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Article

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Abstract: The contamination of soil by potentially toxic elements (PTEs) is a problem resulting from various anthropic activities including the exploitation of mines, which determines an accumulation of metal(loid)s in the surrounding area. It is therefore necessary to use remediation techniques to prevent the potential damage to human health and the ecosystem. One of these techniques is phytoremediation, which involves the revegetation of contaminated areas in such a way as to reduce the spread of contaminants and entry into the groundwater by stabilizing the metal(loid)s in the soil, decreasing their mobility. To increase the ability of plants to grow under the extreme conditions of contaminated soils, it is necessary to use amendments, which can also intervene directly in reducing the mobility of contaminants. In this study, an open-field mesocosm was set up using a former mining technosol contaminated mainly by As. A biochar produced from hardwood was added at two different depths to evaluate the effectiveness of these application modalities for an overall observation duration of 17 months. Iron sulphate was also applied in both non-biochar and biochar amended conditions. In addition, trees of *Alnus* sp. were planted to examine the effectiveness of these plants for their use in soil remediation and the effect of the treatments used. The results showed an increase in soil pH induced by the biochar, which decreased over time. During the period examined, the application of biochar in the deepest layer was able to retain As more effectively. The *Alnus* sp. showed similar growth rates among the various treatments, resulting from its tolerance towards arsenic.

Keywords: alder; phytoremediation; soil restoration; arsenic

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1. Introduction

Soil contamination by pollutants is one of the major problems of our modern society, which is correlated with industrial development. This pollution is responsible for the alteration of biogeochemical cycles, causing the degradation of ecosystems and risks to human health through multiple exposure routes such as entry into the food chain. The European Environment Agency [1] reported that over 300,000 contaminated sites have already been identified in Western Europe, and across Europe the overall estimated number is even higher. The main activities that cause soil contamination are related to human activities and urbanization such as the use of pesticides for agricultural purposes, the oil industry, incorrect waste disposal, the combustion processes, the use of solvents and the extraction and refining of metals and metalloids [2,3]. These activities can deteriorate the soil and cause irreversible damage.

Metal(loid)s are a major cause of environmental contamination and are mainly related to human activities such as mining, which has existed for many years and is still growing [4]. During mining activities, extraction residues are produced and deposited on site. These residues contain large quantities of potentially toxic elements (PTEs) including heavy metals (HM) such as Zn, Ni, Pb and Cu and metalloids such as As, which, due to leaching, diffusion and infiltration, are transported to the surface and/or ground water, resulting sometimes in acid drainage [5]. Heavy metals and many metalloids are toxic and can have negative effects, even at very low concentrations, such as the induction of oxidative stress through the production of free radicals. When dispersed in the environment, they can lead to a lack of soil capacity to host vegetation [6] by altering the chemico-physical properties of the soil and the soil pore water. Arsenic is one of the most dangerous metalloids for human health [7]. It can leach into groundwater, contaminating drinking water, and damaging the digestive and the cardio-respiratory systems. Thus, according to European Union legislation, total As concentration in drinking water must not exceed 10 $\mu g\ L^{-1}$ [8].

Various methods have been developed for the reduction of metal(loid) risks towards the environment and human health, through the removal or the stabilization of metal(loid)s. For this, different chemical and physical techniques have been developed, such as mechanical and pyrometallurgical separation, soil flushing, and soil washing [9]. However, a more sustainable and cost-effective technique has attracted attention over recent decades: phytoremediation [4,10]. Phytoremediation is an eco-friendly, green-based solution, which uses plants, associated or not with amendments, to reduce the risks induced by the pollution present in a soil. It includes a series of techniques, which can be used and selected according to the type of contamination and the nature of the contaminant, the characteristics of the site to be treated and the plant species used. One of the possibilities is the use of amendments to reduce the mobility and bioavailability of metals (stabilization) in a soil, to improve its agronomic qualities in order to allow the growth of plants that will limit the dispersion of pollutants by the wind or by leaching to the neighboring environmental compartments [11]. This technique, called assisted-phytostabilization, will allow, on a soil unsuitable for the plant development, the installation of a vegetation cover during successive growth seasons and the restoration of an ecosystem in which the pollution is sequestered. It will also have a positive aesthetic impact [12]. This technique is proposed for large areas of pollution where it is impossible to consider a physical and/or chemical technique that would be too expensive and disruptive to the soil.

In most contaminated soils, such as those of former mines, there are problematic conditions for the establishment of vegetation, especially the extreme pH and the low content of nutrients [13] and, therefore, the use of amendments is required to allow for plant growth [14,15]. An amendment that has shown excellent capabilities in this regard is biochar. It is an organic porous compound obtained mainly from the pyrolysis of vegetal biomass produced from agricultural and forest waste, manure or mineral sources, that can be used as soil improver. Biochar appears as a structured carbon matrix with a high surface/mass ratio, which allows it to absorb certain charged elements on its surface [16]. Adding biochar to soil improves the overall soil health and quality, root growth, soil biodiversity, and helps in the immobilization of contaminants. It allows a better water retention capacity, a pH increase, and long-term carbon sequestration. This finally permits the implementation and growth of plants [17]. The increase in pH and the biochar negative charges can cause a decrease in the bioavailability of positively charged ions like Pb but it has been shown to have no effect or to mobilize negatively charged anions like As [18]. To immobilize As in the presence of biochar, an Fe-based compound, such as iron sulphate, can be used due to the high affinity between As and Fe, and thus As mobility is reduced through adsorption or surface precipitation [19,20]. Mahmood-ul-Hassan et al. [21] proved that the As concentration of brown rice grain is considerably decreased when soils were amended with iron sulphate, which masks the impact of As toxicity on its growth and uptake. Thus, the possibility of combining biochar and iron sulphate on multi contaminated soil (with both anions and cations) could be an interesting solution. Indeed, Fe-containing chemicals are selected because Fe-(hydr)oxides display a high affinity for oxyanions such as arsenate [22]. Thus, in order to allow the stabilization of arsenic and prevent its mobility, iron sulfate could be added to polluted soils at the same time as biochar. It is proposed that the iron will associate with the biochar, which reduces the risk of iron loss by leaching and moreover it has been shown that once fixed on the biochar, the iron allows the fixation of arsenic by adsorption and precipitation processes [23–25]. Finally, it has been shown that the application of Fe-enriched adsorbents can alter the chemical and physical properties of soils and reduce the As chemo-availability [26].

Among the different plants proposed for phytoremediation, the *Alnus* tree genus could be used as it is a pioneer plant presenting rapid growth that is capable of adapting to soils with a low amount of nutrients. Moreover, through root symbiosis with bacteria, it can improve its assimilation of atmospheric nitrogen [27] and is able to produce an expanded root system [28].

The aim of this study was to evaluate the potential of a biochar + iron sulphate amendment associated with *Alnus* plant establishment for the phytostabilization of a former tin mine located in Abbaretz (Pays-de-la-Loire, France), highly contaminated with As. In more detail, the objectives were (i) to evaluate the biochar + iron sulphate effect and biochar depth incorporation effect on the physico-chemical properties of the soil pore water sampled at two depths; (ii) to assess *Alnus'* effect on the soil pore water; (iii) to observe the changes over time in the soil pore water's physico-chemical properties and concentration of As and Fe; and (iv) to determine the stem plant biomass and its concentration in As and Fe.

2. Material and Methods

2.1. Site of Study

The contaminated soil used for the present study was a post-mining soil situated at Abbaretz, (47°33′42″ North, 1°32′38″ West). The extractive activities were carried in the Gallo-Romanesque period and then during the twentieth century, to extract tin (Sn) mineral (Gloaguen et al. 2018). The ore mined consists mainly of cassiterite (tin oxide, SnO_2) in the first few meters, while sulphides appear more frequently at the deepest levels, always in the form of arsenopyrite. The average soil As concentration measured by the French National Geological Service (BRGM) in 2009 corresponds to 291 mg kg^{-1}. Due to As pollution, access to the mine is currently restricted. In addition to the high As soil pollution, acid mine drainage is present on this site: sulphide mineral residuals contaminated in the heaps, in contact with atmospheric air and (rain) water, oxidize and dissolve, generating acidic water (pH = 3 to 4) loaded with trace elements, dissolved metals, and sulphates. The Abbaretz technosol chemico-physical proprieties were obtained from [20] (Table 1).

Table 1. Physico-chemical properties of the soil and the amendments.

	Abbaretz Technosol *	Biochar **	Iron Sulphate **
pH	5.15 ± 0.04	8.46 ± 0.01	2.6 ± 00
Electrical conductivity ($\mu S \cdot cm^{-1}$)	16 ± 0.3	302 ± 1	Nd
Water holding capacity (%)	35 ± 0.1	212 ± 4	Nd
Cation exchange capacity ($cmol(+) \cdot kg^{-1}$)	Nd	<1.05	Nd
Specific surface area ($m^2 \cdot g^{-1}$)	Nd	4.38	Nd
Total pore volume ($cm^3 \cdot g^{-1}$)	Nd	0.01	Nd
Mean pore diameter (nm)	Nd	9.13	Nd
C (%)	Nd	79 ± 1	Nd
H (%)	Nd	1.74 ± 0.07	Nd
N (%)	Nd	2.4 ± 0.8	Nd
Total [As] ($mg \cdot kg^{-1}$)	297 ± 30	Nd	Nd
Phytoavailable [As] ($mg \cdot kg^{-1}$)	3.3 ± 0.7	0.9 ± 0.1	16.5 ± 0.5
Phytoavailable [Fe] ($mg \cdot kg^{-1}$)	9.3 ± 0.9	18 ± 5	23265 ± 299
Phytoavailable [P] ($mg \cdot kg^{-1}$)	9.7 ± 1.7	8 ± 1	25.4 ± 1.2
Phytoavailable [K] ($mg \cdot kg^{-1}$)	Nd	752 ± 30	7.9 ± 0.7

* data from Simiele et al. [20]; ** data from Lebrun et al. [29] and Nandillon et al. [15].

2.2. Biochar and Iron Sulphate

As reported by Simiele et al. [20], the biochar used was provided by La Carbonerie (Crissey, France) and derives from the slow pyrolysis (temperature of 500 °C with a heating rate of 2.5 $°C \cdot min^{-1}$ and a 3 h residence time) of oak, beech and charm wafers and chips. The pyrolysed material was crushed to obtain a final granulometry between 0.5–1 mm. The iron sulphate used was a commercial product START® from Star-Jardin (Nouâtre, France). The chemico-physical characterization of these materials as calculated in previous studies are shown in Table 1 [15,29].

2.3. *Alnus*

The alder plants used (*Alnus* sp.) were obtained from seedlings produced from seeds collected on a tree in a contaminated area located in Pontgibaud (Puy-de-Dôme, France) (Lambert II coordinates: X: 637,898.27 and Y: 2,087,992.92). Plants were grown for two years on a regular soil (compost 1/2 and garden soil 1/2) before they were transferred to the tested soils. The selected plants were of similar size and had comparable root systems. At the beginning of the experiment, the aerial parts of the plants were pruned at 10 cm from the ground.

2.4. Mesocosms Settlement

The present experiment was the continuation of the work undertaken by Simiele et al. [20]. It consisted of a mesocosm open-field settlement started in April 2017 at the National Forestry Office in Guéméné-Penfao (France). The experiment took place within 10 km of the polluted area so that the climatic and altimetric conditions were as close as possible to those observed on the site from which the polluted soils originated. Three conditions were set up in PVC tubes (100 cm height, 30 cm diameter). The first tested condition consisted of the contaminated soil collected from the mine (Abbaretz soil alone, AB), the second corresponded to AB soil mixed with 2% biochar on the 1/3 upper part of the cylinders (AB + 1/3 BC), the third tested condition corresponded to the cylinders filled with AB soil mixed with 2% biochar on the entire length (AB + BC). On March 2018, 25 g of iron sulphate was added on the top of each tube. After 1 year of soil maturation, one alder tree was planted per cylinder (seven replicates per treatment). For each of the three treatments, one batch of tubes was left without vegetation (seven replicates per treatment). The water supply was provided by rain and,, if necessary, the soils were watered with groundwater. A representation of the experimental design is given Figure 1a.

2.5. Soil pore Water (SPW) and Plant Organs Analysis

SPW was collected using Rhizons® (model MOM, Rhizosphere Research Products, Wageningen, The Netherlands) samplers placed at two depths, i.e., upper level (17 cm from above) and lower level (83 cm from above). The sampling at two depths was performed in order to evaluate the leaching potential of metal(loid)s and thus the possible composition of acid mine drainage. For each SPW sample, pH and electrical conductivity (EC) were measured (multimeter, Mettler-Toledo, Seven Excellence) and 5 mL of subsample were taken, acidified with 83 µL of 65% HNO_3 and stored at 4 °C before As and Fe quantification (Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), ULTIMA 2, HORIBA, Labcompare, San Francisco, CA, USA). The first set of SPW was collected in October 2019 (T7) and the second in October 2020 (T19). The monitoring of SPW is summarized in Figure 1b.

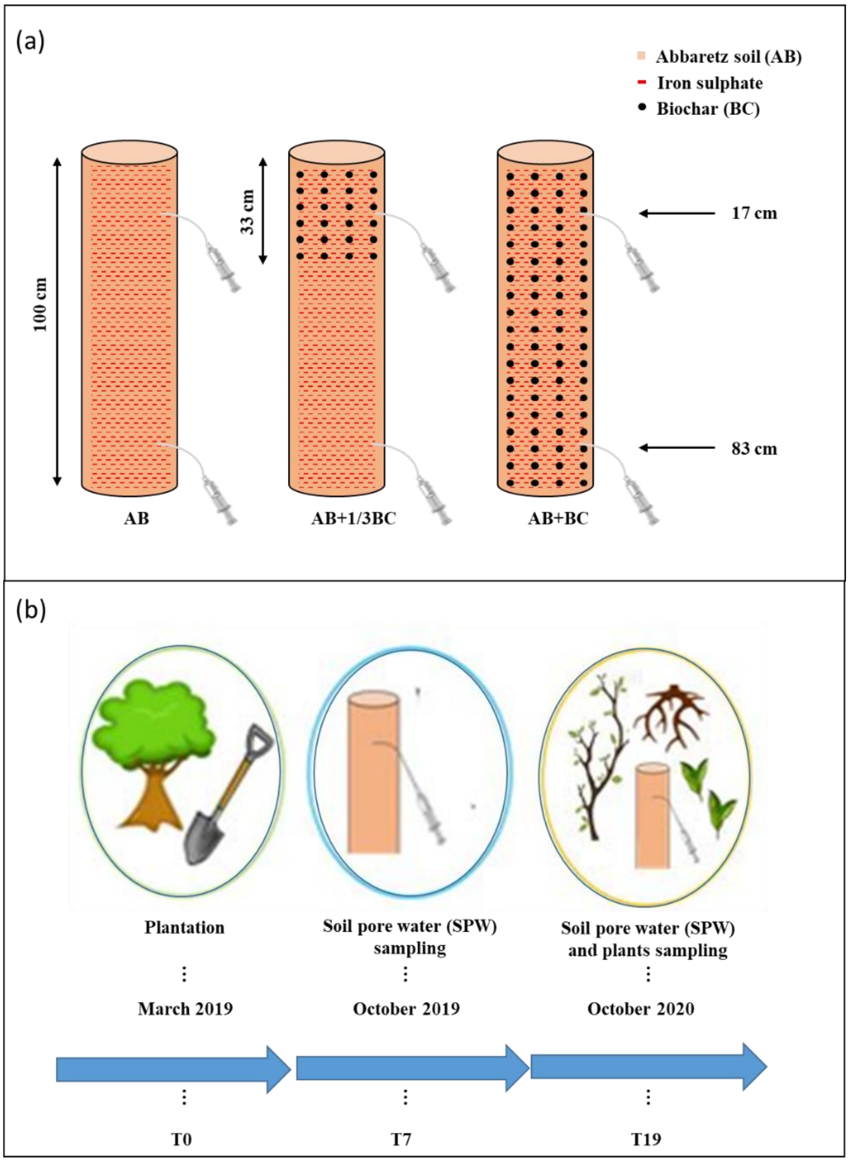


Figure 1. Representation of the experimental design (**a**) and monitoring (**b**) (adapted from [20]).

Plant organs were collected at T19 and were washed twice with tap water and once with distilled water (Figure 1b). Subsequently, the roots, stems and leaves were separated and dried at 50 °C for two days. The dried material was weighed for biomass determination and the different organs were crushed before mineralization. The mineralization procedure was obtained using 200 mg of plant material mixed with 3 mL of HCl (37%) and 6 mL of HNO_3 (65%) in a teflon tube. The tubes were then placed in a microwave (Multiwave 5000, Anton Paar, Courtaboeuf, France), the mineralization conditions were as follows: increase in temperature for 15 min to reach 160 °C, hold temperature for 30 min at 160 °C, and cool to room temperature for 30 min. The digested samples were then recovered and diluted with distilled water up to 50 mL. Finally, the solutions were filtered under vacuum with cellulose nitrate filter (0.45 µm). The As and Fe content was measured using ICP-AES.

2.6. Statistical Analysis

All statistical analyses were carried out using R (3.6.1) software (Vienna, Austria) [30]. In all cases, the Shapiro test was used to verify the normality of the data, then the Bartlett (if p-value was > 0.05) or Fligner (if p-value $\leq$ 0.05) tests were applied to verify the homogeneity of variance. Following this, the means of the three treatments (AB, AB + 1/3BC, and AB + BC) were compared for the vegetated and unvegetated conditions separately using the ANOVA (for parametric data) or Kruskal–Wallis (for non-parametric data) tests, followed by a post hoc test, i.e., Tukey HSD or pairwise Wilcox tests, respectively. Moreover, vegetation, time and level effects were evaluated for the soil pore water data. For this, the same procedure as described previously was used, except means were compared using the Student test (if p-value > 0.05) or the Wilcox test (if p-value $\leq$ 0.05). Difference was considered significant at $p < 0.05$.

3. Results and Discussion

3.1. SPW Chemico-Physical Properties

At T7, the pH of the control treatment AB was the most acidic, with values averaging from 6.1 to 6.5 for the unvegetated and vegetated condition, respectively, at the up level and 5.7 and 5.9 at the down level (Table 2).

Table 2. Soil pore water (SPW) chemico-physical properties (pH and electrical conductivity) determined on Abbaretz (AB) technosol non-amended or amended with 2% biochar added for the top 33 cm of the tube (AB + 1/3 BC) or for the entire tube (AB + BC) in the unvegetated tubes and in the tubes vegetated with Alnus sp. SPW was sampled both at the top (17 cm) and at the bottom (83 cm) of the tubes in October 2019 (T7) and in October 2020 (T19).

	Top			**Bottom**			**Level Effect**		
	AB	**AB + 1/3 BC**	**AB + BC**	**AB**	**AB + 1/3 BC**	**AB + BC**	**AB**	**AB + 1/3 BC**	**AB + BC**
pH-T7									
Unvegetated	6.1 ± 0.2b,A	7.1 ± 0.2a,A	7.0 ± 0.4ab,A	5.7 ± 0.4b,A	7.1 ± 0.2a,A	7.0 ± 0.1a,A	ns	ns	ns
Vegetated	6.5 ± 0.1b,A	6.9 ± 0.1a,A	6.8 ± 0.1ab,A	5.9 ± 0.1b,A	6.8 ± 0.1a,A	7.0 ± 0.1a,A	**	ns	ns
pH-T19									
Unvegetated	6.0 ± 0.1b,A	6.4 ± 0.1a,A	6.7 ± 0.1a,A	5.8 ± 0.1c,A	6.3 ± 0.1b,A	6.9 ± 0.1a,A	ns	ns	ns
Vegetated	6.1 ± 0.1a,A	6.1 ± 0.1a,B	6.3 ± 0.1a,B	6.0 ± 0.1b,A	6.2 ± 0.1b,A	6.7 ± 0.1a,A	ns	ns	ns
pH-Time effect									
Unvegetated	ns	*	ns	ns	**	ns			
Vegetated	ns	***	*	ns	***	*			
EC-T7									
Unvegetated	213 ± 46a,B	340 ± 88a,A	343 ± 100a,A	333 ± 56 a,A	417 ± 119a,A	360 ± 162a,A	ns	ns	ns
Vegetated	453 ± 47a,A	489 ± 53a,A	681 ± 186a,A	466 ± 68b,A	564 ± 41b,A	939 ± 83a,A	ns	ns	ns
EC-T19									
Unvegetated	176 ± 26a,A	261 ± 36a,A	199 ± 16a,A	231 ± 12c,A	389 ± 17b,A	610 ± 48a,A	ns	*	***
Vegetated	368 ± 11a,A	364 ± 7a,A	371 ± 6a,A	312 ± 26b,A	332 ± 30ab,A	343 ± 8a,A	***	**	***
EC-Time effect									
Unvegetated	ns	ns	ns	ns	ns	ns			
Vegetated	**	***	*	ns	*	**			

SPW pH and EC ($\mu S \cdot cm^{-1}$) values are means of seven values (±SE), the treatment effect in the vegetated and unvegetated conditions (taken separately) is represented by the lower-case letters while the vegetation effect inside a single treatment is represented by the capital letters ($p \leq 0.05$). The time and level effects for each treatment/(un)vegetated condition are given with level of significance: ns = non-significant ($p \geq 0.05$); * ($p \leq 0.05$); ** ($p \leq 0.01$); *** ($p \leq 0.001$).

At the top, for both vegetated and non-vegetated conditions, only the AB + 1/3BC treatment increased SPW pH, while at the bottom, both biochar treatments increased it in the non-vegetated and vegetated pots without a difference between the two biochar treatments (Table 2). At this time, the only level effect was observed for the AB vegetated condition, in which pH was higher at the top than at the bottom of the tube. At T19, the pH of the AB treatment at the upper level still had an acid pH (pH 6 at the top and pH 5.8 at the bottom). For the unvegetated condition, biochar significantly increased the SPW pH at the top level for both amended treatments. At the bottom level, the AB + BC treatment led to a higher pH increase (1.1 units) than the AB + 1/3 BC treatment (0.5 units) compared to the control (Table 2). In the presence of alder (vegetated condition), the pH with respect to the control increased only at the down level of the AB + BC treatment. No difference between top level and down level was observed at that time for all tested conditions (Table 2).

The increase in pH due to the addition of biochar to the contaminated soil could be associated with its alkalinity (pH 8.46), the dissolution of metal oxides, hydroxides and carbonates and the presence of surface functional groups that act as binding sites for H^+ [31,32]. The alkalinisation effect of biochar could have beneficial outcomes on the acid mine drainage found on the site by reducing its acidity as pH in biochar amended conditions were around neutrality.

When evaluating the effect of alder on SPW pH, only two significant modifications were observed: SPW pH was decreased by 0.3 and 0.4 units at the top of the tubes of the vegetated condition of AB + 1/3BC and AB + BC, respectively, at T19 (Table 2). Such a decrease could be due to the presence of the root system at this level [33], which is known to have an acidification effect [34] mediated by organic acid secretion and cation adsorption [14], and whose activity could be emphasized by the presence of biochar.

Finally, it can be seen that, in some conditions, SPW pH evolved with time, but only in the amended conditions: SPW pH decreased at T19 compared to T7 at the top and bottom of the tubes for the AB + 1/3BC (vegetated and unvegetated) and AB + BC (vegetated). Similarly, Cornelissen et al. [35] and Juriga and Šimanský [36] demonstrated that the beneficial action of biochar for pH tended to decrease over time. Such evolution attests to the reaction of the biochar with the soil particles but also the iron sulphate and root activity, which induced biochar oxidation [13,37], and the leaching of the alkaline materials [35,36].

At T7, the SPW EC values of AB treatment were 213 and 453 µS cm^{-1} at the top of the tubes for the vegetated and non-vegetated conditions, respectively, and 333 and 466 µS cm^{-1} at the bottom of the tubes, respectively (Table 2). In the unvegetated conditions, no biochar effect was observed, whatever the sample level; while in the vegetated condition, SPW EC was doubled in the AB + BC treatment compared to the control, but only at the bottom of the tube (Table 2). At the time of sampling, no difference in SPW EC was observed between the top and the bottom of the tube.

At T19, SPW EC values were still low on AB and did not evolve at the top of the tubes, in either vegetated or unvegetated tubes (Table 2). However, at the bottom of the tubes, in the unvegetated condition, both biochar treatments increased SPW EC but the treatment AB + BC led to a higher increase (610 µS cm^{-1}) than AB + 1/3BC (389 µS cm^{-1}) (Table 2). At that time, in the unvegetated condition, SPW EC was higher at the bottom than at the top of the tubes in AB + 1/3BC and AB + BC, while in the vegetated condition, SPW EC was lower at the bottom of the tubes than at the top. Biochar is known to have excess electrons in the π level of aromatic C and many functional groups, such as hydroxyls, carbonyls, carboxyls and phenolic hydroxyl, on its surface that can be dissolved, dissociated or protonated once biochar is added to the soil [38,39], resulting in higher SPW EC values. Furthermore, the increase in pH that occurred due to the addition of biochar probably increased salt dissolution and nutrient leaching [13]. The higher EC value at the bottom of the tubes, in the unvegetated condition, could be related to the leaching of the dissolved salts with soil watering, while the fact that EC values were higher at the top of the tubes in the vegetated condition could be attributed to the excretion of compounds by the roots, which are more developed at the top of the tubes, and a reduction of salt leaching through the presence of roots.

When assessing the evolution of the SPW EC with time, the unvegetated condition did not show different EC between T7 and T19, whatever the biochar treatment and the level, while in the vegetated condition, SPW EC decreased with time (from T7 to T19) in all cases except the bottom of AB (Table 2).

3.2. SPW Metal(Loid)s Concentration

The As concentration in the non-amended Abbaretz SPW at T7 (Figure 2a) was 0.60 mg·L^{-1} at the top level and 2.57 mg·L^{-1} at the bottom level for the non-vegetated condition, while for the vegetated condition, concentrations were 0.38 mg·L^{-1} and 1.23 mg·L^{-1}, respectively. At that time, there was no effect of the biochar amendment on SPW As concentration at either the top or bottom level in both vegetated and non-vegetated conditions, except for the increase in the SPW at the top level in the vegetated AB + 1/3BC compared to the vegetated AB. At T7, SPW As concentrations were higher at the bottom of the tubes compared to the top for the three tested conditions: unvegetated AB, vegetated AB and vegetated AB + BC (Figure 2a). A previous study showed that biochar did not possess a sorption site for As [40]. Furthermore, biochar was shown to induce As mobilization in the studies of [20] and [41]. Such an effect was related to the increase in soil pH, as observed here, which induces a decrease in the number of positively charged sites, and thus less sorption sites for As [42].

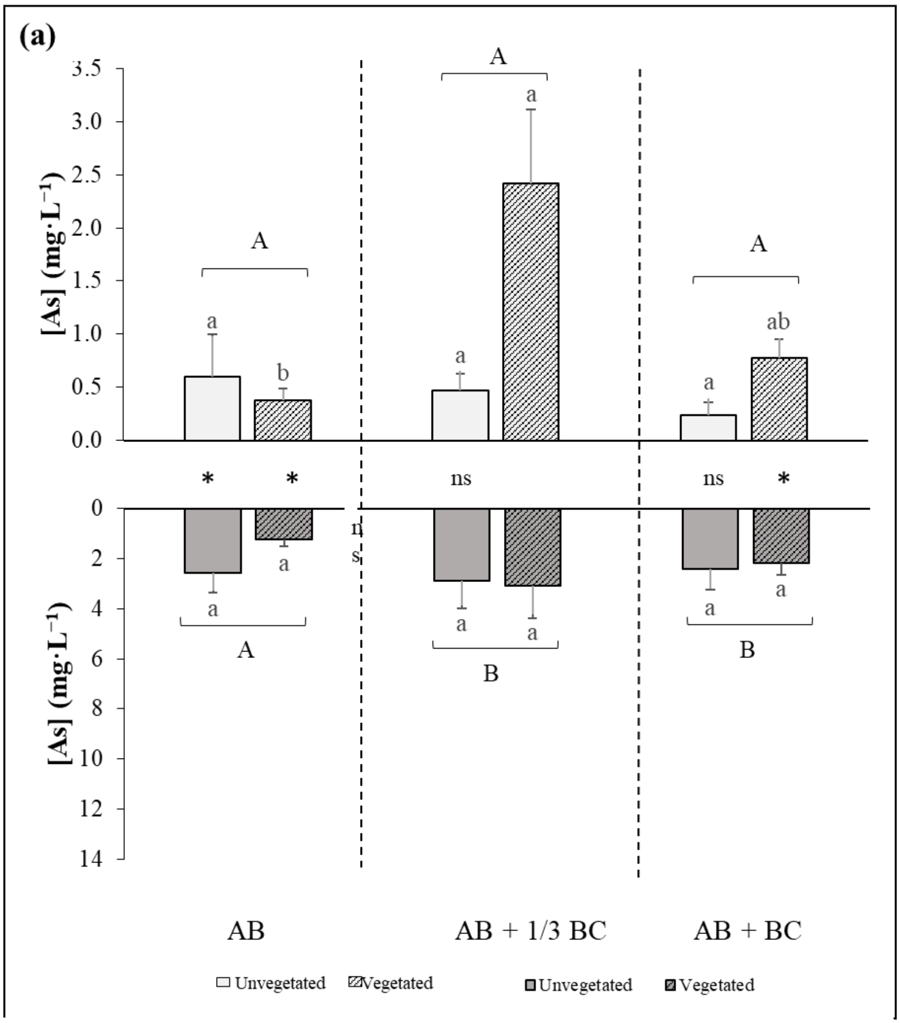
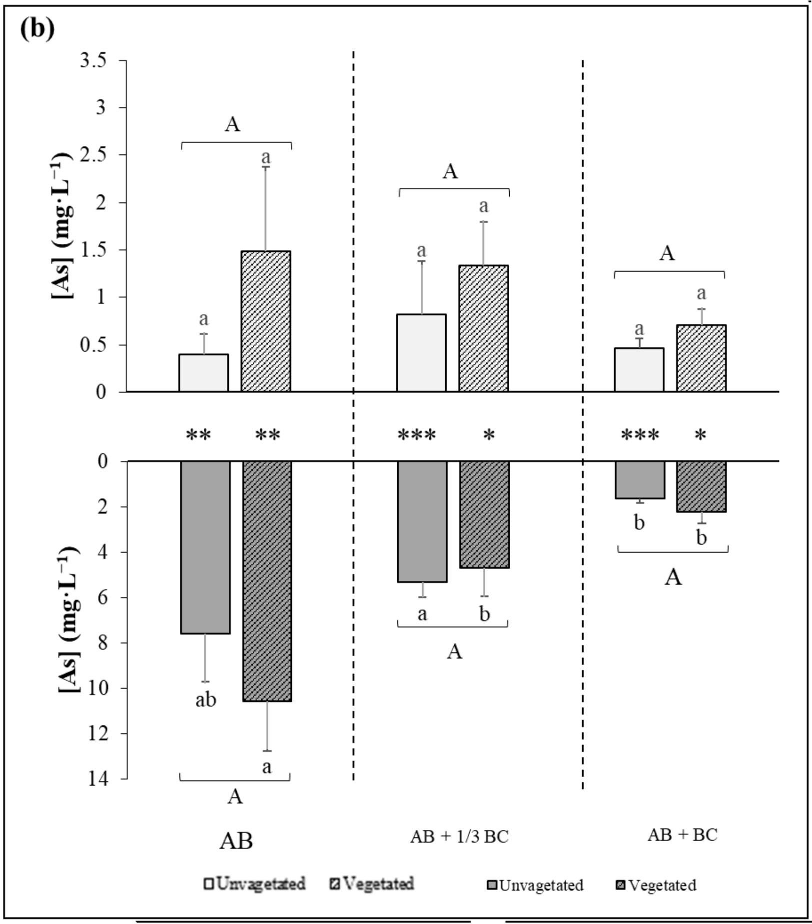


Figure 2. *Cont.*



(c)	Top			Bottom		
	AB	AB + 1/3	AB + BC	AB	AB + 1/3	AB + BC
Time effect						
Unvegetated	ns	ns	ns	*	ns	ns
Vegetated	ns	ns	ns	*	ns	ns

Figure 2. Soil pore water (SPW) arsenic concentration ([As] = mg·L $^{-1}$) is mean of seven values (±SE), determined on Abbaretz (AB) technosol non amended or amended with 2% biochar added to the top 33 cm of the tube (AB + 1/3 BC) or the entirety of the tube (AB + BC) in the unvegetated tubes and in the tubes vegetated with Alnus sp. SPWs were sampled both at the top (light gray box) and at the bottom (gray box) of the tubes in (**a**) October 2019 (T7) and (**b**) October 2020 (T19). The treatment effect in the vegetated and unvegetated conditions (taken separately, the level effect for each treatment/(un)vegetated condition and the (**c**) time effect between T7 and T9 are given with level of significance: ns = non-significant ($p \geq 0.05$); * ($p \leq 0.05$); ** ($p \leq 0.01$); *** ($p \leq 0.001$).

At T19, SPW As concentrations on non-amended AB were: 0.40 mg·L^{-1} at the top of the non-vegetated tube, 7.58 mg·L^{-1} at the bottom of the non-vegetated tube, 1.49 mg·L^{-1} at the top of the vegetated tube, and 10.58 mg L^{-1} at the bottom of the vegetated tube (Figure 2b). For the SPW sampled at the top of the tubes, biochar amendment had no effect in either vegetated or non-vegetated conditions. For the bottom sampling, biochar amendment decreased SPW [As]. At that sampling time (T19), in all cases, SPW [As] was higher at the bottom of the tubes compared to the top. This coincided with the SPW pH increase due to the presence of biochar, which could have induced As mobilization. However, here, an immobilization of As was observed in the biochar amended treatment. This could have been linked to a sorption of As on the iron sulphate, and the sorption of Fe on the biochar surface, creating an indirect As sorption. Indeed, Fresno et al. [43]

showed that arsenic was associated with a higher proportion of Fe oxides in the treatment involving biochar and Fe. Similarly, Lebrun et al. [40] demonstrated that biochar could be functionalized by fixing Fe onto its surface, which ameliorated its capacity to sorb As in sorption tests. Some studies showed that biochar was capable of sorbing As [32,44], showing inconsistent effects of biochar on As mobility depending on soil type, biochar feedstock and time.

Finally, from the comparison of As concentrations at the top and the bottom of the tubes, results showed that As was highly leached from the top towards the bottom of the tubes in all treatments. This showed that, although biochar was capable of reducing As mobility after 17 months, a high proportion of As still leached from the tubes. Thus, biochar amendment was not able to significantly reduce the risk of As leaching in acid mine drainage.

Since iron sulphate was added to all tubes, Fe concentrations were measured in SPW. At T7, in the non-vegetated AB, concentration was 0.32 mg·L^{-1} at the top and 4.27 mg·L^{-1} at the bottom, while in the vegetated AB treatments, concentration was 0.07 mg·L^{-1} and 6.25 mg·L^{-1}, respectively (Figure 3a). At the top of the tubes, there was no effect of biochar amendment on SPW Fe concentration in neither non-vegetated nor vegetated conditions. At the bottom of the tube, the addition of biochar decreased SPW Fe concentration, in the absence and presence of *Alnus* plants. At that time, SPW Fe concentration was higher at the bottom of the tube compared to the top only in the AB treatment, with and without plants.

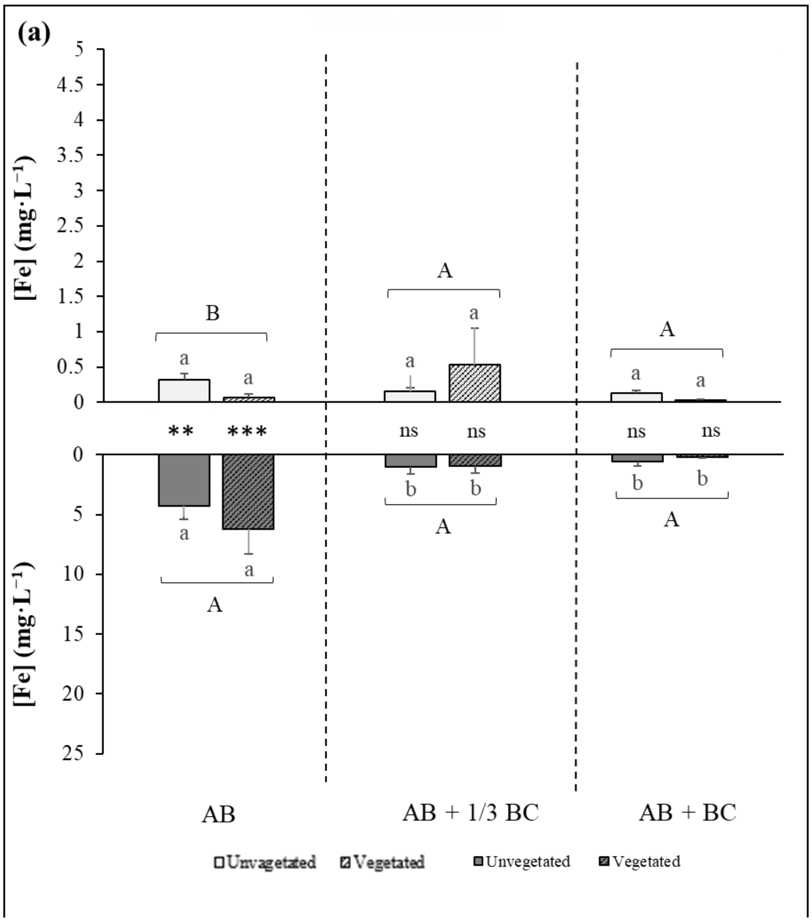
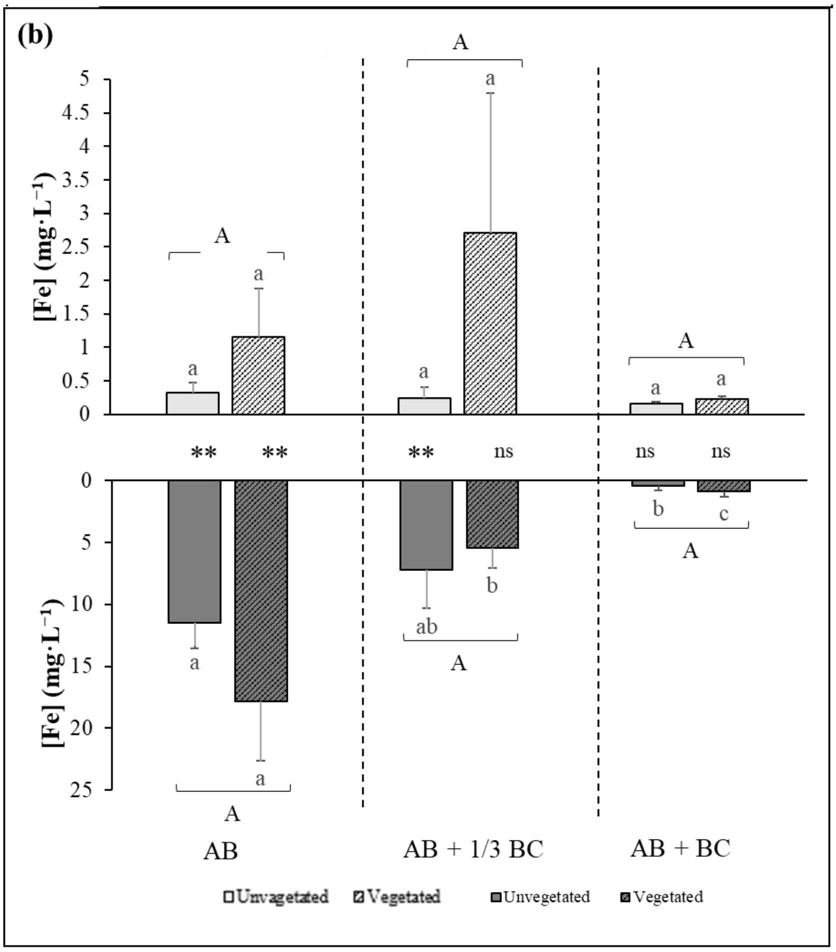


Figure 3. *Cont.*



(c)

	Top			Bottom		
	AB	AB + 1/3 BC	AB + BC	AB	AB + 1/3 BC	AB + BC
Time effect						
Unvegetated	ns	ns	ns	*	ns	ns
Vegetated	*	ns	**	ns	*	ns

Figure 3. Soil pore water (SPW) iron concentration ([Fe] = mg·L $^{-1}$) is mean of seven values (±SE), determined on Abbaretz (AB) technosol non amended or amended with 2% biochar added on the top 33 cm of the tube (AB + 1/3 BC) or on the tube entirety (AB + BC) in the unvegetated tubes and in the tubes vegetated with Alnus sp. SPWs were sampled both at the top (light gray box) and at the bottom (gray box) of the tubes, in (**a**) October 2019 (T7) and in (**b**) October 2020 (T19). The treatment effect in the vegetated and unvegetated conditions (taken separately, the level effect for each treatment/(un)vegetated condition and the (**c**) time effect between T7 and T9 are given with level of significance: ns = non-significant ($p \geq 0.05$); * ($p \leq 0.05$); ** ($p \leq 0.01$); *** ($p \leq 0.001$).

At T19, AB treatment contained 0.32 mg·L^{-1} Fe and 11.49 mg·L^{-1} Fe at the top and bottom of the non-vegetated tubes and 1.16 mg·L^{-1} Fe and 17.87 mg·L^{-1} Fe, respectively, for the vegetated condition (Figure 3b). Similarly to T7, the biochar amendment had no effect on SPW Fe concentration measured at the top of the tubes. At the bottom of the tube, in the non-vegetated condition, only AB + BC had lower Fe concentration compared to AB, whereas in the vegetated condition, AB + BC induced a higher decrease in Fe concentration than AB + 1/3BC, compared to AB. At that time (T19), SPW Fe concentration was higher at the bottom of the tubes than at the top, for the AB treatment, vegetated and non-vegetated, and AB + 1/3BC non-vegetated.

The reduction of Fe concentration in SPW with biochar could be related to the capacity of biochar to sorb positively charged cations such as Fe and the induced pH increase, as demonstrated in the study of Lebrun et al. [13], in which SPW [Fe] was negatively correlated with SPW pH. Moreover, at both times, Fe concentration was higher at the bottom of the tube than at the top, demonstrating the great leaching potential of Fe. However, this was only significant in the non-amended Abbaretz technosol, demonstrating that biochar was capable of reducing the risk of Fe leaching.

The presence of the *Alnus* plant increased SPW [As] at the top of the tubes for the two biochar amended conditions in T7. However, at T19, there was no effect of *Alnus* growth on SPW As concentration. Regarding SPW Fe concentration, there was no effect of plant growth, at either sampling times or in any condition.

The mobilization of As in the presence of plants was observed in the study of Lebrun et al. [45] with *Salix viminalis* and with alder and birch [14]. It may be related to the activity of the roots, as demonstrated in [33] and [46]. This is supported by the fact that As mobilization only happened at the top of the tubes where the root system was the most developed and active. When assessing time effect, the only significant evolution in SPW As concentration was observed at the bottom of the AB treatment, in both vegetated and non-vegetated pots (Figure 2c). Similarly, SPW Fe concentrations were higher at T19 compared to T7 for unvegetated AB (bottom), vegetated AB (up), vegetated AB + 1/3BC (down) and vegetated AB + BC (up) (Figure 3c). These results are consistent with the studies of Lebrun et al. [13], and Lebrun et al. [29] and demonstrate its capacity to immobilize metal(loid) changes with time and biochar oxidation.

3.3. Stems Dry Weight (DW)

In the investigated time (T19), no significant difference was detected in the dry weight of the alder stems between the various treatments (Figure 4). Such neutral effect of biochar on stem biomass production was also observed in Lebrun et al. [13] and could be explained by the fact that *Alnus* plants were capable of growing on the non-amended AB due to their tolerance to metal(loid)s and their capacity to grow on degraded unfertile soils [47,48].

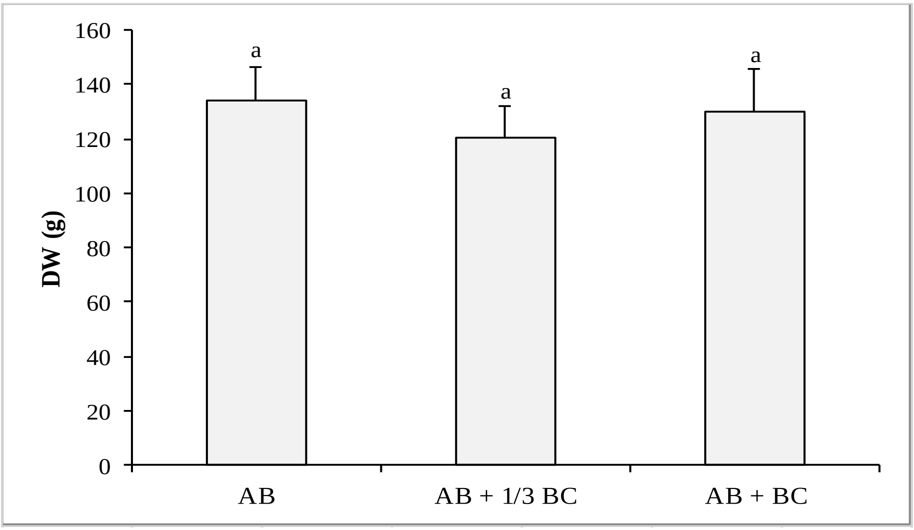


Figure 4. Stems dry weight (DW = g) of the *Alnus* planted in March 2019 (T0) and sampled in October 2020 (T19), grown on Abbaretz (AB) technosol non amended or amended with 2% biochar added on the top 33 cm of the tube (AB + 1/3 BC) or on all the tube (AB + BC). The treatment effect represented by the letters ($p \leq 0.05$) is mean of seven values (±SE).

3.4. Plant Organs Metal(Loid)s Concentrations

For the concentration of As and Fe in the plant organs (roots, stems and leaves), no differences were found at T19 among the treatments (Figure 5), similarly to what was observed in [13].

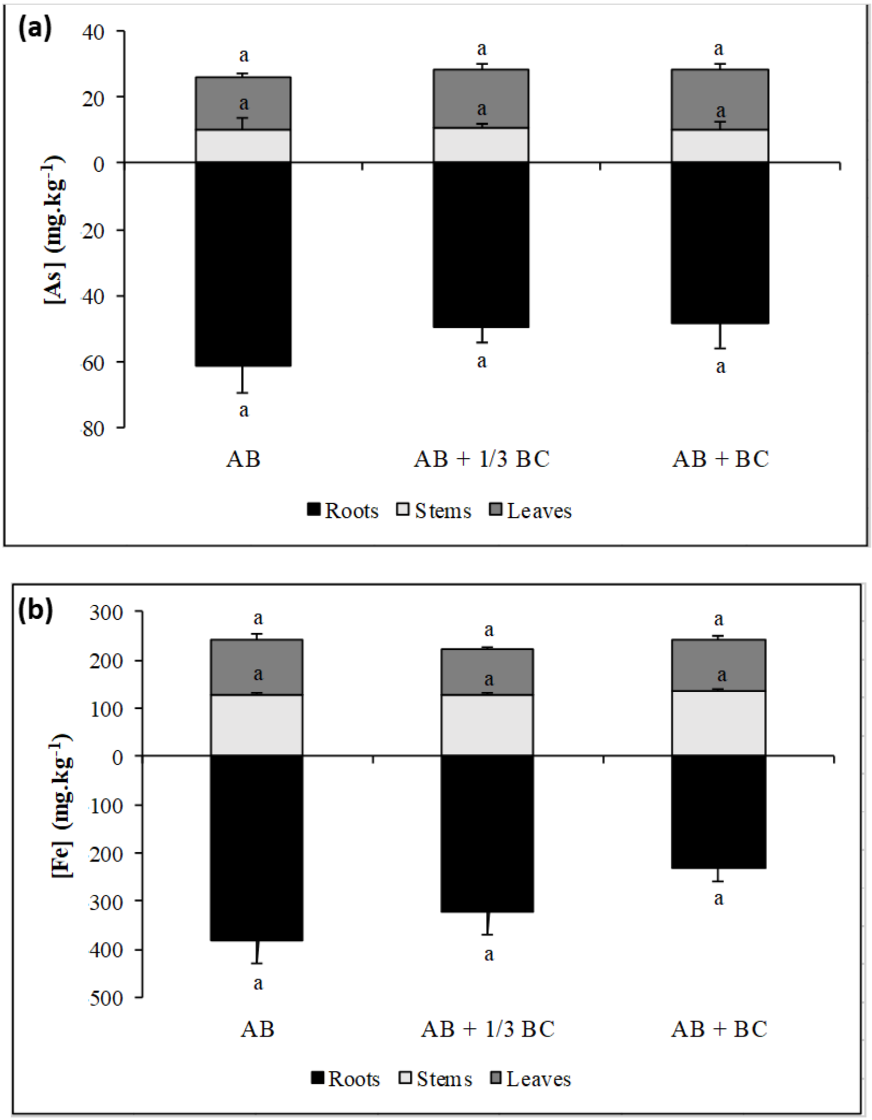


Figure 5. (**a**) Arsenic ([As] = mg·kg $^{-1}$) and (**b**) iron ([Fe] = mg·kg $^{-1}$) concentrations in the different organs (gray box, leaves; light gray box, stems; black box, roots) of the Alnus planted in March 2019 (T0) and sampled in October 2020 (T19), grown on Abbaretz (AB) technosol non amended or amended with 2% biochar added to the top 33 cm of the tube (AB + 1/3 BC) or all of the tube (AB + BC). Plant organs were sampled in October 2020. The treatment effect represented by the letters ($p \leq 0.05$) is mean of seven values (±SE).

Such results, however, were unexpected, since biochar tended to decrease SPW As and Fe concentrations in some cases. The decrease in As and Fe mobility was only observed at the bottom of the tubes, while at the top, where roots developed and took up their necessary water and nutrients, biochar had no effect on As and Fe mobility. Furthermore, As and Fe concentrations were only slightly higher in the roots compared to the aerial parts, showing that translocation of As and Fe occurred in *Alnus* plants, making them not the best choice for phytostabilization process and more particularly within the framework of the installation of processes of phytomanagement where it is preferable to privilege species which limit the translocation of pollutants to the aerial parts, which thus make the produced biomass hardly valorizable without a stage of depollution.

4. Conclusions

This study evaluated the potential of a biochar amendment, associated with iron sulphate, and alder growth for the reduction of As toxicity and environmental risks in a former tin mine. Results showed that biochar amendment was capable of increasing soil pH—an effect that decreased with time. Moreover, a reduction in As mobility was measured, but only after 17 months and at the bottom of the tubes. In most cases, no difference was found among the application methods, demonstrating the potential of biochar surface application in the field. Moreover, alder was able to grow on the contaminated soil, even without biochar, which shows its tolerance towards As and thus its potential as a phytostabilizor plant. However, the study also showed that As and Fe were still highly leached towards the bottom of the tube, although it was reduced by biochar, and that alder growth tended to mobilize metal(loid)s. Therefore, it is important to monitor the biochar–alder association over a longer time period in order to evaluate the possible negative outcomes on metal(loid) leaching and acid mine drainage.

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