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Physiological and Molecular responses of flax (*Linum usitatissimum* L.) cultivars under a multicontaminated technosol amended with biochar

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Abstract

Soil pollution is a worldwide issue and has a strong impact on ecosystems. Metal(loid)s have toxic effects on plants and affect various plant life traits. That is why metal(loid) polluted soils need to be remediated. As a remediation solution, phytoremediation, which uses plants to reduce the toxicity and risk of polluted soils, has been proposed. Moreover, flax (*Linum usitatissimum* L.) has been suggested as a potential phytoremediation plant, due to its antioxidant systems, which can lower the production of reactive oxygen species and can also chelate metal(loid)s. However, the high metal(loid) toxicity associated with the low fertility of the polluted soils render vegetation difficult to establish. Therefore, amendments, such as biochar, need to be applied to improve soil conditions and immobilize metal(loid)s. Here, we analyzed the growth parameters and oxidative stress biomarkers (ROS production, membrane lipid peroxidation, protein carbonylation and 8-oxoGuanine formation) of five different flax cultivars when grown on a real contaminated soil condition, and in the presence of a biochar amendment. Significant correlations were observed between plant growth, tolerance to oxidative stress, and reprogramming of phytochemical accumulation. A clear genotype-dependent response to metal(loid) stress was observed. It was demonstrated that some phenylpropanoids such as benzoic acid, caffeic acid, lariciresinol, and kaempferol played a key role in the tolerance to the metal(loid)-induced oxidative stress. According to these results, it appeared that some flax genotypes, *i.e.* Angora and Baikal, could be well adapted for the phytoremediation of metal(loid) polluted soils as a consequence of their adaptation to oxidative stress.

Highlights

Flax cultivars were used in a biochar assisted phytoremediation process

Flax plants were able to grow on the amended contaminated mine soil

Cultivars showed different responses to metal(loid)-induced oxidative stress

The cultivar Eurodor was sensitive whereas Angora and Baikal were tolerant

Keywords

Flax; Soil pollution; Metal/Metalloid; Technosol; Biochar; Oxidative stress makers

1. Introduction

Nowadays, soil pollution is a worldwide issue. The discharge of wastes into the environment by industries, as well as mining activities, and the use of fertilizers in agriculture, have led to multiple and highly contaminated areas (Ahmad et al., 2015). In Europe, there are 2.8 million sites where polluting activities took place or are taking place (Paya-Perez and Rodriguez-Eugenio 2018). Among pollutants encountered, metal(loid)s are the most abundant ones. In addition to their abundance, metal(loid)s cannot be degraded, and therefore accumulate in the environment, and the majority are toxic to plants and humans (Ahmad et al., 2015).

Metal(loid)s have toxic effects on plants, they can affect the permeability of the cell membrane, the biochemical activities and also reduce the growth and reproduction of the cells (Taran et al. 2020). In particular, metal(loid)s can shift the balance of free-radical metabolism, which can result in oxidative stress (Bajguz, 2011). The formation of reactive oxygen species (ROS), which is one of the first signs of toxicity under stress conditions, can be counteracted by both enzymatic and non-enzymatic antioxidant defense systems in plants (Raja et al., 2017; Jaskulak et al., 2018). Both antioxidant defense systems may be outpaced by the ROS production, leading to cellular damage such as DNA mutation, protein oxidation or lipid membrane peroxidation (Briffa et al. 2020), which are considered to be markers of oxidative stress-induced toxicity in non-tolerant species (Posmyk et al., 2009; Wang et al., 2008). In addition, other markers such as chlorophyll, flavonoid and/or phenolic contents may also provide information on the plant adaptation to this abiotic stress (Esteban et al., 2008). To illustrate this point, Singh et al. (2020) grew *S. polyrhiza* plants in a hydroponic experiment and showed that it had an important biochemical strategy to cope with the toxicity induced by copper (Cu) and mercury (Hg). As antioxidant, phenolic compounds (*e.g.*, flavonoids, lignans or hydroxycinnamic acids) may be involved in both ROS scavenging and metal(loid) chelation (Dresler et al., 2017b). For example, chamomile roots under Cu and cadmium (Cd) stress have shown increased caffeic, ferulic and *p*-coumaric acid content (Kováčik et al., 2009; Kováčik and Klejdus, 2008). Interestingly, *p*-coumaric acid is a known precursor to flavonoids, which are antioxidants, while ferulic acid is a precursor to lignans, known as metal chelators. Indeed, Fucassi et al., (2014) have shown that the lignan from flax, secoisolariresinol diglucoside, is capable of chelating many metals, such as Cu^{2+}, Pb^{2+}, Fe^{2+}, Ni^{2+} and Ag^{+}, with different affinities. However, few studies exist on the interaction between flax and metal(loid)s in real soil conditions.

Therefore, the ability of a plant to cope with oxidative stress is of primary importance for its adaptation and survival during a stress period (Verma and Dubey, 2003). Indeed, Dazy et al., (2008) studied three plant species growing on both non-contaminated and contaminated areas, and concluded that a plant species which exhibited an efficient defense system could cope with pollution, and therefore grow on polluted soils. Similarly, Dresler et al., (2017a) compared two ecotypes of *Echium vulgare*, metallicolous (M) and non-metallicolous (NM), and found that the M population presented a higher ability to respond to the contamination than the NM population through a more efficient up-regulation of secondary metabolites. Such studies show that plant metabolism has an important role in stress tolerance.

Moreover, pollution of soils induces a loss of biodiversity and its ecosystemic functions. These bare contaminated soils thus present a risk of wind erosion as well as water leaching and run-off. Therefore, the contamination can spread to non-contaminated environments, and possibly agricultural areas. Toth et al., (2016) analyzed soil samples all over the European Union and found that 6.24 % of agricultural lands needed local assessment and may require remediation, which corresponded to 137 000 km^2 of agricultural land. Consequently, crop culture on polluted soils can induce the transfer of the pollution into the food chain (Kabata-Pendias, 2004), thereby impacting human health. For this reason, it is important to prevent the spreading of contamination, and to remediate and valorize such contaminated soils. One remediation solution which has been gathering attention over the last decades is phytoremediation, which uses plants and their associated microbiota to reduce the toxic potential of contaminants (Gómez et al., 2019). In the phytoremediation process, it is important to select the right plant species that can tolerate metal(loid)s present on the site and produce a sufficient biomass. Flax (*Linum usitatissimum* L.) was demonstrated to possess a tolerance towards diverse metal(loid)s, and more particularly Cd (Douchiche et al., 2010;

Smykalova et al., 2010). Moreover, flax plants produce a lignan, secoisolariciresinol diglucoside, that can chelate metal(loid)s (Fucassi et al., 2014). Therefore, flax could have potential in the phytoremediation of metal(loid) polluted soils (Angelova et al., 2004). In addition, the plantation of flax on polluted soils will allow the vegetation and valorization of such areas, and preserve agricultural soils. Flax is well known for producing fiber, which can be integrated into the production of biomaterials. Therefore, this study tested several flax cultivars, representative of the existing flax diversity.

However, plant establishment on contaminated soils is often difficult due to the unfavorable conditions (*i.e.* acidic pH, low nutrient quantity and availability) (Alvarenga et al., 2014; Lebrun et al., 2017). Therefore, amendments have to be applied. In terms of the choice of amendment, biochar has gathered attention in the last few years for its use in both uncontaminated agricultural soils and contaminated sites (Barrow, 2012). Biochar is a carbon-rich, porous product obtained from the pyrolysis of organic materials under low oxygen conditions and at relatively high temperatures (Barrow, 2012; Paz-Ferreiro et al., 2014). Biochar is usually characterized by an alkaline pH, a high cation exchange capacity (CEC), an elevated porosity, and a large specific surface area (Paz-Ferreiro et al., 2014; Tan et al., 2017). Several studies have shown the positive effects of biochar application on soil properties: increase in pH, electrical conductivity (EC), CEC, organic carbon content, water holding capacity (WHC) and nutrient concentrations and availabilities (Forján et al., 2016; Herath et al., 2015; Janus et al., 2015), associated with a decrease in the soil bulk density (Janus et al., 2015). Biochar can also be efficient in decreasing metal(loid) concentration and mobility (Houben et al., 2013), due to its sorption capacity (Wiszniewska et al., 2016; Zhang et al., 2017). These soil condition ameliorations were shown to improve the general plant growth (Wiszniewska et al., 2016; Lebrun et al., 2017, 2018a, 2020; Herath et al., 2015).

To the best of our knowledge, no studies have analyzed the stress biomarkers in a real contaminated soil condition and in presence of amendments. Moreover, no studies compared metal(loid) tolerance and accumulation of different flax cultivars when grown on a biochar amended contaminated soil. Therefore, the aim of this study was firstly to evaluate the effect of a biochar amendment on (i) the soil physico-chemical properties, (ii) flax growth and metal(loid) accumulation and (iii) biochemical profiles (oxidative stress biomarkers). Secondly, the study goal was to compare five flax cultivars in terms of their growth, metal(loid) accumulation and biochemical profiles. Finally, the ultimate goal was to understand and explain the difference in metal(loid) tolerance and accumulation of the flax cultivar by their ability to cope with oxidative stress.

2. Materials and Methods

2.1. Soil collection site

A former mine extraction site was studied. This mine, located at Roure-les-Rosiers (St Pierre le Chastel), belonged to the Pontgibaud mining district (Puy-de-Dôme, France), one of the most important mining districts in Europe during the nineteenth century. The mining activities, which ended in 1897, led to hundreds of tons of sandy technosol highly contaminated by arsenic (539 $mg.kg^{-1}$) and lead (11453 $mg.kg^{-1}$). The soil has been characterized in previous studies, which showed that it had an acidic pH (pH 4.6) (Lebrun et al. 2017), low organic matter content (2.6 %) (Lebrun et al. 2019) and low nutrient content and availability (465 $mg.kg^{-1}$ organic phosphorus, 6 $mg.kg^{-1}$ available phosphorus and 75 $mg.kg^{-1}$ nitrogen) (Lebrun et al. 2020). An analysis of ten of the most found metal(loid)s in soil revealed that As and Pb were the two pollutants above permissible limits (Lebrun et al. 2020).

2.2. Compost and biochar used

A commercial compost was used as a control. The biochar used as an amendment was provided by La Carbonerie (Crissey, France). It was obtained from the slow pyrolysis of lightwood biomass (birch biomass) under the following parameters: heating rate of 2.5 $°C.min^{-1}$, pyrolysis temperature of 500 °C and residence time of 3 h. The pyrolysis product was then sieved to obtain a particle size between 0.2 and 0.4 mm. Biochar characteristics and methods used were described in a previous paper (Lebrun et al., 2018a). All amendment properties are presented in Table 1.

2.3. Plant

Different cultivars of flax (*L. usitatissimum*) were used in this study: Angora, Baladin, Baikal, Drakkar and Eurodor. Seeds were provided by Laboulet Semences (Airaines, France), Coopérative Linière Terre de Lin (Saint-Pierre-le-Viger, France), and Arvalis-Institut Technique du Lin (Boigneville, France). These cultivars were selected because they represent the flax diversity found in France and could thus give information on the mechanisms of tolerance, through the oxidative stress response, and of how different cultivars have contrasting toxicity responses towards metal(loid) contaminated soils.

2.4. Treatments and growth conditions

Three different treatments were prepared: (i) a commercial compost used as a "non-contaminated control", named C, (ii) Pontgibaud technosol, collected between 0 and 20 cm depth and sieved at 2 mm, named P0% and (iii) Pontgibaud technosol amended with 5 % biochar, named PB5%.

Mixtures were put into 16 plastic pots (13*13*12.5 cm) per treatment. Twenty-five seeds of each flax cultivar were sown in three pots per treatment, and one pot was left unvegetated. Flax growth was conducted for 21 days under greenhouse conditions (temperature 22 ± 2 °C, light intensity 800 $mmol.m^{-2}.s^{-1}$, photoperiod 16 h), and irrigation was provided every two days based on the water lost through evapotranspiration. The 21 days of growing was chosen in order to evaluate, in the middle of the growing phase, the roots and shoots answer of flax plants to metal(loid) pollution and amendments, before the remobilization of resources during the flowering phase comes to disturb this partition.

2.5. Soil pore water (SPW) sampling and analysis

The soil pore water sampling was performed in three pots from each treatment before the seed sowing (T0), using soil moisture samplers (Rhizon) (model MOM, Rhizosphere Research Products, Wageningen, The Netherlands) as described in Lebrun et al., (2017). SPW was used directly (i) to measure pH using a pHmeter (FE20/EL20, Metler-Toledo AG 2007) and electrical conductivity (EC) using a conductivity meter (CDM210, MeterLab), as well as (ii) for performing a toxicity test using *Photobacterium phosphoreum* (Qu et al., 2013). The toxicity test was performed in microplates, by adding 50 µL of phosphate buffer (100 mM, pH 7.0) to 50 µL of SPW sample, followed by an addition of 50 µL of bacterial suspension (absorbance at 600 nm adjusted to 1). The plate was then covered and incubated at room temperature. After 30 min, luminescence was measured with a luminometer (PolarStar Omega, BMG Labtech). The relative toxicity was calculated using the control (C) as a reference and as follows: relative luminescence (%) = $luminescence_{sample}/luminescence_{control}$ x 100.

In addition, As and Pb concentrations were measured after sample acidification, using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (ULTIMA 2, HORIBA, Labcompare, San Francisco, USA).

2.6. Soil collection and analysis

At harvest time, soil was collected from both the vegetated and non-vegetated pots. Soil from the three vegetated pots of each cultivar (rhizosphere soil) was collected by shaking the roots inside a sterile bag (Lebrun et al., 2019). The soils were air-dried prior to a microtoxicity test (adapted from Kołtowski et al., 2017): 3 g of dried soil was mixed with 30 mL NaCl 2% and agitated at ambient temperature. After 10 minutes, the mixtures were centrifuged (10 minutes, 500 rpm) and the supernatants were collected and used for the toxicity test, following the same procedure as for the SPW toxicity assessment. The relative toxicity was calculated using non-vegetated compost (C) as a reference.

2.7. Plant harvest

After 21 days of growth, almost no growth was observed on the P0% substrate, as shown in figures 1 and S1, and consequently, no further analyses were performed. On the other two treatments (C and PB5%), all seedlings were harvested and counted. The seedlings were rinsed twice in tap water and once in distilled water to remove any soil particles. Five seedlings were separated into above- and belowground parts and immediately frozen and stored at -80 °C until further analysis. The other seedlings were grouped into lots (4-6 plants).

2.8. Physiological analyses

Above- and belowground parts of each lot were separated, and then dried at 60 °C for 72 h in order to determine dry weight (Lebrun et al., 2017). As and Pb concentrations were determined using ICP-AES, and As and Pb mineralomass was calculated according to Lebrun et al. (2017): 0.2 g of sample was mixed with 6 mL HNO_3 65% and 3 mL HCl 35%, and digested in a microwave (program: 15 min temperature increase up to 180 °C, 15 min at 180 °C and 15 min cool down); digested solution samples were recovered and diluted to 30 mL with distilled water.

Finally, three indices were calculated based on the SPW and plant metal(loid) concentrations. Translocation factor (TF) (equation 1) was the ratio of root metal(loid) concentrations to shoot metal(loid) concentration.

$$(1)\ TF = \frac{shoot\ metal(loid) concentration}{root\ metal(loid) concentration}$$

Bioconcentration factor (BF) (equation 2) was the ratio of root metal(loid) concentration to SPW metal(loid) concentration, while the biological accumulation factor (BAF) (equation 3) was the ratio of shoot metal(loid) concentration to SPW metal(loid) concentration.

$$(2)\ BF = \frac{root\ metal(loid)\ concentration}{SPW\ metal(loid)\ concentration}$$

$$(3)\ BAF = \frac{shoot\ metal(loid)\ concentration}{SPW\ metal(loid)\ concentration}$$

2.9. Phytochemical analysis

2.9.1. Extraction

The frozen-dried vegetal material was used for these analyses. *L. usitatissimum* above and belowground extracts were obtained by grinding 5 mg of material (using a balance precise at 0.1 mg) in 1 mL of 50 % (v/v) aqueous ethanol (HPLC grade solvents, Thermo Scientific) using ultrasound-assisted extraction in a USC1200TH (Prolabo) ultrasonic bath operating at a 30 kHz frequency, and a temperature set at 45 °C for 60 min. After extraction, the extract was centrifuged for 15 min at 5000 x *g* and the supernatant extract was filtered through 0.45 µm of nylon syringe membranes.

2.9.2. Determination of total phenolic and total flavonoid content

The total phenolic content was analyzed using the Folin-Ciocalteu (Sigma) reagent method and the total flavonoid content was determined using the $AlCl_3$ colorimetric method as described by Lopez et al. (2016).

For total phenolic content (TPC), 20 µL of flax extract, 90 µL Na_2CO_3 and 90 µL Folin-Ciocalteu reagent (Sigma-Aldrich) were mixed and incubated for 15 min at room temperature (25 ± 2 °C). The absorbance at 630 nm was measured and the TPC was expressed in $mg.g^{-1}$ dry weight (DW) of gallic acid equivalent, using a 5-point calibration curve (0–40 µg/mL; gallic acid; $R^2 = 0.998$).

For total flavonoid content (TFC), 20 µL of flax extract and 180 µL $AlCl_3$ (5% w/v prepared in methanol) were mixed and incubated for 30 min at room temperature (25 ± 2 °C). The absorbance at 415 nm was measured and the TFC was determined in $mg.g^{-1}$ DW quercetin equivalent using a 5-point calibration curve (0–40 µg/mL; quercetin; $R^2 = 0.998$).

2.9.3. HPLC analysis

The quantification of compounds was carried out using a Varian high-performance liquid chromatographic (HPLC) system as described by Corbin et al. (2015). The HPLC system is composed of: a Varian Prostar 230 pump, a Metachem Degasit, Varian Prostar 410 autosampler, and a Varian Prostar

335 Photodiode Array Detector (PAD). Analysis was driven using Galaxie version 1.9.3.2 software. The separation was performed at 35 °C using a Purospher (Merck) RP-18 column (250 x 4.0 mm i.d.; 5 μm) with the detection set at 280 nm. The mobile phase was composed of solvent A (0.2% (v/v) acetic acid in HPLC grade water) and solvent B (HPLC grade methanol). A nonlinear gradient was applied for the mobile phase variation with a flow rate of 0.8 ml/min as follows: from 0 to 40 min of A–B: 90:10 (v/v) to 30:70 (v/v), from 41 to 50 min of A–B: 30:70 (v/v) to 0:100 (v/v), and A–B: 0:100 (v/v) from 51 to 60 min. Each compound was identified by comparison of its retention times and UV spectrum to those of authentic standard. Quantification was performed using calibration curves of each standard ranging from 0.0125 to 0.5 mg/ml, with a correlation coefficient of at least 0.999, and using *o*-coumaric acid as an internal standard (add at a 0.05 mg/mL final concentration in the extract prior extraction).

2.10. Antioxidant free radical scavenging activity

The antioxidant free radical scavenging activity (FRSA) of flax extracts was evaluated by their ability to scavenge the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical as described by Lopez et al. (2016). For this, 20 µL of extract sample were mixed with 180 µL of 6.10^{-5} M DPPH solution in a 96-well microplate. The plate was then placed in the dark for 1 h at room temperature (25 ± 2 °C). The absorbance at 515 nm was determined using a microplate reader. Extraction solvent (HPLC grade 50 % (v/v) aqueous ethanol solution) was used as a blank. The formula used for calculating the FRSA was: FRSA (%) = 100 × (1 − AE/AB), with AE the mixture absorbance at 515 nm, while AB represents the absorbance of the blank.

2.11. Oxidative stress markers analysis

2.11.1. ROS quantification

ROS (hydrogen peroxide) content was estimated as described in Hano et al. (2008) by using fluorescent dihydrofluorescein diacetate. The fluorescence intensity was measured with VersaFluor fluorimeter (Biorad) using λex= 490 nm and λem= 514 nm.

2.11.2. Membrane lipid peroxidation

Membrane lipid peroxidation was conducted as described in Hano et al. (2008). A thiobarbituric acid (TBA) reactive substances (TBARS) assay was employed to determine the membrane lipid peroxidation level. A sample fraction (75 µL) was then mixed with 25 µL of SDS (3 % (w/v)), 50 µL of TBA (3 % (w/v) in a 50 mM NaOH solution) and 50 µL of HCl (23 % (v/v)) with vigorous mixing after each addition. The mixture was incubated at 80 °C for 20 min and then cooled on ice. The TBARS value was determined by measuring absorbance at 532 nm, and subtracting non-specific absorbance at 600 nm using UV-Vis spectrophotometer (Varian).

2.11.3. Protein carbonyl content

Total proteins were extracted as described in Hano et al. (2008), and their carbonylation content was determined using the ELISA method (OxiSelect™ Protein Carbonyl ELISA Kit, Cell Biolabs). Total protein content was determined using the Quant-iT Protein Assay Kit (Invitrogen) using the Qubit fluorometer (Invitrogen). The protein carbonylation level was determined as described by the manufacturer's instructions, by measuring the absorbance value at 405 nm, and the relative protein carbonylation level was expressed as a percentage relative to the A_{405} value measured for the control.

2.11.4. DNA 8-oxo-Guanine content

DNA was extracted as described in Hano et al. (2008) using cetyl-trimethyl-ammonium bromide (CTAB), and the 8-oxoGuanine level was determined using the ELISA method (Oxiselect oxidative DNA damage ELISA kit, Cell Biolabs). DNA content was determined using the Quant-iT DNA BR Assay Kit (Invitrogen) using the Qubit fluorometer (Invitrogen). The 8-oxoGuanine level was determined using the ELISA method (Oxiselect oxidative DNA damage ELISA kit, Cell Biolabs, San Diego, CA, USA) as described by the manufacturer's instructions, by measuring the absorbance value at 405 nm, and the relative protein carbonylation level was expressed as a percentage relative to the A_{405} value measured for the control.

2.12. Statistical analyses

The data regarding soil, soil pore water and plant physiological parameters were analyzed using the R software (R Development Core Team, 2009). Shapiro followed by Bartlett tests were used to assess the normality and homoscedasticity of the data. Then, for each treatment, the cultivar effect was analyzed by comparing the means using an Anova or Kruskal test, depending on the result from the previous tests, followed by a post-hoc test (TukeyHSD test and Pairwise-Wilcox test, respectively). The results of the Anova and Kruskal tests to assess cultivar and treatment effects are shown in Table S1. Finally, for each cultivar, the treatment effect was studied using the Student test, for normal data, or Wilcox test, for non-normal data. Differences were considered significant when $p < 0.05$. For the phytochemical analysis, data were the results of two biological and two technical replicates, therefore only a comparison between the two treatments (C and PB5%) was performed for each cultivar using Student tests (XL-STAT 2019). For a better discrimination of the cultivars and the treatments, Pearson correlations were performed using R software version 3.0.2 and a PCA was performed with PAST software, with each parameter considered as a discrete variable. The initial dataset was then converted into principal components (PCs), and it was possible to graphically display the relationships among the considered parameters. HCA was visualized with MeV4 software.

3. Results and Discussion

3.1. Physico-chemical characterization of the soil pore water (SPW) properties

Soil pore water was sampled at the beginning of the experiment in the three conditions. The pH measurements showed that control SPW presented a pH around neutrality (6.25) (Table 2). However, SPW of the contaminated soil P0% presented an acidic pH (4.23), which increased by 2.41 units when 5 % biochar was added. Similar results were found by Oustriere et al., (2016), with two commercial biochars (prepared from pine bark and chicken manure) applied to a Cu-contaminated topsoil. Similarly, Beesley et al., (2010) amended a multi-contaminated soil (As, Cd, Cu, Pb, Zn) with hardwood biochar and observed a 2.1 unit pH increase. Finally, 5% *Miscanthus* biochar application increased the pH of an acidic mine soil (Novak et al., 2018). This acidity correction can be attributed to the alkaline nature of the biochar (pH 9.56) (Masulili et al., 2010) and to a decrease in competition between H^+ ions and metal ions for cation exchange sites (Lomaglio et al., 2017).

The SPW electrical conductivity followed the same trend as the pH (Table 2): the control condition had a high EC while the contaminated soil showed a low EC, at 274 $\mu S.cm^{-1}$. Biochar addition increased SPW EC by almost five times, which is consistent with previous works (Alburquerque et al., 2014; Nigussie et al., 2011). However, SPW EC was lower for PB5% than the control. Three mechanisms could explain such an EC rise with biochar: (i) the ashes contained in the biochar (Fellet et al., 2014), (ii) the enhancement of nutrient leaching into the soil solution (Lomaglio et al., 2017), and (iii) the dissolution of soluble salts and cations present on the biochar's surface (Chintala et al., 2014). SPW As concentrations were really low for the three conditions, below 0.03 $mg.L^{-1}$ (Table 2). No difference in As concentrations was found between the three conditions. A non-biochar effect on SPW As concentration has already been observed when applying 10 % orchard biochar to a multi-contaminated mine soil (Beesley et al., 2014), and could be due to the limited sorption ability of biochar towards As anions (Wang et al., 2015). Regarding SPW Pb concentrations, a high concentration was found in the non-amended condition (9.32 $mg.L^{-1}$) (Table 2). Biochar application to P0% induced an 86 % decrease in Pb concentration, up to levels similar to the ones observed in the control. Such results have been observed previously and explained by: (i) a pH increase (Houben et al., 2013; Mahar et al., 2015), and (ii) Pb sorption onto the biochar surface (Lebrun et al., 2018b; Shen et al., 2015).

3.2. Soil and soil pore water toxicity

At the beginning of the experiment, substrate toxicity was assessed for the soil pore water samples. The results were expressed in relative toxicity compared to the control: samples showing a value inferior to 1 (value of the non-vegetated compost) were considered more toxic than the control while samples with a value above 1 were less toxic. The results showed that P0% was more toxic than C (Table 3). However, biochar application decreased the toxicity. Indeed, SPW toxicity values of PB5% and C were not statistically different. This toxicity reduction was in accordance with the study of Rees et al., (2017), which showed a decrease in the potential genotoxicity of contaminated soils after biochar addition. In this case, the toxicity reduction can be associated with the decrease in SPW Pb concentrations (Sáez et al., 2016).

At the end of the experiment time course, substrate toxicity was measured for the soil samples, as SPW could not be sampled. Relative toxicity was expressed using the non-vegetated compost as a reference (value 1). On the soil C, Baikal and Eurodor growth increased the soil toxicity, by 2-fold on average (Table 3), compared to the non-vegetated condition. On the PB5% substrate, all five cultivars reduced the soil toxicity compared to the non-vegetated condition, with Angora having the highest effect. When Baikal, Baladin and Eurodor seedlings were grown, there was no significant difference in soil toxicity between the compost and the amended-contaminated soil. However, after Angora and Drakkar growth, the two soil conditions were significantly different. These differences observed among the treatments and the cultivars can be attributed to a plant root effect. Indeed, Punz and Sieghart, (1993) demonstrated that in response to metal stress, plants were inducing: (i) biochemical and enzymatic changes on the root surface, (ii) extracellular deposition, and (iii) binding to the cell wall components. The difference between cultivars at the phytochemical level will be explored in the next sections. In addition, plants can exudate low molecular weight organic acids that can chelate metals, thus reducing their toxicity (Dong et al., 2008; Montiel-Rozas et al., 2016). Those root excretions have been shown to depend on species and growth conditions (Kidd et al., 2009). Finally, in the non-vegetated condition, both metal(loid) contaminated soils, P0% and PB5%, presented a higher toxicity than the control, due to the soil contamination. However, biochar had no effect on the soil toxicity.

3.3. Plant growth parameters

On the contaminated condition P0% and for all the five cultivars, the seedling growth was not sufficient to allow harvest and further analysis (Figures 1 and S1). The application of 5 % biochar to the contaminated

Pontgibaud soil increased seedling growth for all five cultivars (Figures 1 and S1): on this treatment, flax plants produced between 5 and 11 mg of aerial tissues and between 2 and 3 mg of roots. Likewise, Yue et al., (2017) observed an increase in dry matter production when using sewage sludge biochar and a mix of grass seeds. Such growth amelioration can be attributed to: (i) an increase in WHC, organic matter content and nutrient content and bioavailability (Agegnehu et al., 2015; Lebrun et al., 2019); (ii) a decrease in metal(loid) availability (Puga et al., 2015), and (iii) a general improvement in the soil conditions (Agegnehu et al., 2016), *i.e* increase in pH, EC and decrease in metal(loid) SPW concentrations, as shown in Table 1.

When comparing the control and the amended-contaminated soil PB5%, two contrasting behaviors were observed, depending on the organs (Figure 2). Firstly, the shoot dry weights were two to three times lower when plants were grown on PB5% compared to C, for the five cultivars. This decline in shoot DW on PB5% could be due to soil toxicity, as demonstrated by the toxicity test performed on the substrates, as well as metal(loid) toxicity, when metal(loid)s are transported into the aerial parts. Secondly, contrary to the shoot, the root DW was increased, by two to five-folds, in the amended Pontgibaud soil compared to the compost. This is consistent with the rhizobox experiment performed by Rees et al., (2016). Several mechanisms can explain such an improvement. Firstly, the biochar-induced decrease in bulk density lowers soil resistance, which promotes root development and growth (Alburquerque et al., 2014; Rees et al., 2016). Secondly, biochar amendment can decrease or increase nutrient availability and increase water availability (Rees et al., 2016; Xiang et al., 2017). Such amelioration of the soil conditions improved root growth, due to the direct contact with the soil, whereas the aerial parts were less positively affected, probably due to a lower translocation of nutrients associated with a translocation of metal(loid)s.

3.4. As and Pb above- and belowground concentrations

For the five cultivars and both elements, As and Pb, higher above- and belowground concentrations were found when seedlings were grown on the amended contaminated soil compared to the control, which was consistent with the metal(loid) concentrations found in the polluted soil (Figure 3).

Additionally, on the contaminated soil PB5%, above- and belowground concentrations were different depending on the flax cultivar: the lowest aerial and root As and Pb concentrations were found in the Drakkar cultivar, while the Baladin cultivar presented the highest concentrations. In more details, on PB5% Drakkar accumulated 129 $mg.kg^{-1}$ As and 962 $mg.kg^{-1}$ Pb in its aerial parts, whereas root concentrations were 918 $mg.kg^{-1}$ As and 7,949 $mg.kg^{-1}$; Baladin plants presented 241 $mg.kg^{-1}$ As and 2,096 $mg.kg^{-1}$ Pb in its aerial parts and 3,308 $mg.kg^{-1}$ and 26,138 $mg.kg^{-1}$ Pb in its roots (Figure 3). These results demonstrated the diverse accumulation ability among the flax cultivars. As and Pb quantities (mineralomasses) were calculated based on DW and metal(loid) concentrations. It showed that Baladin was the cultivar with the highest As quantity in both shoots (1.7 µg) and roots (8.1 µg) (Figure 4A), while Drakkar had the lowest (0.9 and 2.2 µg, respectively). Regarding Pb accumulation, aerial quantity was the highest in Baikal (20.2 µg), followed by Baladin (14.8 µg), Eurodor (10.6 µg) and Angora (7.8 µg), and Drakkar (6.9 µg). However, Baladin harbored the highest root Pb quantity (64.2 µg), followed by Baikal (39.0 µg), Eurodor (36.0 µg) and Angora (32.4 µg), and finally Drakkar (18.8 µg) (Figure 4B). It should be noted that Drakkar presented the lowest As and Pb quantities in both plant parts, mainly due to its reduced As and Pb concentrations, as DW production was similar to other cultivars.

A higher metal(loid) accumulation in the roots compared to the shoots has been observed in several studies for diverse plant species (Beesley et al., 2013; Marchiol et al., 2007). The present study also showed higher As and Pb concentrations and quantities in the roots compared to the shoots, for all the five cultivars. Indeed, all the TFs were low and below 1 (Table 4), showing the poor translocation of As and Pb to the aerial parts (Fayiga and Ma, 2006). Among the five cultivars, only Baladin and Eurodor differed from the others, showing the lowest TF values. Moreover, all five cultivars showed a similar translocation of As and Pb, except for Baikal, which presented a higher translocation of Pb than As. This testified for a limited metal(loid) movement along the plant conductive system (Angelova et al., 2004). Such results can be explained by the high toxicity of the metal(loid)s and their low solubility once inside the root cells (Tanhan et al., 2007). In addition, in general, plants can limit the translocation of toxic elements in order to reduce the adverse effects on the biological processes. Indeed, the Casparian strip in the endodermis acts as a barrier (Pourrut et al., 2011), and therefore metal(loid)s are compartmentalized in the roots (Douchiche et al., 2010). Moreover, the main accumulation of metal(loid)s in the roots, associated to their reduced translocation in the aerial part, can indicate the suitability of all five cultivar for phytostabilization over phytoextraction process.

BF values (Table 4) were the highest in the case of Baladin for both metal(loid)s (Table 4). BF_{As} was similar for Baikal and Eurodor, which presented higher values than Angora and Drakkar. However, BF_{Pb} values were similar for the four other cultivars and were all lower than Baladin.

BAF values (Table 4) showed a different pattern. In the case of arsenic, two groups can be formed: Drakkar and Eurodor presented lower BAF_{As} values than Angora, Baikal and Baladin. For Pb, again Drakkar and Eurodor showed the lowest BAF_{Pb} values, followed by Baladin and Angora, and finally Baikal, which presented the highest value. Moreover, BF and BAF values were very high, due to the fact that these indices were calculated using SPW values on PB5%, and not soil, and that biochar greatly decreased metal(loid) mobility. Therefore, even though BF and BAF values are very high, in this case, flax cannot be considered as a hyperaccumulator due to its low TF values and low concentrations in plant tissues compared to soil concentrations usually found in Pontgibaud soil, around 11,000 $mg.kg^{-1}$ Pb and 500 $mg.kg^{-1}$ As (Lebrun et al., 2019, 2017). However, such indices showed that plants still accumulated As and Pb in concentrations much higher than SPW concentrations. There are two possible explanations for these results: (i) Pontgibaud soil was still able to re-supply As and Pb to the soil, which was directly taken up by the plants, and/or (ii) flax plants were able to uptake metal(loid)s, even when bound to soil particles. Finally, based on these indices, Baladin seems a better choice for phytostabilization as it showed the highest As and Pb root accumulation (BF values) associated to the lowest translocation to upper parts (TF values).

3.5. Oxidative stress and its consequences on membrane lipids, proteins and DNA integrity

The relative quantification of ROS (hydrogen peroxide) revealed an induction of oxidative stress observed as a result of the metal(loid) pollution of each flax cultivar (Figure 5B). Measured oxidative stress was not only localized to the root part, which was directly in contact with the metal(loid)s, but also affected the aerial part of the plants, most likely due to As and Pb translocations from the roots to the aerial parts. The production of hydrogen peroxide, for all flax cultivars, was two- to seven-times higher in the presence of metal(loid)s than under control conditions. No major difference was observed between accumulations in root vs aerial tissue, except for Eurodor and Drakkar. The highest level of ROS production was noted in Eurodor and Drakkar cultivars. Additionally, for these two cultivars, the production of ROS in aerial tissue was more intense than in root tissue. Interestingly, these two cultivars also exhibited a low accumulation capacity of As and Pb. The toxicity of metal(loid)s has been related to their ability to induce oxidative stress in many plant species (Dutta et al., 2018; Štolfa et al., 2015). The higher oxidative stress measured for these two flax cultivars could reflect their lower tolerance to the metal(loid) stress. As a result of this increased ROS production, increases in membrane lipid peroxidation, protein carbonylation and 8-oxo-guanine DNA levels were observed (Figure 5C, D, E). These different oxidative stress markers confirmed that the oxidative stress induced by metal(loid)s was more severe for Eurodor and Drakkar cultivars.

Increased membrane lipid peroxidation has been reported in different species of plants as a consequence of metal(loid) stress (Kumari et al., 2018; Mubarak et al., 2016). The reduction in membrane lipid peroxidation appears to be an important parameter for plant adaptation to polluted soils, and also for plant metal extraction capacity. In fact, this feature has been associated with higher plant tolerance and growth in polluted soils as well as higher metal accumulation capacity in plant tissues (Kumari et al., 2018; Mubarak et al., 2016). For example, maintenance of membrane integrity has been reported as one of the specific characteristics of the Cd hyper-accumulator tolerant species *Brassica juncea* compared to the non-tolerant non-hyper-accumulator *Brassica napus* (Mourato et al., 2015). Membrane integrity maintenance also allows the tolerant species to maintain ion homeostasis through ion efflux mechanisms that prevent and/or reduce the entry of toxic metal ions into the cells (Kumar and Trivedi, 2016). Several efficient efflux transporter proteins have been identified for some tolerant plant species (Kumar and Trivedi, 2016). Oxidative damage at protein level could therefore hinder this detoxification process and reduce plant tolerance to metal(loid) stress (Bhagyawant et al., 2019; Kumar and Trivedi, 2016). Translocation from the root to the aerial part is another aspect that could also be altered by protein oxidative damages, affecting important carriers and/or channels. Plant exposure to heavy metal(loid)s also resulted in DNA damage, indicating the possible genotoxic effect of metal stress that could lead to cell death (Bhagyawant et al., 2019; Imtiaz et al., 2016). In addition to the classical DNA breaks already reported, here, we reported the formation of 8-oxo-guanine, one of the major mutagenic DNA damages caused by metal(loid)-induced oxidative stress. The highest accumulations of As and Pb were observed in Baikal and Baladin flax cultivars, which were the most efficient in circumventing ROS production and ROS damage. These characteristics may be interpreted as a sign of tolerance of these cultivars (Baikal and Baladin). On the contrary, the highest ROS production and oxidative damages were observed with Eurodor and Drakkar cultivars, which failed to accumulate high amounts of metal(loid)s, and can therefore be considered as sensitive. The third strategy of the Angora cultivar could be considered stress avoidance, possibly relying on efficient ion efflux mechanisms to prevent and/or reduce the entry of toxic metal ions into the cells, thus limiting the production of ROS and related oxidative damage. Antioxidant response leading to ROS scavenging is a way of avoiding the toxic effects of metal(loid)s in the cells. To do so, plants have developed a set of antioxidant defense systems relying on enzymatic and/or non-enzymatic mechanisms (Dutta et al., 2018; Štolfa et al., 2015). Our next goal was therefore to evaluate the non-enzymatic antioxidant system, which has been less studied in most plant species and has not been studied in flax to date.

3.6. Accumulation of phenolic compounds and antioxidant response

Among antioxidant compounds, the low molecular weight antioxidants such as the phenolic substances are considered water-soluble. In particular, flavonoids accumulate in the vacuole and are direct scavengers of H_2O_2, singlet O_2 and radical OH (Kolupaev et al., 2020). Flavonoids can trap free radicals and chelate metal ions; all flavonoids have been found to be more or less involved in the antioxidant defense system (Kolupaev et al., 2020). The trends of total phenolic contents (TPC), total flavonoid contents (TFC) and free radical scavenging activities (FRSA) were similar for the five flax cultivars (Figures 5A and 6A, B). For each analyzed tissue, metal(loid) stress resulted in increases in TPCs (2- to 3-fold) and FRSAs (2 to 5 folds) in the five cultivars. Angora and Baikal cultivars presented the highest increases in TPC and FRSA. TPC and FRSA were higher in roots than in aerial parts of Eurodor and Drakkar cultivars, while no significant differences were noted with the three other flax cultivars. TFCs also increased in response to metal(loid) stress but showed some variations in response intensity (Figure 6B). Angora and Baikal showed the highest increase in TFC, but with a distinct pattern of tissue accumulation. TFC showed a preferential accumulation in root parts for all cultivars, with the exception of Baikal for which TFC was higher in aerial parts.

Targeted HPLC analysis focusing on the major compounds accumulated in flax confirmed the effect of metal stress on the activation of the phenylpropanoid pathway. Global increases, from the most abundant to the least abundant, were observed for ferulic acid, lariciresinol, secoisolariciresinol, pinoresinol, *p*-coumaric acid, caffeic acid, benzoic acid, quercetin and kaempferol (Figure 6).

Heatmap representation (Figure 7), normalized with the average accumulation of each compound in the five cultivars was used to depict natural genetic differences *vs* metal stress variations among cultivars. To do this, we used a hierarchical clustering analysis (HCA) using Spearman correlation coefficients to show pairwise comparisons of the effect of metal stress on the phenylpropanoid accumulations of flax cultivars with different genetic backgrounds. A similar ranking was observed for the two analyzed tissues (*i.e.*, roots *vs* aerials). Two distinct clusters were clearly observed. The first cluster, divided into two separate groups, brought together the five flax cultivars grown under control conditions as well as Eurodor under metal stress. This cluster accounted for the natural genetic variations of these cultivars grown under control conditions, with Angora, Eurodor and Drakkar presenting a lower accumulation of phenylpropanoids, whereas Baladin and Baikal accumulated slightly higher amounts. In particular, comparatively, Baladin was naturally rich in kaempferol and benzoic acid in its roots as well as in lariciresinol and kaempferol in its aerial parts. Roots and aerial parts of Baikal presented high TPC under normal conditions, and in particular had relative high amounts of pinoresinol and ferulic acid in its roots. Eurodor grown under metal(loid) stress conditions was linked with Baladin and Baikal grown under control conditions. This could be explained by the low increase in phenylpropanoid accumulation in response to metal(loid) stress observed for this cultivar, particularly in aerial parts. The other cultivars grown under metal(loid) stress conditions were grouped in the second cluster with 2 subgroups: Angora and Baikal on the one hand, and Baladin and Drakkar on the other hand. In particular, in response to metal(loid) exposure, Angora and Baikal roots accumulated higher amounts of benzoic acid, caffeic acid, ferulic acid, lariciresinol and secoisolariciresinol. Under these metal(loid) stress conditions, Angora also accumulated high levels of *p*-coumaric acid in its aerial parts. A marked difference in tissue distribution was also observed for this cultivar in response to metal(loid) stress with a preferential accumulation of ferulic acid and benzoic acid in root tissue compared to aerial parts, whereas the opposite repartition was observed for *p*-coumaric acid and pinoresinol.

Phenolic compounds are of particular interest in the context of metal stress, not only because of their inherent antioxidant capacity, but also because of their ability to chelate metals (Kumar et al., 2017). Some studies have reported an induction of the phenylpropanoid pathway in response to metal stress (Sharma et al., 2019). In particular following exposure to Pb, an increase in TPC in *Brassica juncea* (Kaur Kohli et al., 2018; Kohli et al., 2018), as well as in ferulic acid and caffeic acid in *Prosopis farcta* (Zafari et al., 2016) were reported. The response was more complex in *Zea mays*, for which the increase in TPC was associated with a decrease in caffeic acid and ferulic acid (Kısa et al., 2016). Exposure to As also induced an increase in TPC in *Azolla filiculoides* (Sánchez-Viveros et al., 2011). Flax is of particular interest as a rich source of various classes of antioxidant phenylpropanoid-derived compounds (Garros et al., 2018; Oomah, 2001), which may be involved in chelating processes, such as secoisolariciresinol, ferulic acid, *p*-coumaric acid or quercetin (Fucassi et al., 2014). Here, the differential induction of different classes of phenylpropanoid-derived compounds in response to metal(loid) stress in five flax cultivars with a contrasting response is therefore of particular interest. Correlation analyses were used to evidence possible molecular mechanisms involving these phytochemicals which could contribute to metal(loid) tolerance of flax.

2.7. Correlation analyses

Clear discrimination between the control and metal conditions with principal component analysis (PCA) accounting for 99.37% (F1 + F2) of the initial variability of the data was obtained (Figure 8, S2, S3). The discrimination between the control and metal conditions occurred principally in the first dimension (F1 axis). This F1 axis explained 99.19% of the initial variability, with BF(As), BF(Pb), BAF(As), [As], and [Pb] as the main discriminating contributor for this axis. The second dimension (F2 axis), accounting for only 0.18% of the initial variability, nevertheless gave a clear separation of the different genotypes according to their phytochemical accumulation and antioxidant response to metal stress. The main discriminating contributors of this F2 axis were benzoic acid, caffeic acid, lariciresinol and kaempferol contents as well as the FRSA. The flax genotypes grown under control conditions were circumscribed in the lower left part of the ellipse whereas the genotypes under metal(loid) stress conditions were more largely distributed with Drakkar and Baladin located in the bottom upper part, and Angora and Baikal in the lower right part, whereas Eurodor was the only genotype located in the left part of the biplot. These results confirmed the contrasting response of the 5 flax genotypes to the stress of metal(loid)s with similar discrimination to the one observed on the HCA.

The Pearson correlation analysis study supported this pattern by connecting BF(As), BF(Pb), BAF(As), [As] and [Pb], on the one hand, with the content of benzoic acid, caffeic acid, lariciresinol and kaempferol, and on the other, with RSA.

These results demonstrated the genotype-dependent nature of flax response to metal(loid) stress and the strong impact of resistance to induced oxidative stress, in particular RRSA and accumulation in stress tolerance of certain essential phenylpropanoids such as benzoic acid, caffeic acid, lariciresinol and kaempferol. Based on these results, the different behavior observed for the five flax cultivars indicates that, as a function of this specific phytochemical accumulation capacity, certain genotypes could be well adapted to the phytoremediation of metal(loid) contaminated soils: the cultivar Eurodor was the one having the best growth, but was a sensitive cultivar, while Angora and Baikal were the two most tolerant cultivars. Therefore, Angora and Baikal could be used in associated to biochar for the remediation of an As and Pb contaminated soils.

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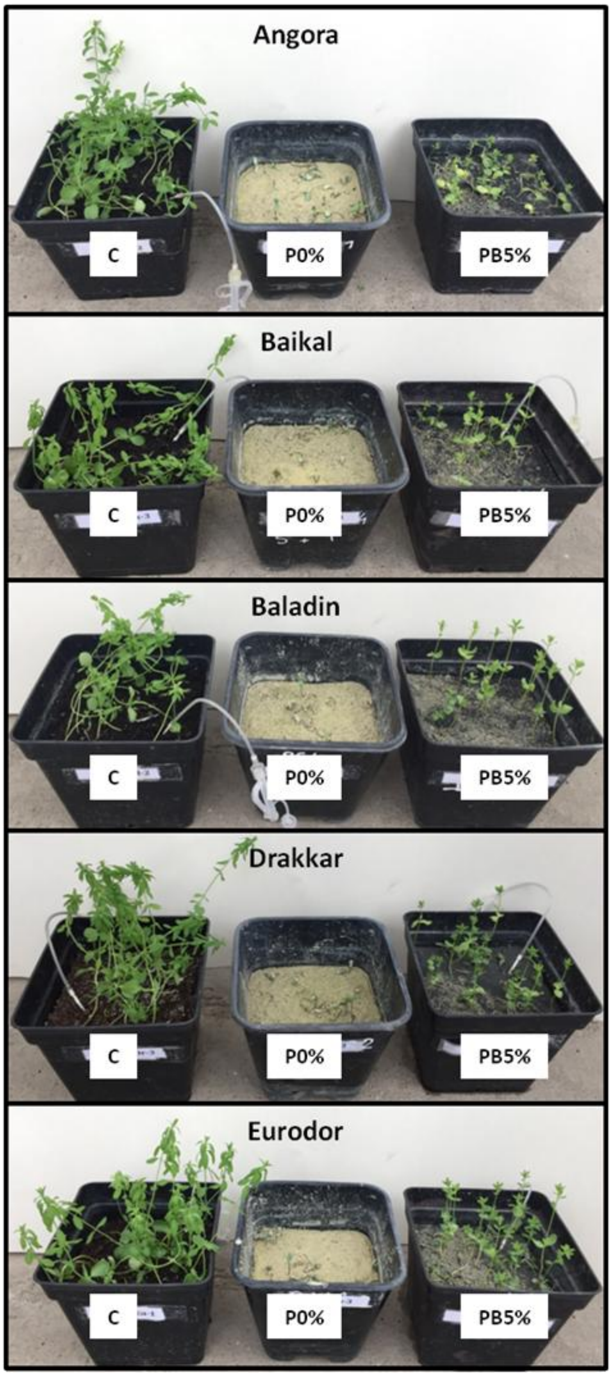
Angora
C
P0%
PB5%
Baikal
C
P0%
PB5%
Baladin
C
P0%
PB5%
Drakkar
C
P0%
PB5%
Eurodor
C
P0%
PB5%

Figure 1: Photographies of *Linum usitatissimum* plants in the pots (13*13*12.5 cm) after 21 days of growth on the different substrates. C = compost (non contaminated control); P0% = Pontgibaud technosol; PB5% = Pontgibaud technosol amended with 5% biochar.

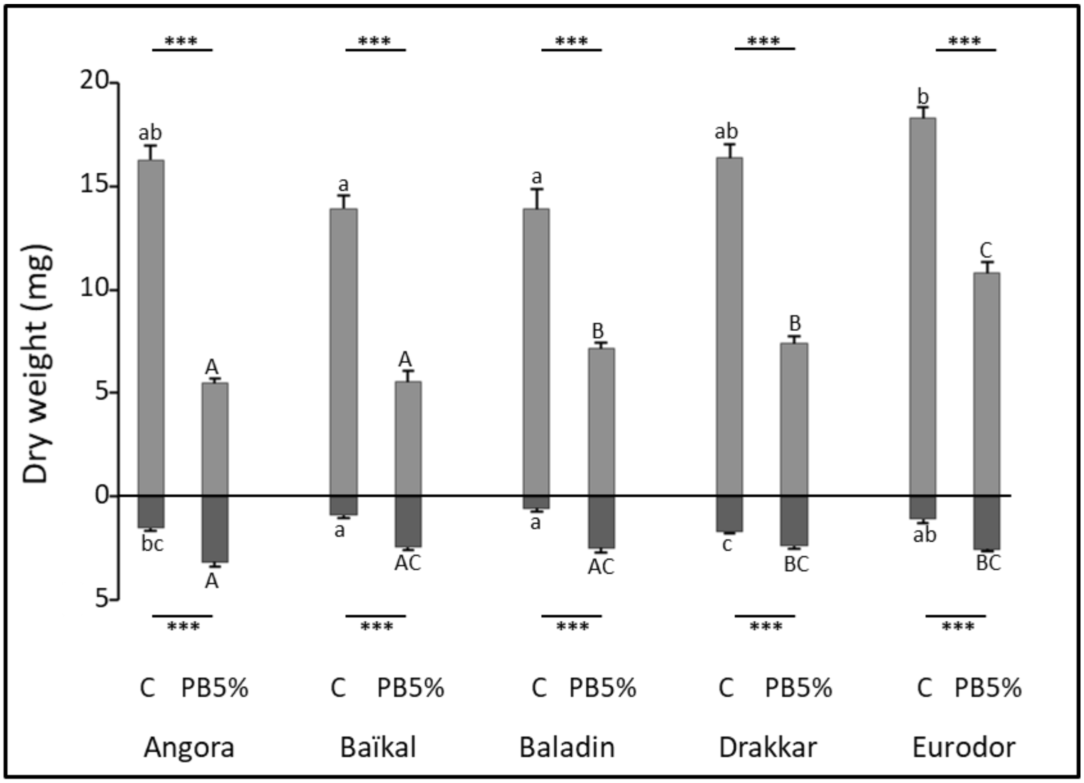

Figure 2: Dry weight (mg) of the aerial (light grey) and root (dark grey) parts of the 5 *Linum usitatissinum* cultivars (Angora, Baïkal, Baladin, Drakkar and Eurodor) after 21 days of growth under 2 conditions. C = compost (non contaminated control); PB5% = Pontgibaud technosol amended with 5% biochar. Capital letters indicate significant difference between the cultivars in the PB5% condition whereas the small letters indicate a singificant difference between the cultivars in the C condition (n = 5-13 ± SD). *** ($p < 0.001$) indicates significant difference between the 2 conditions for one cultivar.

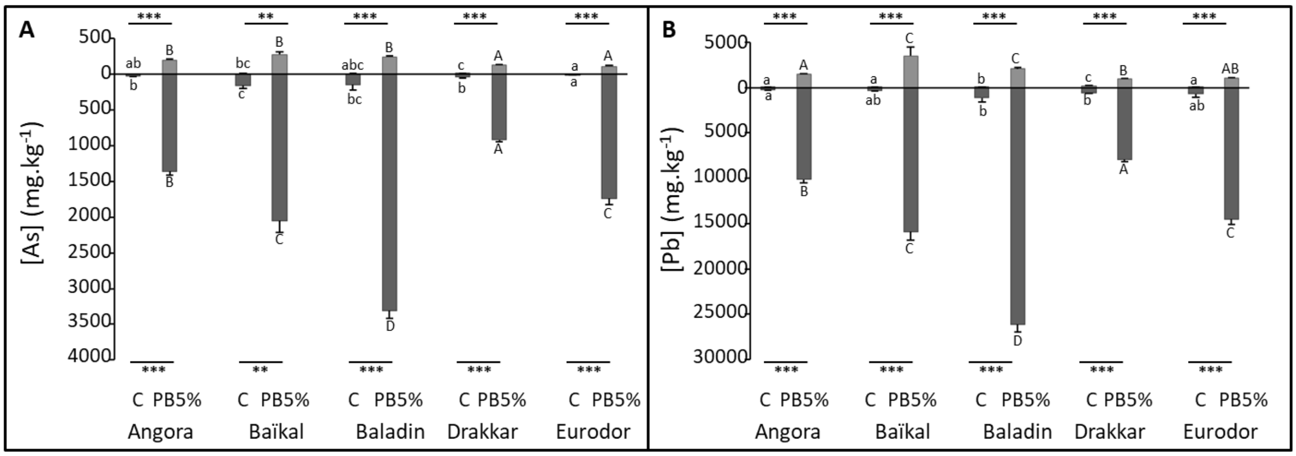

Figure 3: Arsenic (A) and lead (B) concentrations (mg.kg^{-1}) of the aerial (light grey) and root (dark grey) parts of the 5 *Linum usitatissinum* cultivars (Angora, Baïkal, Baladin, Drakkar and Eurodor) after 21 days of growth under 2 conditions. C = compost (non contaminated control); PB5% = Pontgibaud technosol amended with 5% biochar. Capital letters indicate significant difference between the cultivars in the PB5% condition whereas the small letters indicate a singificant difference between the cultivars in the C condition (n = 5-13 ± SD). ** ($p < 0.01$) and *** ($p < 0.001$) indicate significant difference between the 2 conditions for one cultivar.

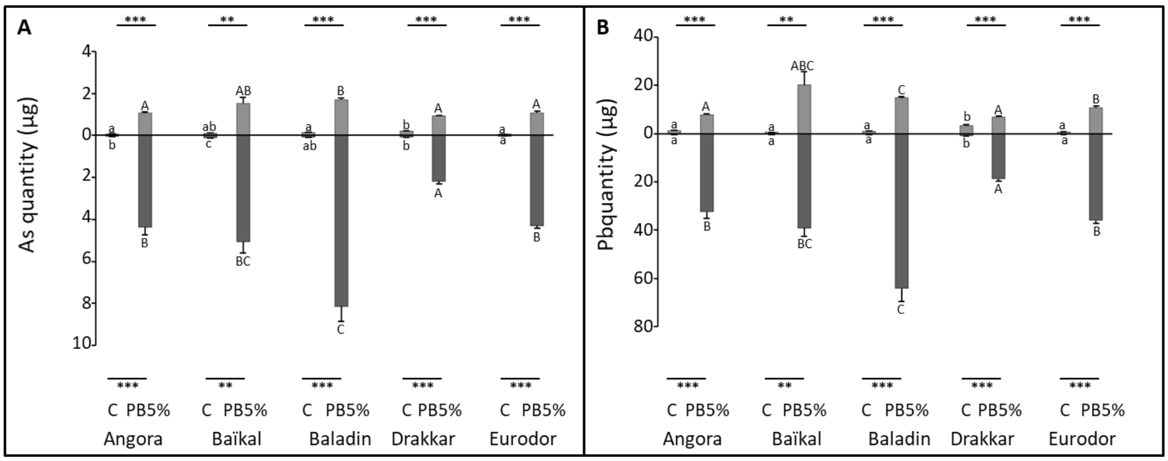

Figure 4: Arsenic (A) and lead (B) quantities (mg.kg^{-1}) of the aerial (light grey) and root (dark grey) parts of the 5 *Linum usitatissinum* cultivars (Angora, Baïkal, Baladin, Drakkar and Eurodor) after 21 days of growth under 2 conditions. C = compost (non contaminated control); PB5% = Pontgibaud technosol amended with 5% biochar. Capital letters indicate significant difference between the cultivars in the PB5% condition whereas the small letters indicate a singificant difference between the cultivars in the C condition (n = 5-13 ± SD). ** ($p<0.01$) and *** ($p<0.001$) indicate significant difference between the 2 conditions for one cultivar.

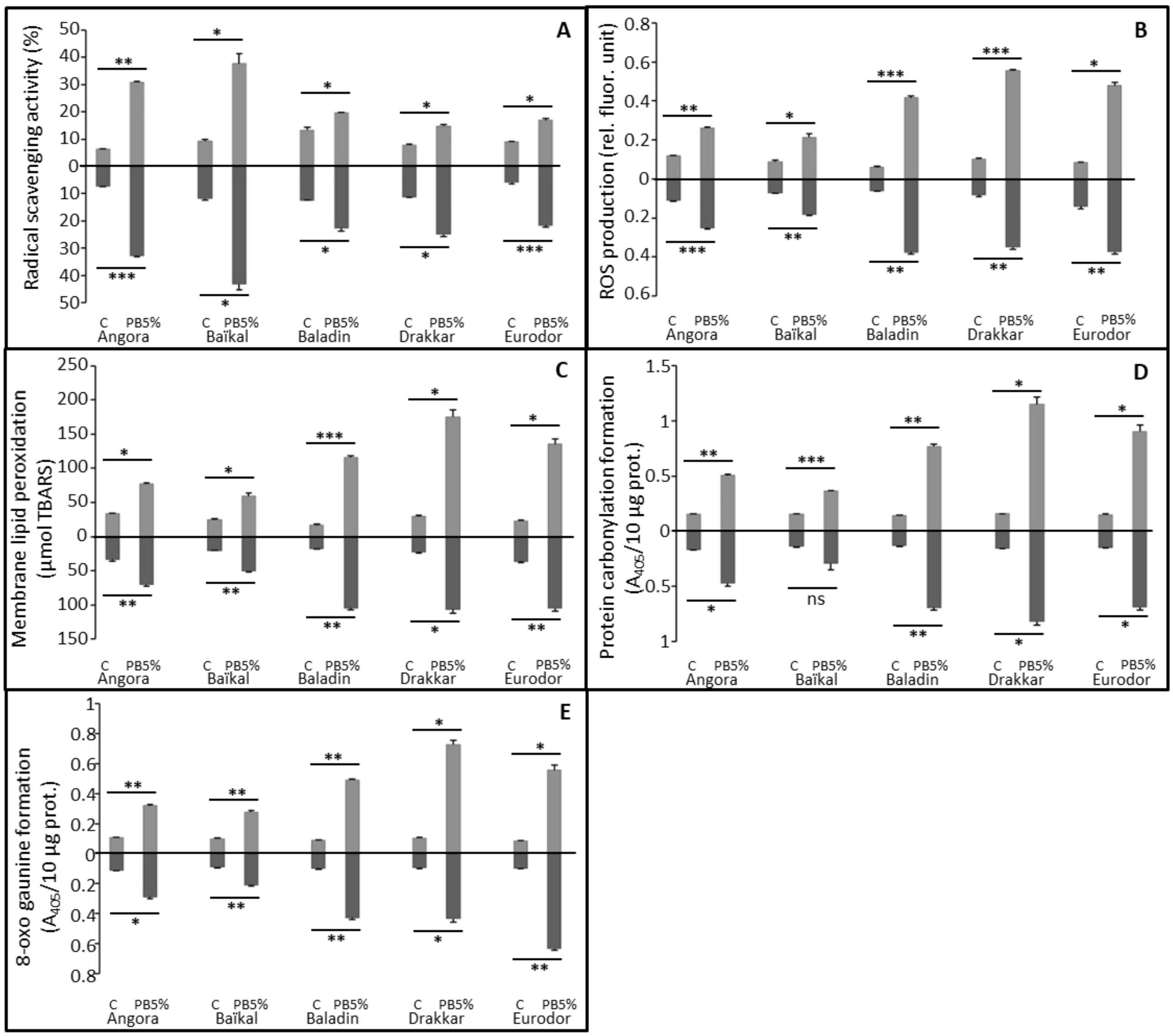

Figure 5: Radical scavenging activity (%) (A), reactive oxygen species (ROS) formation (relative fluorescence unit) (B), membrane lipid peroxidation (µmol TBARS) (C), protein carbonylation formation (A_{405}/10 µg protein) (D) and 8-oxo guanine formation (A_{405}/10 µg protein) (E) measured in the aerial (light grey) and root (dark grey) parts of the 5 *Linum usitatissinum* cultivars (Angora, Baïkal, Baladin,

Drakkar and Eurodor) after 21 days of growth under 2 conditions. C = compost (non contaminated control); PB5% = Pontgibaud technosol amended with 5% biochar. Values are means ± SD of 2 independent experiments (2 biological and 2 technical replicates); * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (comparison with the corresponding compost condition).

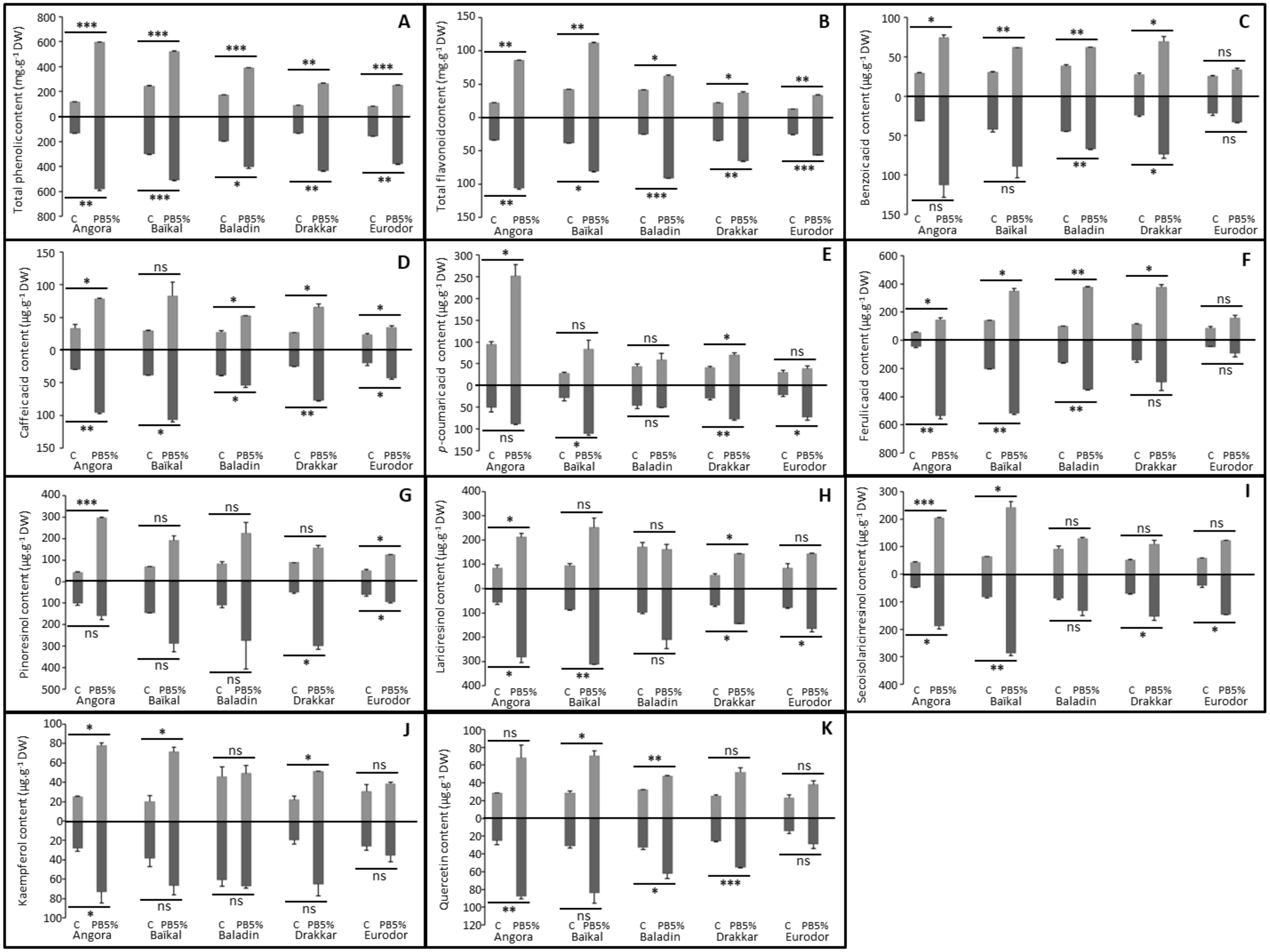
A
Total phenolic content (mg.g-1 DW)
B
Total flavonoid content (mg.g-1 DW)
C
Benzoic acid content (µg.g-1 DW)
D
Caffeic acid content (µg.g-1 DW)
E
p-coumaric acid content (µg.g-1 DW)
F
Ferulic acid content (µg.g-1 DW)
G
Pinoresinol content (µg.g-1 DW)
H
Lariciresinol content (µg.g-1 DW)
I
Secoisolariciresinol content (µg.g-1 DW)
J
Kaempferol content (µg.g-1 DW)
K
Quercetin content (µg.g-1 DW)
C PB5%
Angora
Baïkal
Baladin
Drakkar
Eurodor
ns

Figure 6: Content ($mg.g^{-1}$ or $\mu g.g^{-1}$ dry weight) in phytochemical compounds (total phenolic (A), total flavonoid (B), benzoic acid (C), caffeic acid (D), p-coumaric acid (E), ferulic acid (F), pinoresinol (G), lariciresinol (H), secoisolariciresinol (I), kaempferol (J) and quescetin (K)) of the aerial (light grey) and root (dark grey) parts of the 5 *Linum usitatissinum* cultivars (Angora, Baïkal, Baladin, Drakkar and Eurodor) after 21 days of growth under 2 conditions. C = compost (non contaminated control); PB5% = Pontgibaud technosol amended with 5% biochar. Values are means ± SD of 2 independent experiments (2 biological and 2 technical replicates); * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns: not significant at $p<0.05$ (comparison with the corresponding compost condition).

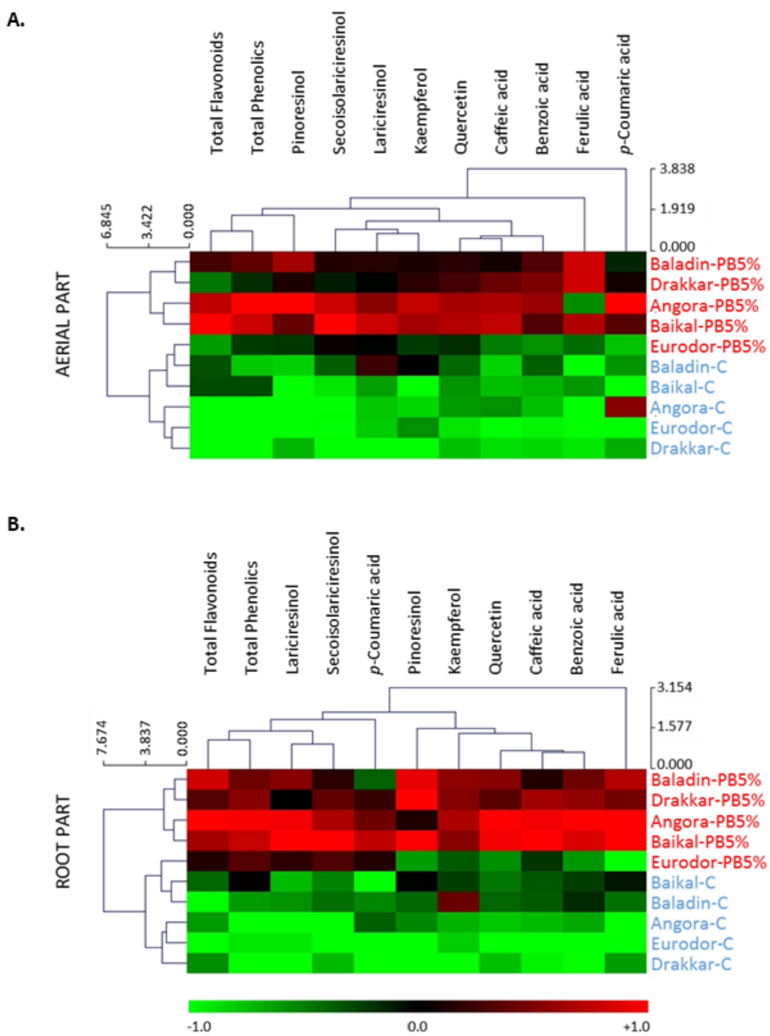

Figure 7: Hirarchical clustering analysis of the phytochemical compounds measured in the aerial (A) and root (B) parts of the 5 *Linum usitatissinum* cultivars (Angora, Baïkal, Baladin, Drakkar and Eurodor) after 21 days of growth under 2 conditions. C = compost (non contaminated control); PB5% = Pontgibaud technosol amended with 5% biochar.

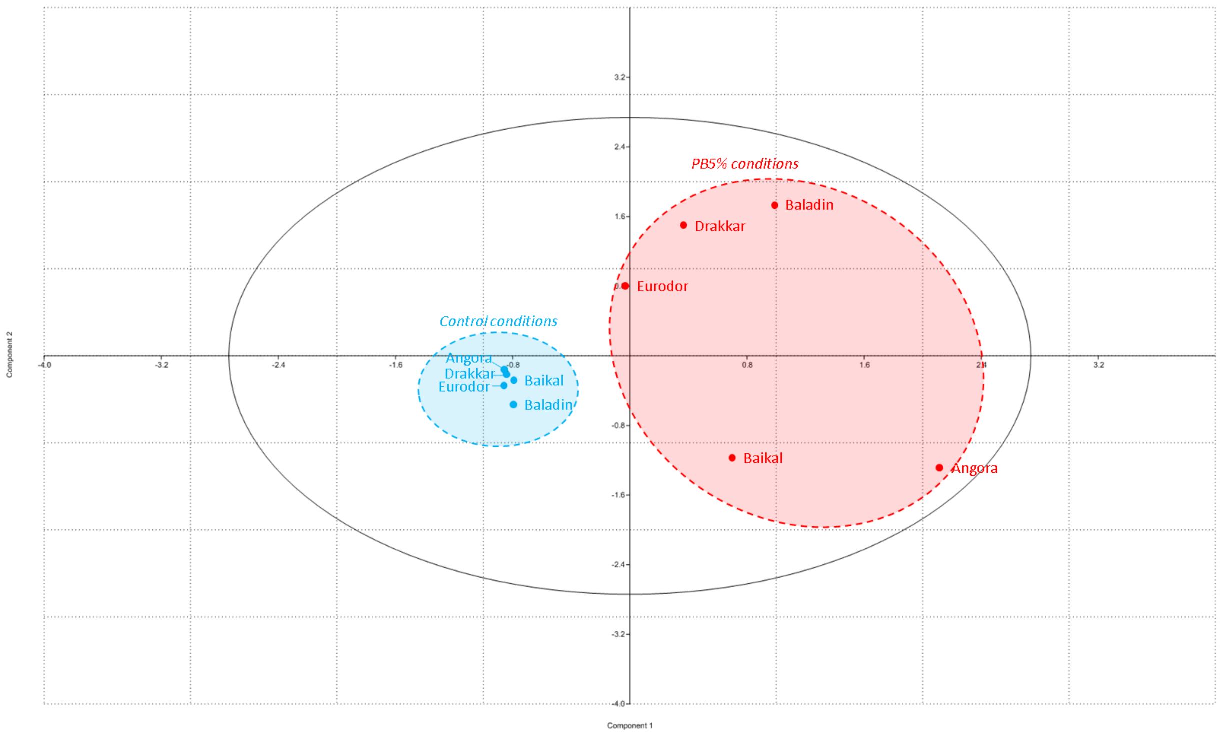

Figure 8: Principal component analysis of the different parameters measured the 5 *Linum usitatissinum* cultivars (Angora, Baïkal, Baladin, Drakkar and Eurodor) after 21 days of growth under 2 conditions (non amended control = blue, PB5% = pink). The Hotelling's ellipse confines the confidence region (95%)of the score plot.

Table 1: Biochar and compost characteristics.

	Compost	Biochar
pH	6.5[1]	9.56 ± 0.01[2]
Electrical conductivity ($\mu S.cm^{-1}$)	520[1]	849 ± 4[2]
Water holding capacity (% mass)	60[1]	194 ± 2
Organic matter (% dry)	68[1]	nd
Particle size (mm)	nd	0.2-0.4[1]
Specific surface area ($m^2.g^{-1}$)	251.42	nd
Total pore volume ($m^3.g^{-1}$)	0.1486	nd
Pore diameter	2.3639	nd

(1) Data provided by the supplier
(2) From Lebrun et al. (2018a)

Table 2: Soil pore water physico-chemical properties (pH and electrical conductivity (EC) ($mg.L^{-1}$)) and metal(loid)s (As and Pb) concentrations ($mg.L^{-1}$), at the beginning of the experiment for the 3 conditions. C = compost (control); P0% = Pontgibaud technosol; PB5% = Pontgibaud technosol amended with 5% biochar. Letters indicate significant difference between the 3 treatments (n=3 ± SD).

	pH	EC ($\mu S.cm^{-1}$)	[As] ($mg.L^{-1}$)	[Pb] ($mg.L^{-1}$)
C	6.25 ± 0.05 **b**	5263 ± 446 **c**	0.023 ± 0.014 **a**	0.131 ± 0.043 **a**
P0%	4.23 ± 0.19 **a**	274 ± 23 **a**	0.025 ± 0.002 **a**	9.322 ± 2.150 **b**
PB5%	6.64 ± 0.07 **b**	1302 ± 73 **b**	0.011 ± 0.007 **a**	1.304 ± 0.014 **a**

Table 3: Relative toxicity of the soil pore water, determined at the beginning of the experiment (T0), and of the soil, determined at the end of the experiment (T21) . C = compost (control); P0% = Pontgibaud technosol; PB5% = Pontgibaud technosol amended with 5% biochar. NV = non-vegetated pots. Minuscule letters indicate significant difference between the 3 treatments (n=3), while capital letters indicate significant difference between cultivar (n = 6 ± SD).

	Soil pore water	Soil					
	T0	T21					
		NV	Angora	Baikal	Baladin	Drakkar	Eurodor
C	1 **b**	1 **bB**	1.21 ± 0.07 **aB**	0.53 ± 0.04 **aA**	0.95 ± 0.05 **aB**	1.50 ± 0.06 **bB**	0.50 ± 0.06 **aA**
P0%	0.79 ± 0.04 **a**	0.29 ± 0.02 **a**	-	-	-	-	-
PB5%	0.96 ± 0.05 **b**	0.25 ± 0.02 **aA**	1.46 ± 0.09 **bE**	0.45 ± 0.02 **aBC**	0.87 ± 0.02 **aD**	0.42 ± 0.02 **aB**	0.51 ± 0.02 **aC**

Table 4: Translocation factor, bioconcentration factor and biological accumulation factor determined for each cultivar in the PB5% condition. Letters indicate significant difference ($p < 0.05$) ($n = 5\text{-}13 \pm SD$).

	Translocation factor (TF)		Bioconcentration factor (BF)		Biological accumulation factor (BAF)	
	As	Pb	As	Pb	As	Pb
Angora	0.15 ± 0.01 **b**	0.15 ± 0.01 **b**	135872 ± 6138 **b**	7809 ± 312 **a**	20055 ± 1768 **b**	1123 ± 100 **ab**
Baikal	0.14 ± 0.03 **b**	0.23 ± 0.07 **b**	204984 ± 18461 **c**	12218 ± 834 **a**	27367 ± 4051 **b**	2712 ± 855 **c**
Baladin	0.07 ± 0.00 **a**	0.08 ± 0.00 **a**	330842 ± 11818 **d**	20106 ± 703 **b**	24108 ± 1607 **b**	1612 ± 107 **b**
Drakkar	0.14 ± 0.01 **b**	0.12 ± 0.01 **b**	91828 ± 3443 **a**	6114 ± 177 **a**	12946 ± 886 **a**	740 ± 50 **a**
Eurodor	0.06 ± 0.01 **a**	0.07 ± 0.01 **a**	173667 ± 9088 **c**	11160 ± 477 **a**	10681 ± 1226 **a**	802 ± 86 **a**