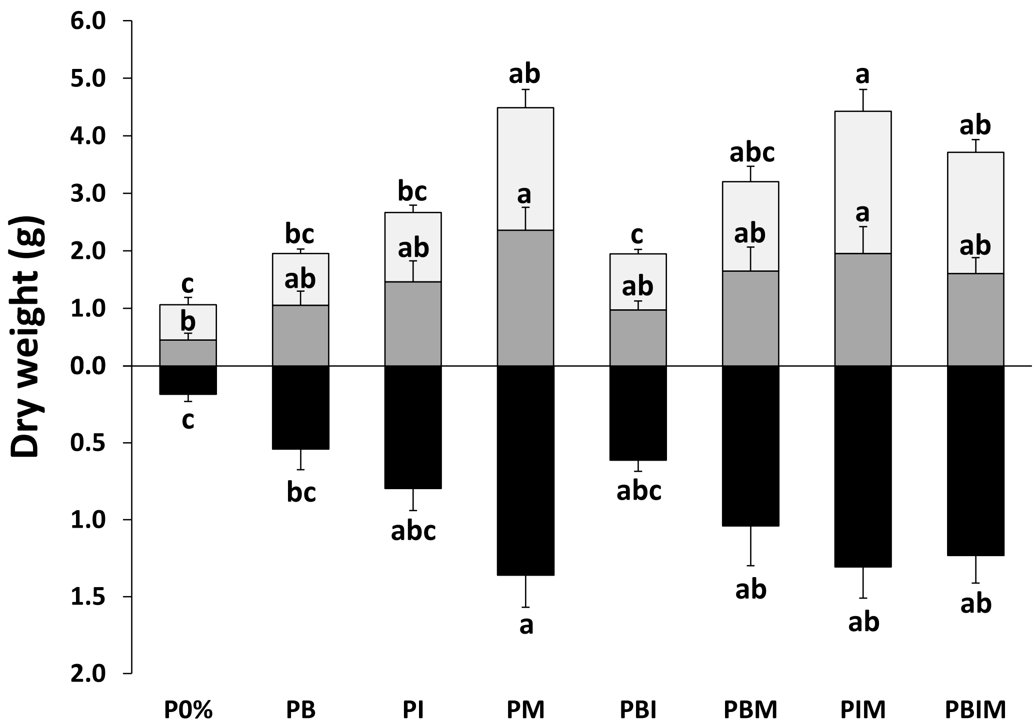

Figure 2. Dry weight (g) of roots (black box), stems (dark grey box) and leaves (light grey box) determined after 60 days of *Salix triandra* growth (T60) in the eight studied treatments. Different letters indicate significant difference among the treatments ($p < 0.05$) ($n = 5$). The letter "a" is used for the highest value found. Different treatments with at least one letter in common show no significant difference. Thus, different treatments not having any letter in common are significantly different from each other. P0%—Pontgibaud technosol; PB—Pontgibaud amended with 1% biochar; PI—Pontgibaud amended with 1% ochre; PM—Pontgibaud amended with 1% manure; PBI—Pontgibaud amended with 1% biochar and 1% ochre; PBM—Pontgibaud amended with 1% biochar and 1% manure; PIM—Pontgibaud amended with 1% ochre and 1% manure; PBIM—Pontgibaud amended with 1% biochar, 1% ochre and 1% manure.

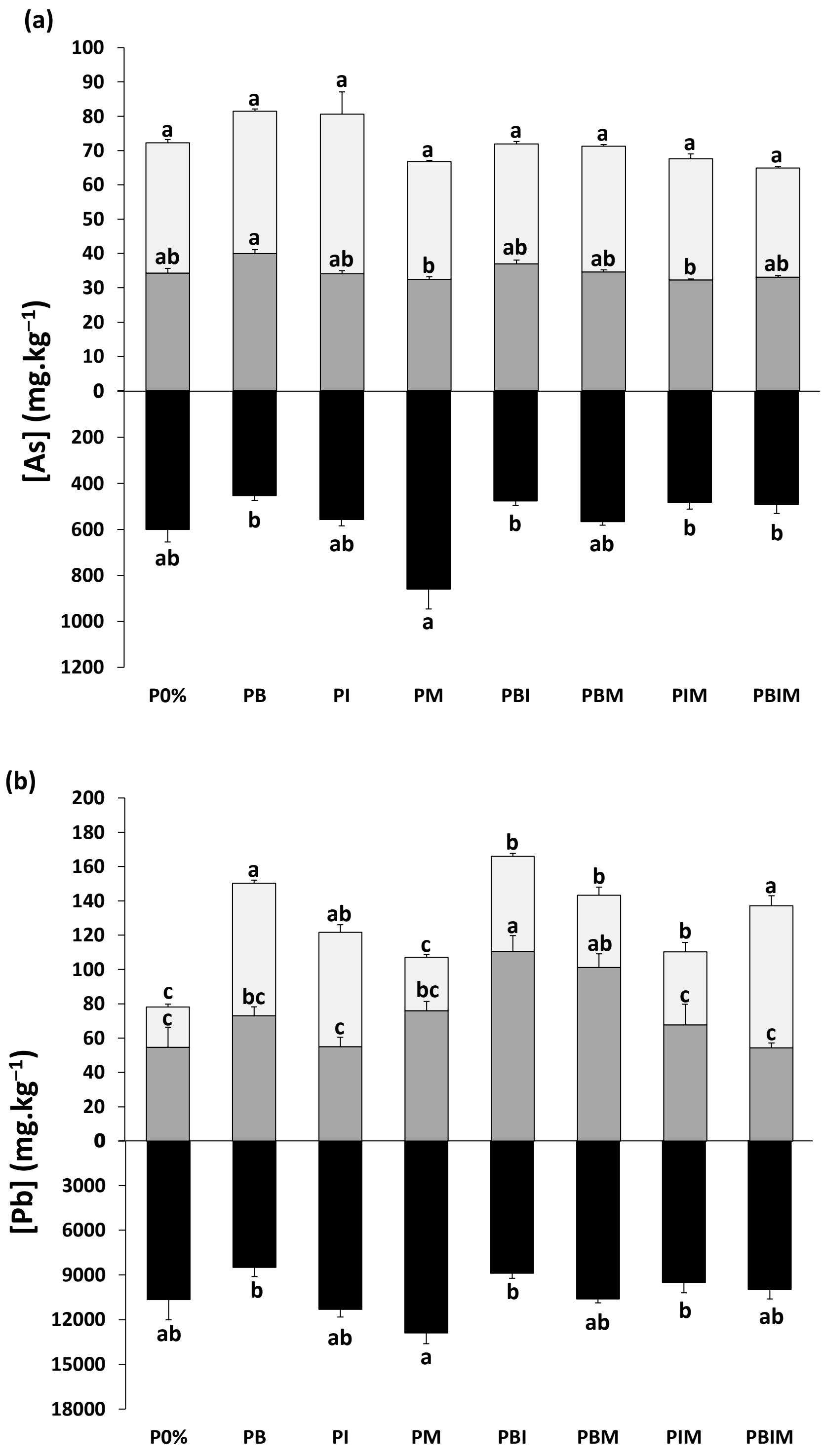

Figure 3. (**a**) Arsenic (mg·kg^{-1}) and (**b**) lead (mg·kg^{-1}) concentrations in roots (black box), stems (gray box) and leaves (white box) determined after 60 days of *Salix triandra* growth (T60) in the eight studied treatments. Different letters indicate significant difference among the treatments ($p < 0.05$) ($n = 5$). The letter "a" is used for the highest value found. Different treatments with at least one letter in common show no significant difference. Thus, different treatments not having any letter in common are significantly different from each other. P0%—Pontgibaud technosol; PB—Pontgibaud amended with 1% biochar; PI—Pontgibaud amended with 1% ochre; PM—Pontgibaud amended with 1% manure; PBI—Pontgibaud amended with 1% biochar and 1% ochre; PBM—Pontgibaud amended with 1% biochar and 1% manure; PIM—Pontgibaud amended with 1% ochre and 1% manure; PBIM—Pontgibaud amended with 1% biochar, 1% ochre and 1% manure.

3.4. *Salix triandra* Plants

3.4.1. Dry Weight

At the end of the experiment (T60), *Salix triandra* plants were harvested and the dry weight (DW) of the leaves, stems, and roots was determined (Figure 2).

Leaf DW of the plants grown on three soils added with manure (PM, PIM, and PBIM) was higher compared to the *S. triandra* plants grown on the untreated Pontgibaud soil (P0%). In detail, the leaf DW of the plants grown on the P0% treatment was 0.62 g, and the manure amendment, both alone (PM) and in combination with the biochar and ochre (PBIM), led to a three-fold increase of the leaf DW, while when the Pontgibaud soil was amended with the combination of the manure and ochre (PIM), the DW of leaves raised by four times (Figure 2).

The addition of the manure, in the PM and PIM conditions, also increased the stem DW of *S. triandra* as compared to the P0% control soil (Figure 2). The stem DW of the plants grown on the P0% soil was 0.45 g and it was raised to 2.36 g in the PM treatment, and to 1.95 g in the PIM condition (Figure 2).

In addition, the manure amendment also improved the root growth of the *S. triandra* plants in the PM, PBM, PIM and PBIM treatments with respect to the P0% soil. Indeed, the root DW increased from 0.18 g in the P0% condition to 1.36 g, 1.04 g, 1.31 g, and 1.23 g in the PM, PBM, PIM, and PBIM soils, respectively (Figure 2).

This improvement in biomass production was also reported in Lebrun et al. [38] and may be explained by (i) the observed amelioration in the soil physicochemical properties (Table 3), (ii) the reduction metal(loid) mobility and availability (Table 4, Figure 1), and (iii) the provision of nutrients by manure [38,81]. As described above, the addition of manure, alone or in combination with biochar and/or ochre, improved soil characteristics by increasing pH and electrical conductivity, and decreasing concentrations of phytoavailable lead and, therefore, the plants, being subjected to reduced metal toxicity may have had a rise in their growth and development. An enhanced growth of the *S. triandra* plants is also probably ascribed to the added manure either used alone or in combinations with the other two soil amendments. The cow manure could have improved soil nutrient availability for plant uptake and change soil microbial communities with positive effect on the *S. triandra* plant growth. Indeed, many studies reported that manure application to land played an important role in strongly and positively affecting soil properties and, consequently, plant growth by increasing soil organic carbon storage [52], soil nutrient content (e.g., nitrogen, phosphorus, and potassium), and soil pH [52,64]. Ozlu and Kumar [64], for example, found that manure application increased by 64% soil organic carbon concentration, and led to a higher nitrogen content (3.45 $g \cdot kg^{-1}$) with respect to control soil (2.24 $g \cdot kg^{-1}$). Moreover, soil alkalinization due to manure can raise the availability of soil nutrients to plants and it can thereby increase plant growth [52]. High values of soil pH may also induce variations in soil enzyme activity, increases in microbial biomass, and changes in microbial community structure, which might be important mechanisms for alleviating acid stress and metal(loid) toxicity into soil by providing a better environment for the growth of plants [82]. Thus, in the present study, the better growth of *S. triandra* plants found in the different treatments with respect to the control condition is almost certainly attributable to the manure use, and manure capacity to raise nutrient availability into soil.

3.4.2. Arsenic and Lead Concentrations

As shown in Figure 3a, the arsenic (As) concentrations in leaves, stems and roots of *S. triandra* plants grown on all the seven different amended soils were not significantly different compared with plants grown on the untreated Pontgibaud soil.

However, some differences were found in stem and root As concentrations among soils added with the three amendments (Figure 3a).

Figure 3b shows that, apart from the plants grown in the soil amended with manure alone (PM), *S. triandra* grown in all the other amended soils had an average of 2.5 times higher lead (Pb) concentrations in leaves than the plants in the P0% treatment (24 $mg \cdot kg^{-1}$).

Moreover, in the PB and PBIM conditions, *S. triandra* plants accumulated higher leaf Pb concentrations (77 mg·kg^{-1} and 83 mg·kg^{-1}, respectively) compared to the plants grown on the PBI (55 mg·kg^{-1}), PBM (42 mg·kg^{-1}) and PIM soil (43 mg·kg^{-1}) (Figure 3b).

In the PBI and PBM treatments, the plants had higher Pb concentrations in stems with respect to the P0%, PI, PIM, and PBIM soils (Figure 3b). Specifically, the stem Pb concentration in the plants grown on PBI and PBM (111 mg·kg^{-1} and 101 mg·kg^{-1}, respectively) was two-fold higher than the one registered in the other four above-mentioned treatments (Figure 3b).

Regarding Pb concentrations in *S. triandra* root system, no significant difference was found in the amended soils with respect to the P0% condition (Figure 3b). Nonetheless, the plants in the PM treatment accumulated a higher amount of Pb (12,895 mg·kg^{-1}) compared to the Pb concentration of about 8000 mg·kg^{-1} detected in the roots of *S. triandra* plants grown on the PB, PBI and PIM soils (Figure 3b).

Overall, the data obtained about the concentrations of arsenic and lead in the different organs (leaves, stems, and roots) of *S. triandra* plants show that there were no significant differences in the soils amended with the biochar, ochre, and cow manure compared to the untreated soil of Pontgibaud. However, these results should be analyzed by considering the data reported on the mobility, and phytoavailability of metal(loid)s (Figure 1 and Table 4) and what was observed regarding *S. triandra* growth (Figure 2). Indeed, on one hand, it was found that the different combinations of the three soil amendments did not have positive results on stabilizing arsenic in the soil, on the other hand these amendments decreased the lead phytoavailability (Figure 1). In addition, as shown in Figure 2, the plants grew better on the amended soils than on the Pontgibaud soil and better growth was observed especially in the treatments with the manure (PM, PBM, PIM and PBIM). It is likely that the higher plant growth and the reduced phytoavailability of metal(loid)s in the amended soils offset the lower biomass of the plants in the Pontgibaud control soil that had greater amounts of phytoavailable metal(loid)s.

In order to better understand the capacity of *S. triandra* to remove and accumulate arsenic and lead from the soil and transfer them from roots to stems and leaves, the bioconcentration factor (BCF) and the translocation factor (TF) were calculated, respectively (Table 5).

The BCF values in leaves, stems, and roots, for both As and Pb, reflected the data concerning the As and Pb concentrations in the different organs of *S. triandra* plants (Figure 3). Indeed, among the eight treatments, there were no differences in the leaf BCF of As, whereas stem BCF of As was higher in the PB treatment with respect to the PM and PIM conditions (Table 5a). Moreover, the plants grown on the PM soil had a higher As BCF in roots compared to the plants in the PB-, PBI-, PIM-, and PBIM-amended soils (Table 5a). As with arsenic, the values of the lead accumulation index in the different organs of *S. triandra* (Table 5b) also showed differences among the various treatments that closely followed the results observed in Figure 3b.

Table 5 also shows that the TF values, for both the metal(loid)s, had no significant difference in the amended soils compared to P0%. However, for As, the TF was two times higher in the PB condition than in the PM treatment (Table 5a).

It is interesting to see that, for both As and Pb, the BCF values were higher in roots than in stems and leaves and, moreover, the TF values in *S. triandra* did not exceed one (TF < 1) (Table 5). The BCF and TF values indicated that *S. triandra* plants immobilized arsenic and lead in roots and limited their translocation from roots to shoots. The accumulation of metal(loid)s in roots is considered an avoidance mechanism against metal(loid) toxicity [83] and in this way *S. triandra* may prevent the transport of non-essential metal(loid)s to the plant's most sensitive aboveground part, thus suggesting the utility of this plant species for phytostabilization of arsenic and lead in contaminated soils. Metal(loid) restriction in roots with a low translocation towards upper parts is consistent with the results of Simiele et al. [32], Bart et al. [47] and Lebrun et al. [84] who observed that *Salix* spp., growing on contaminated lands, are able to stabilize metal(loid)s at root system level. However, in our study, the low values for BCF and TF could be related to: (i) the concentrations of As and Pb in the soil that were significantly higher (1501 and 19,228 mg·kg^{-1} of As and Pb respectively, Table 1) than those in plant tissues; and (ii) the relatively low concentrations of phytoavailable As and Pb (Figure 1) compared to their total concentration in the Pontgibaud soil.

Table 5. Translocation factor (TF) and organ-specific bioconcentration factor (BCF) of arsenic (As) (a) and lead (Pb) (b) calculated for *Salix triandra* grown for 60 days on the eight studied treatments. For each parameter, different letters indicate significant difference among treatments ($p < 0.05$) ($n = 5$). The letter "a" is used for the highest value found. Different treatments with at least one letter in common show no significant difference. Thus, different treatments not having any letter in common are significantly different from each other.

(a)	TF As	Leaf BCF As	Stem BCF As	Root BCF As
P0%	0.14 ± 0.03 ab	0.036 ± 0.002 a	0.032 ± 0.002 ab	0.56 ± 0.09 ab
PB	0.18 ± 0.01 a	0.039 ± 0.001 a	0.037 ± 0.002 a	0.42 ± 0.03 b
PI	0.15 ± 0.03 ab	0.044 ± 0.011 a	0.032 ± 0.001 ab	0.54 ± 0.05 ab
PM	0.09 ± 0.02 b	0.032 ± 0.001 a	0.030 ± 0.001 b	0.81 ± 0.14 a
PBI	0.15 ± 0.01 ab	0.033 ± 0.001 a	0.035 ± 0.002 ab	0.45 ± 0.03 b
PBM	0.13 ± 0.01 ab	0.034 ± 0.001 a	0.032 ± 0.001 ab	0.53 ± 0.03 ab
PIM	0.14 ± 0.01 ab	0.033 ± 0.002 a	0.029 ± 0.001 b	0.45 ± 0.05 b
PBIM	0.15 ± 0.03 ab	0.030 ± 0.001 a	0.031 ± 0.001 ab	0.46 ± 0.06 b
(b)	**TF Pb**	**Leaf BCF Pb**	**Stem BCF Pb**	**Root BCF Pb**
P0%	0.008 ± 0.001 a	0. 001 ± $6.7 \cdot 10^{-5}$ a	0.0023 ± 0.0004 c	0.46 ± 0.05 ab
PB	0.018 ± 0.002 a	0.003 ± $6.9 \cdot 10^{-5}$ a	0.0031 ± 0.0002 bc	0.36 ± 0.02 b
PI	0.011 ± 0.001 a	0.003 ± $1.7 \cdot 10^{-4}$ a	0.0024 ± 0.0002 c	0.48 ± 0.02 ab
PM	0.008 ± 0.000 a	0.001 ± $5.9 \cdot 10^{-5}$ a	0.0032 ± 0.0002 bc	0.55 ± 0.03 a
PBI	0.019 ± 0.001 a	0.002 ± $6.6 \cdot 10^{-5}$ a	0.0047 ± 0.0004 a	0.38 ± 0.01 b
PBM	0.014 ± 0.000 a	0.002 ± $1.8 \cdot 10^{-4}$ a	0.0043 ± 0.0003 b	0.45 ± 0.01 ab
PIM	0.012 ± 0.001 a	0.002 ± $2.1 \cdot 10^{-4}$ a	0.0029 ± 0.0005 c	0.41 ± 0.03 b
PBIM	0.014 ± 0.001 a	0.004 ± $2.3 \cdot 10^{-4}$ a	0.0023 ± 0.0001 c	0.43 ± 0.02 ab

P0%—Pontgibaud technosol; PB—Pontgibaud amended with 1% biochar; PI—Pontgibaud amended with 1% ochre; PM—Pontgibaud amended with 1% manure; PBI—Pontgibaud amended with 1% biochar and 1% ochre; PBM—Pontgibaud amended with 1% biochar and 1% manure; PIM—Pontgibaud amended with 1% ochre and 1% manure; PBIM—Pontgibaud amended with 1% biochar, 1% ochre and 1% manure.

4. Conclusions

A mesocosm study was set up in order to evaluate the effect of different amendment combinations on soil properties, *Salix triandra* growth and metal(loid) accumulation, and thus their potential use in restoration of arsenic- and lead-contaminated mining soil. Results showed that several treatments with biochar, ochre, and manure improved soil characteristics, by reducing soil acidity. All the three amendments, both alone and in combination, were also able to immobilize lead into the soil. However, the treatments had no positive effects on the stabilization of arsenic. Moreover, plants grew better on the amended soils with respect to the acidic Pontgibaud technosol, especially when manure was used alone or in combination with the other soil amendments. In general, metal(loid) accumulation abilities of *Salix triandra* did not discriminate among amended soils. Nonetheless, the use of manure, both alone and in combination with biochar and ochre, would appear to have made greater improvements than the other treatments in both plant growth and ability to accumulate and store metal(loid)s at root system level. In summary, improved growth of *Salix triandra*, jointly with lower lead accumulation in the aerial part of the plants, was observed in soils amended with the manure. Therefore, this research showed that manure could be used together with biochar and/or ochre in contaminated areas to decrease lead leaching, availability and toxicity and improve soil fertility, plant growth and effectiveness in stabilizing metal(loid)s in soil.

In addition, the present study investigated how the aging time of soil amendments may affect the performance of phytostabilization strategies. In this regard, our results seem to confirm that aging time causes drastic changes in the physicochemical properties of organic soil conditioners and this, consequently, can positively or negatively affect their abilities to improve soil fertility and reduce the toxicity of certain metal(loid)s. However, pot studies cannot accurately simulate external factors such as rain, temperature variation, and sunlight which influence natural aging mechanisms. Long-term field trials to study the aging effects of soil amendments would be needed to provide good evidence of their temporal evolution. In addition, the microbial communities characterizing the different treatments should be studied in order to understand the correlations in the soil-metal(loid)-amendment-plant-microorganism system since it is known that microorganisms also play an important role in contaminant stabilization processes. Finally, future studies should be conducted to test different concentrations and combinations of soil improvers in order to find an effective strategy in the phytostabilization of arsenic, considering the failure obtained in this study for the immobilization of this metalloid.

Author Contributions: Conceptualization, S.B., D.T., G.S.S. and D.M.; methodology and formal analysis, M.L., S.B. and D.M.; software, validation and data curation, M.S.; writing—original draft preparation, M.S. and M.L.; writing—review and editing, M.S., M.L., S.B., D.T., G.S.S. and D.M.; supervision, S.B. and D.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Qayyum, S.; Khan, I.; Meng, K.; Zhao, Y.; Peng, C. A review on remediation technologies for heavy metals contaminated soil. *Cent. Asian J. Environ. Sci. Technol. Innov.* **2020**, *1*, 21–29.
2. He, Z.; Shentu, J.; Yang, X.; Baligar, V.C.; Zhang, T.; Stoffella, P.J. Heavy metal contamination of soils: Sources, indicators and assessment. *Ecol. Indic.* **2015**, *9*, 17–18.
3. Fajardo, C.; Costa, G.; Nande, M.; Botıas, P.; Garcıa-Cantalejo, J.; Martın, M. Pb, Cd, and Zn soil contamination: Monitoring functional and structural impacts on the microbiome. *Appl. Soil Ecol.* **2019**, *135*, 56–64. [CrossRef]
4. Ye, S.; Zeng, G.; Wu, H.; Zhang, C.; Dai, J.; Liang, J.; Yu, J.; Ren, X.; Yi, H.; Cheng, M.; et al. Biological technologies for the remediation of co-contaminated soil. *Crit. Rev. Biotechnol.* **2017**, *37*, 1062–1076. [CrossRef]
5. Jun, L.; Wei, H.; Aili, M.; Juan, N.; Hongyan, X.; Jingsong, H.; Yunhua, Z.; Cuiying, P. Effect of lychee biochar on the remediation of heavy metal-contaminated soil using sunflower: A field experiment. *Environ. Res.* **2020**, *188*, 109886. [CrossRef]
6. Zine, H.; Midhat, L.; Hakkou, R.; El Adnani, M.; Ouhammou, A. Guidelines for a phytomanagement plan by the phytostabilization of mining wastes. *Sci. Afr.* **2020**, *10*, e00654. [CrossRef]
7. Fiorentino, N.; Mori, M.; Cenvinzo, V.; Duri, L.G.; Gioia, L.; Visconti, D.; Fagnano, M. Assisted phytoremediation for restoring soil fertility in contaminated and degraded land. *Ital. J. Agron.* **2018**, *13*, 34–44.
8. Bajraktari, D.; Petrovska, B.B.; Zeneli, L.; Dimitrovska, A.; Kavrakovski, Z. Soil chemical evaluation and power plant ash impact on chemical properties of *Salix alba* L. (Fam. *Salicaceae*): The impact of bioaccumulation. *Toxicol. Res. Appl.* **2020**, *4*, 2397847320924849. [CrossRef]
9. Sabedot, S.; Bordignon, S.A.L.; Cunha, A.C.B. Ex situ method and *Salix* spp. to treat polluted soil with hydrocarbon. *Ciência e Nat.* **2019**, *41*, 13. [CrossRef]
10. Yang, W.; Wang, Y.; Liu, D.; Hussain, B.; Ding, Z.; Zhao, F.; Yang, X. Interactions between cadmium and zinc in uptake, accumulation and bioavailability for *Salix integra* with respect to phytoremediation. *Int. J. Phytoremediat.* **2020**, *22*, 628–637. [CrossRef]
11. Wani, K.A.; Sofi, Z.M.; Malik, J.A.; Wani, J.A. Phytoremediation of heavy metals using *Salix* (Willows). *Bioremediat. J.* **2020**, *2*, 161–174.
12. Mourinha, C.; Palma, P.; Alexandre, C.; Cruz, N.; Rodrigues, S.M.; Alvarenga, P. Potentially toxic elements' contamination of soils affected by mining activities in the Portuguese sector of the Iberian pyrite belt and optional remediation actions: A review. *Environments* **2022**, *9*, 11. [CrossRef]
13. Ashraf, S.; Ali, Q.; Zahir, Z.A.; Ashraf, S.; Asghar, H.N. Phytoremediation: Environmentally sustainable way for reclamation of heavy metal polluted soils. *Ecotoxicol. Environ. Saf.* **2019**, *174*, 714–727. [CrossRef]

14. Ahmad, M.; Rajapaksha, A.U.; Lim, J.; Zhang, M.; Bolan, N.; Mohan, D.; Vithanage, M.; Lee, S.; Ok, Y. Biochar as a sorbent for contaminant management in soil and water: A review. *Chemosphere* **2014**, *99*, 19–33. [CrossRef] [PubMed]
15. Lehmann, J.; Rillig, M.; Thies, J.; Masiello, C.; Hockaday, W.; Crowley, D. Biochar effects on soil biota—A review. *Soil Biol. Biochem.* **2011**, *43*, 1812–1836. [CrossRef]
16. Oustriere, N.; Marchand, L.; Rosette, G.; Friesl-Hanl, W.; Mench, M. Wood-derived-biochar combined with compost or iron grit for in situ stabilization of Cd, Pb, and Zn in a contaminated soil. *Environ. Sci. Pollut. Res.* **2017**, *24*, 7468–7481. [CrossRef]
17. Cheng, S.; Chen, T.; Xu, W.; Huang, J.; Jiang, S.; Yan, B. Application research of biochar for the remediation of soil heavy metals contamination: A review. *Molecules* **2020**, *25*, 3167. [CrossRef]
18. Rizwan, M.R.; lmriaz, M.; Huang, G.; Chhajro, M.A.; Liu, Y.; Fu, Q.; Zhu, J.; Ashraf, M.; Zafar, M.; Bashir, S.; et al. Immobilization of Pb and Cu in polluted soil by superphosphare, multi walled carbon nanotube, rice straw and its derived biochar. *Environ. Sci. Pollut. Res.* **2016**, *23*, 15532–15543. [CrossRef]
19. Egamberdieva, D.; Wirth, S.; Behrendt, U.; Abd_Allah, E.F.; Berg, G. Biochar treatment resulted in a combined effect on soybean growth promotion and a shift in plant growth promoting rhizobacteria. *Front. Microbiol.* **2016**, *7*, 209. [CrossRef]
20. Tiberg, C.; Kumpiene, J.; Gustafsson, J.P.; Marsz, A.; Persson, I.; Mench, M.; Kleja, D.B. Immobilization of Cu and As in two contaminated soils with zero-valent iron-Long-term performance and mechanisms. *J. Appl. Geochem.* **2016**, *67*, 144–152. [CrossRef]
21. Olimah, J.A.; Shaw, L.J.; Hodson, M.E. Does ochre have the potential to be a remedial treatment for As-contaminated soils? *Environ. Pollut.* **2015**, *206*, 150–158. [CrossRef] [PubMed]
22. Thouin, H.; Norini, M.P.; Le Forestier, L.; Gautret, P.; Motelica-Heino, M.; Breeze, D.; Gassaud, C.; Battaglia-Brunet, F. Microcosm-scale biogeochemical stabilization of Pb, As, Ba and Zn in mine tailings amended with manure and ochre. *J. Appl. Geochem.* **2019**, *111*, 104438. [CrossRef]
23. Zhou, R.; Liu, X.; Luo, L.; Zhou, Y.; Wei, J.; Chen, A.; Tang, L.; Wu, H.; Deng, Y.; Zhang, F.; et al. Remediation of Cu, Pb, Zn and Cd-contaminated agricultural soil using a combined red mud and compost amendment. *Int. Biodeterior. Biodegrad.* **2017**, *118*, 73–81. [CrossRef]
24. Kiran, Y.K.; Barkat, A.; Cui, X.; Feng, Y.; Pan, F.; Tang, L.; Yang, X. Cow manure and cow manure-derived biochar application as a soil amendment for reducing cadmium availability and accumulation by *Brassica chinensis* L. in acidic red soil. *J. Integr. Agric.* **2017**, *16*, 725–734. [CrossRef]
25. Huang, L.M.; Yu, G.W.; Zou, F.Z.; Long, X.X.; Wu, Q.T. Shift of soil bacterial community and decrease of metals bioavailability after immobilization of a multi-metal contaminated acidic soil by inorganic-organic mixed amendments: A field study. *Appl. Soil Ecol.* **2018**, *130*, 104–119. [CrossRef]
26. Tang, X.; Li, X.; Liu, X.; Hashim, M.Z.; Xu, J.; Brookes, P.C. Effects of inorganic and organic amendments on the uptake of lead and trace elements by *Brassica chinensis* grown in an acidic red soil. *Chemosphere* **2015**, *119*, 177–183. [CrossRef]
27. Deng, Y.; Huang, S.; Laird, D.A.; Wang, X.; Dong, C. Quantitative mechanisms of cadmium adsorption on rice straw-and swine manure-derived biochars. *Environ. Sci. Pollut. Res.* **2018**, *25*, 32418–32432. [CrossRef]
28. Simiele, M.; Lebrun, M.; Del Cioppo, G.; Scippa, S.G.; Trupiano, D.; Bourgerie, S.; Morabito, D. Evaluation of different amendment combinations associated with *Trifolium repens* to stabilize Pb and As in a mine-contaminated soil. *Water Air Soil Pollut.* **2020**, *231*, 539. [CrossRef]
29. Zhou, H.; Zhou, X.; Zeng, M.; Liao, B.H.; Liu, L.; Yang, W.T.; Wu, Y.M.; Qiu, Q.Y.; Wang, Y.J. Effects of combined amendments on heavy metal accumulation in rice (*Oryza sativa* L.) planted on contaminated paddy soil. *Ecotoxicol. Environ. Saf.* **2014**, *101*, 226–232. [CrossRef]
30. Garau, M.; Garau, G.; Diquattro, S.; Roggero, P.P.; Castaldi, P. Mobility, bioaccessibility and toxicity of potentially toxic elements in a contaminated soil treated with municipal solid waste compost. *Ecotoxicol. Environ. Saf.* **2019**, *186*, 109766. [CrossRef]
31. Beesley, L.; Moreno-Jiménez, E.; Gomez-Eyles, J.L.; Harris, E.; Robinson, 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]
32. Simiele, M.; Lebrun, M.; Miard, F.; Trupiano, D.; Poupart, P.; Forestier, O.; Scippa, G.S.; Bourgerie, S.; Morabito, D. Assisted phytoremediation of a former mine soil using biochar and iron sulphate: Effects on as soil immobilization and accumulation in three *Salicaceae* species. *Sci. Total Environ.* **2020**, *710*, 136203. [CrossRef]
33. Wu, C.; Cui, M.; Xue, S.; Li, W.; Huang, L.; Jiang, X.; Qian, Z. Remediation of arsenic-contaminated paddy soil by iron-modified biochar. *Environ. Sci. Pollut. Res.* **2018**, *25*, 20792–20801. [CrossRef]
34. Basalirwa, D.; Sudo, S.; Wacal, C.; Oo, A.Z.; Sasagawa, D.; Yamamoto, S.; Masunaga, T.; Nishihara, E. Impact of fresh and aged palm shell biochar on N_2O emissions, soil properties, nutrient content and yield of Komatsuna (*Brassica rapa* var. *perviridis*) under sandy soil conditions. *Soil Sci. Plant Nutr.* **2020**, *66*, 328–343. [CrossRef]
35. Jauregi, L.; Epelde, L.; Alkorta, I.; Garbisu, C. Antibiotic resistance in agricultural soil and crops associated to the application of cow manure-derived amendments from conventional and organic livestock farms. *Front. Vet. Sci.* **2020**, *8*, 153. [CrossRef] [PubMed]
36. Habibiandehkordi, R.; Quinton, J.N.; Surridge, B.W. Enhancing soluble phosphorus removal within buffer strips using industrial by-products. *Environ. Sci. Pollut. Res.* **2014**, *21*, 12257–12269. [CrossRef] [PubMed]
37. Trigo, C.; Spokas, K.A.; Cox, L.; Koskinen, W.C. Influence of soil biochar aging on sorption of the herbicides MCPA, nicosulfuron, terbuthylazine, indaziflam, and fluoroethyldiaminotriazine. *J. Agric. Food Chem.* **2014**, *62*, 10855–10860. [CrossRef]

38. Lebrun, M.; Nandillon, R.; Miard, F.; Le Forestier, L.; Morabito, D.; Bourgerie, S. Effects of biochar, ochre and manure amendments associated with a metallicolous ecotype of *Agrostis capillaris* on As and Pb stabilization of a former mine technosol. *Environ. Geochem. Health* **2021**, *43*, 1491–1505. [CrossRef]
39. WRB, IUSS WORORKINGKING GROROUPUP. World reference base for soil resources 2014: International soil classification system for naming soils and creating legends for soil maps. *World Soil Resour. Rep.* **2014**, *106*, 12–21.
40. Nandillon, R.; Lebrun, M.; Miard, F.; Gaillard, M.; Sabatier, S.; Morabito, D.; Bourgerie, S. Contrasted tolerance of *Agrostis capillaris* metallicolous and non-metallicolous ecotypes in the context of a mining technosol amended by biochar, compost and iron sulphate. *Environ. Geochem. Health* **2021**, *43*, 1457–1475. [CrossRef]
41. Lebrun, M.; Miard, F.; Nandillon, R.; Hattab-Hambli, N.; Scippa, G.S.; Bourgerie, S.; Morabito, D. Eco-restoration of a mine technosol according to biochar particle size and dose application: Study of soil physico-chemical properties and phytostabilization capacities of *Salix viminalis*. *J. Soils Sediments* **2018**, *18*, 2188–2202. [CrossRef]
42. Lebrun, M.; Miard, F.; Nandillon, R.; Scippa, G.S.; Bourgerie, S.; Morabito, D. Biochar effect associated with compost and iron to promote Pb and As soil stabilization and *Salix viminalis* L. growth. *Chemosphere* **2019**, *222*, 810–822. [CrossRef] [PubMed]
43. Lebrun, M.; Van Poucke, R.; Miard, F.; Scippa, G.S.; Bourgerie, S.; Morabito, D.; Tack, F.M. Effects of carbon-based materials and redmuds on metal (loid) immobilization and growth of *Salix dasyclados* Wimm. on a former mine technosol contaminated by arsenic and lead. *Land Degrad. Dev.* **2021**, *32*, 467–481. [CrossRef]
44. Pueyo, M.; Lopez-Sanchez, J.F.; Rauret, G. Assessment of $CaCl_2$, $NaNO_3$ and NH_4NO_3 extraction procedures for the study of Cd, Cu, Pb and Zn extractability in contaminated soils. *Anal. Chim. Acta* **2004**, *504*, 217–226. [CrossRef]
45. Joseph, S.; Cowie, A.L.; Van Zwieten, L.; Bolan, N.; Budai, A.; Buss, W.; Cayuela, M.L.; Graber, E.R.; Ippolito, J.A.; Kuzyakov, Y.; et al. How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. *GCB Bioenergy* **2021**, *13*, 1731–1764. [CrossRef]
46. Jaiswal, A.K.; Frenkel, O.; Elad, Y.; Lew, B.; Graber, E.R. Non-monotonic influence of biochar dose on bean seedling growth and susceptibility to *Rhizoctonia solani*: The shifted Rmax-effect. *Plant Soil* **2015**, *395*, 125–140. [CrossRef]
47. Bart, S.; Motelica-Heino, M.; Miard, F.; Joussein, E.; Soubrand, M.; Bourgerie, S.; Morabito, D. Phytostabilization of As, Sb and Pb by two willow species (*S. viminalis* and *S. purpurea*) on former mine technosols. *Catena* **2016**, *136*, 44–52.
48. Qasim, B.; Motelica-Heino, M.; Joussein, E.; Soubrand, M.; Gauthier, A. Potentially toxic element phytoavailability assessment in technosols from former smelting and mining areas. *Environ. Sci. Pollut. Res.* **2015**, *22*, 5961–5974. [CrossRef]
49. Nielsen, S.S.; Petersen, L.R.; Kjeldsen, P.; Jakobsen, R. Amendment of arsenic and chromium polluted soil from wood preservation by iron residues from water treatment. *Chemosphere* **2011**, *84*, 383–389. [CrossRef]
50. Doi, M.; Warren, G.; Hodson, M.E. A preliminary investigation into the use of ochre as a remedial amendment in arsenic-contaminated soils. *J. Appl. Geochem.* **2005**, *20*, 2207–2216. [CrossRef]
51. Ding, J.; Jiang, X.; Guan, D.; Zhao, B.; Ma, M.; Zhou, B.; Cao, F.; Yang, X.; Li, L.; Li, J. Influence of inorganic fertilizer and organic manure application on fungal communities in a long-term field experiment of Chinese Mollisols. *Appl. Soil Ecol.* **2017**, *111*, 114–122. [CrossRef]
52. Cai, A.; Xu, M.; Wang, B.; Zhang, W.; Liang, G.; Hou, E.; Luo, Y. Manure acts as a better fertilizer for increasing crop yields than synthetic fertilizer does by improving soil fertility. *Soil Tillage Res.* **2019**, *189*, 168–175. [CrossRef]
53. Palansooriya, K.N.; Ok, Y.S.; Awad, Y.M.; Lee, S.S.; Sung, J.K.; Koutsospyros, A.; Moon, D.H. Impacts of biochar application on upland agriculture: A review. *J. Environ. Manag.* **2019**, *234*, 52–64. [CrossRef]
54. Purakayastha, T.J.; Bera, T.; Bhaduri, D.; Sarkar, B.; Mandal, S.; Wade, P.; Kumari, S.; Biswas, S.; Menon, M.; Pathak, H.; et al. A review on biochar modulated soil condition improvements and nutrient dynamics concerning crop yields: Pathways to climate change mitigation and global food security. *Chemosphere* **2019**, *227*, 345–365. [CrossRef]
55. O'Connor, D.; Peng, T.; Zhang, J.; Tsang, D.C.W.; Alessi, D.S.; Shen, Z.; Bolan, N.S.; Hou, D. Biochar application for the remediation of heavy metal polluted land: A review of in situ field trials. *Sci. Total Environ.* **2018**, *619*, 815–826. [CrossRef] [PubMed]
56. Zhao, R.; Coles, N.; Kong, Z.; Wu, J. Effects of aged and fresh biochars on soil acidity under different incubation conditions. *Soil Tillage Res.* **2015**, *146*, 133–138. [CrossRef]
57. Yuan, C.; Gao, B.; Peng, Y.; Gao, X.; Fan, B.; Chen, Q. A meta-analysis of heavy metal bioavailability response to biochar aging: Importance of soil and biochar properties. *Sci. Total Environ.* **2021**, *756*, 144058. [CrossRef]
58. Liu, Z.; Demisie, W.; Zhang, M. Simulated degradation of biochar and its potential environmental implications. *Environ. Pollut.* **2013**, *179*, 146–152. [CrossRef]
59. Ahmad, M.; Ahmad, M.; El-Naggar, A.H.; Usman, A.R.; Abduljabbar, A.; Vithanage, M.; Elfaki, J.; Al-Faraj, A.; Al-Wabel, M.I. Aging effects of organic and inorganic fertilizers on phosphorus fractionation in a calcareous sandy loam soil. *Pedosphere* **2018**, *28*, 873–883. [CrossRef]
60. Zhao, R.; Coles, N.; Wu, J. Carbon mineralization following additions of fresh and aged biochar to an infertile soil. *Catena* **2015**, *125*, 183–189. [CrossRef]
61. Bhatt, M.K.; Labanya, R.; Joshi, H.C. Influence of long-term chemical fertilizers and organic manures on soil fertility—A review. *Univers. J. Agric. Res.* **2019**, *7*, 177–188. [CrossRef]
62. Ghorbani, M.; Asadi, H.; Abrishamkesh, S. Effects of rice husk biochar on selected soil properties and nitrate leaching in loamy sand and clay soil. *Int. Soil Water Conserv. Res.* **2019**, *7*, 258–265. [CrossRef]

63. Molahid, V.L.M.; Mohd Kusin, F.; Madzin, Z. Role of multiple substrates (spent mushroom compost, ochre, steel slag, and limestone) in passive remediation of metal-containing acid mine drainage. *Environ. Technol.* **2019**, *40*, 1323–1336. [CrossRef]
64. Ozlu, E.; Kumar, S. Response of soil organic carbon, pH, electrical conductivity, and water stable aggregates to long-term annual manure and inorganic fertilizer. *Soil Sci. Soc. Am. J.* **2018**, *82*, 1243–1251. [CrossRef]
65. Amarawansha, E.A.G.S.; Kumaragamage, D.; Flaten, D.; Zvomuya, F.; Tenuta, M. Phosphorus mobilization from manure-amended and unamended alkaline soils to overlying water during simulated flooding. *J. Environ. Qual.* **2015**, *44*, 1252–1262. [CrossRef]
66. Lambkin, D.C.; Gwilliam, K.H.; Layton, C.; Canti, M.G.; Piearce, T.G.; Hodson, M.E. Soil pH governs production rate of calcium carbonate secreted by the earthworm *Lumbricus terrestris*. *J. Appl. Geochem.* **2011**, *26*, S64–S66. [CrossRef]
67. Wang, L.; O'Connor, D.; Rinklebe, J.; Ok, Y.S.; Tsang, D.C.; Shen, Z.; Hou, D. Biochar aging: Mechanisms, physicochemical changes, assessment, and implications for field applications. *Environ. Sci. Technol.* **2020**, *54*, 14797–14814. [CrossRef]
68. Kunz, A.; Steinmetz, R.L.R.; Ramme, M.A.; Coldebella, A. Effect of storage time on swine manure solid separation efficiency by screening. *Bioresour. Technol.* **2009**, *100*, 1815–1818. [CrossRef]
69. Guo, X.X.; Liu, H.T.; Zhang, J. The role of biochar in organic waste composting and soil improvement: A review. *J. Waste Manag.* **2020**, *102*, 884–899. [CrossRef]
70. Husson, O.; Brunet, A.; Babre, D.; Charpentier, H.; Durand, M.; Sarthou, J.P.J.; Temp, M. Conservation agriculture systems alter the electrical characteristics (Eh, pH and EC) of four soil types in France. *Soil Tillage Res.* **2018**, *176*, 57–68. [CrossRef]
71. Garau, G.; Silvetti, M.; Deiana, S.; Deiana, P.; Castaldi, P. Long-term influence of red mud on as mobility and soil physico-chemical and microbial parameters in a polluted sub-acidic soil. *J. Hazard. Mater.* **2011**, *185*, 1241–1248. [CrossRef] [PubMed]
72. Álvarez-Benedí, J.; Bolado, S.; Cancillo, I.; Calvo, C.; Garcia-Sinovas, D. Adsorption–desorption of arsenate in three Spanish soils. *Vadose Zone J.* **2005**, *4*, 282–290. [CrossRef]
73. Kim, H.B.; Kim, S.H.; Jeon, E.K.; Kim, D.H.; Tsang, D.C.; Alessi, D.S.; Kwon, E.E.; Baek, K. Effect of dissolved organic carbon from sludge, rice straw and spent coffee ground biochar on the mobility of arsenic in soil. *Sci. Total Environ.* **2018**, *636*, 1241–1248. [CrossRef]
74. Wan, H.; Huang, Q.; Wang, Q.; Yu, Y.; Su, D.; Qiao, Q.; Li, H. Accumulation and bioavailability of heavy metals in an acid soil and their uptake by paddy rice under continuous application of chicken and swine manure. *J. Hazard. Mater.* **2020**, *384*, 121293. [CrossRef] [PubMed]
75. Zhang, H.; Selim, H.M. Kinetics of arsenate adsorption−desorption in soils. *Environ. Sci. Technol.* **2005**, *39*, 6101–6108. [CrossRef] [PubMed]
76. Yadav, V.; Jain, S.; Mishra, P.; Khare, P.; Shukla, A.K.; Karak, T.; Singh, A.K. Amelioration in nutrient mineralization and microbial activities of sandy loam soil by short term field aged biochar. *Appl. Soil Ecol.* **2019**, *138*, 144–155. [CrossRef]
77. Khaledian, Y.; Pereira, P.; Brevik, E.C.; Pundyte, N.; Paliulis, D. The influence of organic carbon and pH on heavy metals, potassium, and magnesium levels in Lithuanian Podzols. *Land Degrad. Dev.* **2017**, *28*, 345–354. [CrossRef]
78. Pan, Y.; Rossabi, J.; Pan, C.; Xie, X. Stabilisation/solidification characteristics of organic clay contaminated by lead when using cement. *J. Hazard. Mater.* **2019**, *362*, 132–139. [CrossRef]
79. Hua, Y.; Heal, K.V.; Friesl-Hanl, W. The use of red mud as an immobiliser for metal/metalloid-contaminated soil: A review. *J. Hazard. Mater.* **2017**, *325*, 17–30. [CrossRef]
80. Wang, T.; Sun, H.; Ren, X.; Li, B.; Mao, H. Evaluation of biochars from different stock materials as carriers of bacterial strain for remediation of heavy metal contaminated soil. *Sci. Rep.* **2017**, *7*, 12114. [CrossRef]
81. Jiang, W.; Hou, Q.; Yang, Z.; Zhong, C.; Zheng, G.; Yang, Z.; Li, J. Evaluation of potential effects of soil available phosphorus on soil arsenic availability and paddy rice inorganic arsenic content. *Environ. Pollut.* **2014**, *188*, 159–165. [CrossRef] [PubMed]
82. Peacock, A.D.; Mullen, C.L.; Ringelberg, D.B.; Tyler, C.M.; Hedrick, D.S.; Gale, P.M.; White, A.F. Soil microbial community responses to dairy manure or ammonium nitrate applications. *Soil Biol. Biochem.* **2001**, *33*, 1011–1019. [CrossRef]
83. Edelstein, M.; Ben-Hur, M. Heavy metals and metalloids: Sources, risks and strategies to reduce their accumulation in horticultural crops. *Sci. Hortic.* **2018**, *234*, 431–444. [CrossRef]
84. Lebrun, M.; Miard, F.; Scippa, G.S.; Hano, C.; Morabito, D.; Bourgerie, S. Effect of biochar and red mud amendment combinations on *Salix triandra* growth, metal(loid) accumulation and oxidative stress response. *Ecotoxicol. Environ. Saf.* **2020**, *195*, 110466. [CrossRef]