

Efficiency of metal(loid) phytostabilization by white lupin (*Lupinus albus*), common vetch (*Vicia sativa*), and buckwheat (*Fagopyrum esculentum*).

Gaëlle Saladin

gaelle.saladin@unilim.fr

Université de Limoges: Université de Limoges <https://orcid.org/0000-0002-8000-6239>

Marilyne Soubrand

Université de Limoges Faculté des Sciences et Techniques

Emmanuel Joussein

Université de Limoges Faculté des Sciences et Techniques

Ilham Benjelloun

Université de Limoges Faculté des Sciences et Techniques

Research Article

Keywords: bioavailability , heavy metal , phytomanagement , phytoremediation , metalloid , trace element

Posted Date: March 19th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3797540/v1>

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Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on September 9th, 2024. See the published version at <https://doi.org/10.1007/s11356-024-34911-5>.

Abstract

The accumulation of metal(loid)s in soils, often linked to human activities, is a source of toxicity for living organisms. Erosion and leaching from contaminated sites can spread pollution to adjacent ecosystems. Phytostabilization is a method that consists of selecting plant species accumulating little or no metal(loid)s in aerial parts to establish a vegetation cover and thus to stabilize the soil. Seeds of white lupin, cultivated vetch, and buckwheat were sown in greenhouse on soils from former French mines (Pontgibaud and Vaulry) contaminated with several metal(loid)s including high concentrations of arsenic and lead. After 4 weeks of exposure, the growth of white lupin was less affected than that of the 2 other species probably because the concentration of metal(loid)s measured in the roots and aerial parts of lupins was lower. In addition, the level of antioxidant molecules and metal(loid) scavenging compounds was higher in white lupin suggesting that this species could be a good candidate for metal(loid) phytostabilization. However, the level of oxidation was higher than in control plants, suggesting that amendments could be tested to improve the tolerance of this species to metal(loid)s.

Introduction

Numerous human activities contribute to the accumulation of metal(loid)s in soils such as mining and metallurgical activities, the combustion of coal and fossil fuels, agricultural activities (metal(loid)s are present in phosphate fertilizers and several pesticides), sewage sludge or wastewater (Bradl 2005). The presence of these metal(loid)s in the soil is not without risks for living organisms including human health (Sun et al. 2022; Yang et al. 2023). Indeed, metal(loid)s generate cellular oxidation and cause cancers and other diseases such as arsenic, lead or mercury poisoning (Nag et al. 2022). Physicochemical processes to remove pollutants are costly and increase soil degradation (Pouresmaeli et al. 2022). The use of plants for decontamination (phytoremediation) promotes soil stabilization and is a less costly and more environmentally friendly method. However, for soils with high metal(loid) contamination the extraction process is very time consuming, therefore the method used aims rather at immobilizing the pollutants by phytostabilization (Pouresmaeli et al. 2022). This method consists of establishing a vegetation cover to limit soil erosion and contaminant leaching by wind and water in order to protect adjacent ecosystems (Hbaieb et al. 2018; Sheoran and Choudhary 2021). One of the keys to the effectiveness of this method is the selection of excluding plant species. Excluders have low uptake or non-lethal root storage of metal(loid)s as well as a low rate of metal(loid) translocation to aerial parts (Lin and Aarts 2012).

It has been shown that excluding plants secrete exudates that can modify the pH of rhizosphere as well as the cation exchange capacity (CEC) and can chelate or precipitate metal(loid)s in soil (Ma et al. 2022). As a consequence, metal(loid)s are less soluble and thus less phytoavailable. Many exudates limiting metal(loid)s uptake by root are cited in the literature according to the plant species such as organic acids (malate, citrate, fumarate, aconitate...), amino acids (proline, glutamic acid, alanine, valine...), carbohydrates, mucilage, phenolic compounds, carbonates, sulfates, sulfides (Canarini et al. 2019; Chai and Schachman 2022; Quiñones et al. 2022; Thakur et al. 2022). In some excluding plants the mechanisms of (metal)loid tolerance can be related to a low expression of transporters importing metal(loid)s (ZIP, Nramp1) and a high expression of transporters promoting efflux of metal(loid)s (YSL, HMA4, PCR1 and 2, PDR8) or storage in the vacuole of root cells (HMA3, ZIF1, ABCC1 and 2, MTP1 and 3) (Lin and Aarts 2012). Metal(loid)s that are nevertheless

absorbed are scavenged with mechanisms similar to those present in hyperaccumulating plant species but the storage is mainly root-based whereas it is more stimulated in the aerial parts in hyperaccumulating species (Yan et al. 2020; Thakur et al. 2022). Metal(loid)s can be bound at intracellular level by ligands such as cysteine rich oligopeptides also named non-protein thiols (NPT) (phytochelatins, glutathione), metallothioneins, proline, histidine, polyamines, nicotianamine, organic acids (Thakur et al. 2022; Ghuge et al. 2023). Metal(loid)s can be also stored in the cell wall by binding with pectins, lignin or structural proteins or by precipitation with silicates.

The other requirement in plant species selection is to have plants that can grow on low fertility soils. Indeed metal(loid)-rich sites are often mining waste deposition areas that have acidic pH, low CEC and a low content of organic matter and nutrients (Bonnet et al. 2016; Hbaieb et al. 2018). Local pioneer species are generally preferred as they are adapted to poor soil quality and climatic conditions of the site. Some species improve soil fertility that may be an additional selection criterion for phytostabilization. For example, Fabaceae are known to establish root symbioses with nitrogen fixing bacteria (*Rhizobium*, *Bradyrhizobium*...). This association helps to reduce the contribution of nitrogenous fertilizers on low fertile soils and thus limits the costs of the rehabilitation process.

The aim of our work was to compare the growth and responses of three plant species grown on two soils contaminated with metal(loid)s (mainly As and Pb) in order to select the best performing species for phytostabilization. White lupin (*Lupinus albus*) and common vetch (*Vicia sativa*) belong to the Fabaceae family. White lupin grows most often on soils with a slightly acidic to neutral pH but can tolerate acidic pH (Martínez-Alcalá et al. 2010). It was shown to have exclusionary properties and / or low translocation from roots to shoots for several metal(loid)s such as Al, As, Cd, Cu, Pb, Sb (Vázquez et al. 2006; Garau et al. 2021; Quiñones et al. 2022). In parallel, it can mobilize several essential elements through its exudates such as Fe, Mn, Zn and phosphorus (Martínez-Alcalá et al. 2010). Common vetch (*Vicia sativa*) is a species that grows on acid to alkaline soils with high tolerance to biotic and abiotic stresses and is of interest as a green manure (Tigka et al. 2021). It was recently shown that this species could be used in phytostabilization on soils containing As, Cd, Cu, Ni, and Zn (Zine et al. 2021). Buckwheat (*Fagopyrum esculentum*) which belongs to the Polygonaceae family tolerates acidic soils and improves soil phosphorus solubilization (Chen et al. 2008; Teboh and Franzen 2011). However, its root exudates also promote the solubilization of Al and Pb and these metals can accumulate in the aerial parts of the plant (Chen et al. 2008; Tamura et al. 2005).

Materials and methods

Soil sampling and characterization, plant material, and culture conditions

We collected the first 15–20 cm of soil on two French contaminated sites. The first site is close to a former mine of lead and silver located at Pontgibaud in the Puy-de-Dôme (Pascaud et al., 2014; Bonet et al. 2016; Hbaieb et al. 2018). The second site is close to a former mine of tin and tungsten at Vaulry in Haute-Vienne. Air-dried soils were sieved to 2 mm and stored at ambient temperature in polyethylene containers. A non-contaminated control soil was prepared by mixing horticultural soil and sand (75/25 v/v). The pH of soils was measured in water with a ratio 1:5. The CEC was determined by the 0.05 N cobalt hexamine method and the

organic matter content was determined by the method of loss ignition (Hbaieb et al. 2018). The concentration of P in soil solution was obtained after mixing 1 g of dry soil with 10 mL of 0.01 M CaCl_2 for 2 h. After filtration (0.45 μm) 2.5 mL of sample were mixed with 125 μL of acid ascorbic at 2% (w/v) and 500 μL of reagent. The reagent contained 50% (v/v) sulfuric acid at 15% (v/v), 5% (v/v) of potassium antimony tartrate at 4% (w/v) and 15% (v/v) of ammonium molybdate tetrahydrate 0.28% (w/v). After 30 min at ambient temperature the DO was read at 700 nm and results were expressed in mg P kg^{-1} dry soil with KH_2PO_4 as a standard.

Seeds were purchased from “Graines Baumaux” (Mirecourt, Vosges, France) and sown in greenhouse in pots (12 cm height, 12 cm diameter) containing control and contaminated soils. Pots were watered twice a week with demineralized water. Photoperiod varied from 12 to 14 h corresponding to natural sunlight (no artificial light was used in the experiments), and mean temperature was around 20°C. Plants were harvested after 3 weeks and roots were rinsed with water, then incubated for 1 h in 20 mM EDTA to remove adsorbed metal(loid)s and rinsed again with demineralized water. Stem height and main root length were measured and fresh leaves, stems and roots were weighted. A part of organs was dried at 80°C for 2 days to obtain dry weight (DW) and samples for metal(loid) analysis. The other part of organs (only leaves and roots) was stored at - 20°C until biochemical analyses.

Plant assays

For photosynthetic pigment measurement frozen leaves were ground at 4°C with 80% (v/v) acetone and 0.5% (w/v) MgCO_3 (Saladin et al. 2003). After centrifugation chlorophylls a and b, and carotenoids were measured in the supernatants at 663, 647, and 470 nm, respectively and their concentration was calculated in mg g^{-1} DW according to the equations of Lichtenthaler (1987).

For total soluble protein, total soluble carbohydrate (TSC), and starch content, frozen leaves and roots were ground at 4°C with 0.1 M phosphate buffer at pH 7.5 containing 10 mM MgCl_2 and 5 mM EDTA (Moussavou Moudouma et al. 2013). After centrifugation supernatants were used for protein and TSC assays whereas the pellet was mixed with a solution of 8 N dimethyl sulfoxide and 37% HCl (4:1 v/v) and incubated at 60°C (Bonnet et al. 2016). After centrifugation supernatant was mixed with water and a reagent containing 0.03% I_2 and 0.06% KI in 0.05 N HCl and absorbance was read at 600 nm. The results were expressed in mg g^{-1} DW with starch as standard. For protein assay samples were mixed with a reagent according to manufacturer's instructions (Bio-Rad Protein Assay, Bio-Rad, Munich, Germany) and the results were expressed in mg g^{-1} DW with Bovine Serum Albumin as standard. For TSC, samples were incubated at 95°C with a reagent containing 0.1% anthrone and 0.1% thiourea in 12.5N H_2SO_4 (Saladin et al. 2003). The absorbance was read at 625 nm and the results were expressed in $\mu\text{mol g}^{-1}$ DW with glucose as standard.

The content of total free amino acid (TFAA), total free proline, total non-protein thiols (NPT) and thiobarbituric acid reactive substances (TBARS) was calculated after growing frozen organs with 10% (w/v) trichloroacetic acid containing 6.3 mM diethylenetriaminepentaacetic acid (Bonet et al. 2016). TFAA and proline were quantified after incubation at 95°C with two different reagents containing ninhydrin. Results were expressed in $\mu\text{mol g}^{-1}$ DW with leucine and proline as standards, respectively. NPT were assayed with Ellman's reagent. Absorbance was read at 412 nm and results were expressed in $\mu\text{mol g}^{-1}$ DW with cysteine as standard. TBARS were quantified after incubation of samples at 95°C with 20% (w/v) trichloroacetic acid containing

0.5% (w/v) 2-thiobarbituric acid. Absorbance was read at 532 nm and corrected by subtracting the absorbance read at 600 nm (non-specific absorbance). Results were expressed in $\text{nmol g}^{-1} \text{ DW}$ with the extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

H_2O_2 content was obtained by grounding frozen organs at 4°C with TCA 0.1% (w/w). After centrifugation at 4°C for 15 min at 10 000 g the supernatant was used for the assay according to Junglee et al. (2014) by mixing 125 μL supernatant with 125 μL potassium phosphate buffer (10 mM, pH 7) and 250 μL of 1M KI. After 10 min at room temperature the absorbance was read at 350 nm. The results were expressed in $\mu\text{mol g}^{-1} \text{ DW}$ with H_2O_2 as standard.

The assay of total phenolic compounds (TPC) was performed according to Chandra et al. (2014) with modifications. Frozen organs (100 mg) were ground at 4°C with 2 mL of methanol at 80% (v/v). After centrifugation at 12,000 g for 15 min at 4°C the supernatant was used for assay. A volume of 50 μL was mixed with 400 μL water and 100 μL of Folin-Ciocalteu reagent (Sigma-Aldrich, USA). After 5 min at room temperature, 500 μL sodium carbonate (8% w/v) and 500 μL water were added and the sample was incubated in darkness for 30 min. Absorbance was read at 750 nm and the results were expressed in $\text{mg g}^{-1} \text{ DW}$ with gallic acid as standard.

Metal(loid) concentrations in soils and plants

The concentration of bioavailable metal(loid)s was measured after extraction by 10 mM CaCl_2 for 2 h with a ratio soil / solution of 1:10 (Novozamsky et al. 1993). Metal(loid) concentration in soils, bioavailable fractions, and plant organs were measured according to Bonnet et al. (2016).

Statistical analysis

Results were the mean of five independent experiments. For plant growth parameters, 3 to 10 plants were used per independent experiment and per condition (3 plants for white lupin and 10 plants for the two other plants species). Two-way ANOVA tests were performed with the PAST software (version 2.17) and Tukey's tests were carried out when significant difference was found ($P < 0.05$).

Results

Soil properties and metal(loid) mobilization

The contaminated soils are acidic with a pH of 4.2 for Pontgibaud and 4.7 for Vaulry and the percentage of organic matter was low varying between 3.5 % for Vaulry and 4.3 % for Pontgibaud (Table 1). The CEC and the available phosphorus concentrations were particularly low for both contaminated soils with values lower than 4 cmol kg^{-1} for CEC and around 0.4 mg kg^{-1} for phosphorus. The total metal(loid) concentration was much higher on the Pontgibaud soil than the Vaulry soil for As, Pb, and Sb with 1064, 12340, and 154 mg kg^{-1} , respectively. The exception was Sn for which the total concentration is higher in the Vaulry soil (221 mg kg^{-1}). The CaCl_2 extracts are considered as the exchangeable fraction that is readily available for plants (Hbaieb et al. 2018). The concentrations of phytoavailable metal(loid)s indicated that for the Pontgibaud soil, only Pb was potentially taken up by plants, and at a high concentration around 500 mg kg^{-1} . The concentrations of As, Sb,

and Sn in the CaCl_2 extracts were very low or even lower than the limit of detection for both Pontgibaud and Vaulry soils.

Whatever the soil and the organs the concentrations of As and Pb were the lowest in white lupin and the highest in buckwheat (Table 2). Sb concentrations were higher in buckwheat on Pontgibaud soil but lower than for the other two species on Vaulry soil. Whatever the metal(loid) (Pb, As, Sb) the concentrations were lower in the stems and leaves than in the roots which indicates a weak translocation towards the aerial parts. However, root concentrations of Pb and As remained high for Pontgibaud soil with $392 \text{ mg Pb g}^{-1} \text{ DW}$ and $1618 \text{ mg g}^{-1} \text{ DW}$ in white lupin, which is the species that absorbed these metal(loid)s the least compared to buckwheat and common vetch.

Plant growth and photosynthesis

Leaf DW of white lupin was not modified on the contaminated soils compared to the control as well as stem and root DW on Pontgibaud soil (Fig. 1A). The results showed a decrease of stem DW and an increase of root DW on Vaulry soil. The Leaf DW of common vetch and buckwheat was reduced on contaminated soils compared with controls whereas stem and root DW were significantly reduced on Pontgibaud soil (Fig. 1B, C). Water content in the different organs was mostly similar between control and plants grown on Vaulry soil and reduced on Pontgibaud soil for white lupin (Fig. 1D) and common vetch (Fig. 1E). For buckwheat the water content was globally reduced on both contaminated sites compared to the control (Fig. 1F). Stem length of white lupin and common vetch was reduced on Pontgibaud site whereas it was reduced on both contaminated sites for buckwheat (Fig. 1G). Root length was strongly reduced for the three plant species on both contaminated sites with a stronger effect on Pontgibaud soil (Fig. 1H). The two-way ANOVA analyses showed that there was an effect of the species, the soil, and an interaction between these two parameters (Table 3).

Photosynthesis of white lupin was not affected on Vaulry soil (except an increase of chlorophyll b) unlike Pontgibaud soil (Fig. 2). Indeed, on Pontgibaud soil the results showed a decrease in chlorophyll b and carotenoid contents as well as in starch content in the roots. On the other hand, the TSC content was higher in leaves and roots compared to the control (Fig. 2D, E). The results were similar in common vetch and buckwheat but with stronger differences between the control and the contaminated soils.

Plant nitrogenous compounds and oxidation level

The soluble protein content was significantly reduced in leaves and roots of all species except buckwheat on Vaulry soil (Fig. 3A, B). Differences were observed between species regarding TFAA content (Fig. 3C, D). TFAA levels decreased slightly in the leaves of white lupin on contaminated soils but remained stable in the roots. In common vetch, TFAA content increased significantly in the leaves for both contaminated soils and in the roots for Pontgibaud soil. In buckwheat the values remained unchanged in the leaves and increased at the root level only for Pontgibaud soil. The content of free proline increased significantly in the leaves and roots of white lupin and common vetch on contaminated soils while it decreased slightly or remained unchanged in buckwheat (Fig. 3E, F). The TPN level varied among species (Fig. 3 G, H). In white lupin it increased in leaves on Vaulry soil and in roots on Pontgibaud soil. For common vetch, the TPN concentration increased in leaves on both contaminated soils and in roots on soil Pontgibaud whereas in buckwheat, the TPN content increased strongly in all organs on both contaminated soils.

The results showed an accumulation of H_2O_2 in leaves and roots of the 3 plant species on Pontgibaud soil but not on Vaulry soil (Fig. 4A, B). TBARS content increased significantly in the organs of the 3 species with a stronger effect on Pontgibaud soil than on Vaulry soil (Fig. 4C, D). Overall, the content of TPC increased significantly in the organs of the 3 plant species grown on contaminated soils (Fig. 4E, F). The highest values were for buckwheat not only in response to metal(loid)s but also at a constitutive level on the control soil.

Discussion

Growth was less affected in white lupin

In terms of growth, white lupin had the highest biomass of the three species tested especially at the root level which makes it interesting for phytostabilization. Moreover, its biomass was little or not affected by soil conditions and metal(loid) contamination indicating a good tolerance to stress. Although the main root was shorter on contaminated soils the total root biomass remained unchanged and even increased on Vaulry soil. This phenotype can be interpreted as a stress-induced morphogenic response (SIMR) i.e. a strategy to avoid stress. Indeed, it has been shown that under conditions of metal(loid) contamination or deficiency in essential elements roots can stop their in-depth growth and the plant initiates more lateral roots to find more favorable conditions (Forino et al. 2012; Kolbert 2016). Common vetch and buckwheat also might adopt a similar strategy on Vaulry soil as main root length was reduced but total root biomass remained close to the control. However, the Pontgibaud soil can be considered as toxic for these two species as root biomass and main root length were significantly reduced. Franzaring et al. (2018) have grown buckwheat on mining wastes containing high concentrations of several metal(loid)s such as As and Pb and observed similar results than ours with a reduction of plant height and biomass.

The content of photosynthetic pigments in white lupin has not been modified which allowed the plants to maintain their growth. However, it can be noted on the Pontgibaud soil that white lupin accumulated soluble carbohydrates in both leaves and roots. This accumulation may be due to a hydrolysis of the root starch and a modification of TSC mobilization. Indeed, soluble carbohydrates are part of the molecules involved in osmotic adjustment in response to drought to limit cell plasmolysis and our results showed that white lupin underwent a slight leaf dehydration on the Pontgibaud soil (Sami et al. 2016; Gosh et al. 2021). In common vetch and buckwheat grown on Pontgibaud soil, carbohydrates also strongly accumulated in the leaves (and to a lesser extent in the roots) with a strong decrease in starch and photosynthetic pigment contents suggesting a hydrolysis of starch to counteract the dehydration observed in the organs of these two species. The evolution was quite similar for the plants that grew on Vaulry soil but with a lower intensity of responses which confirms that Vaulry soil was less constraining for the plants than Pontgibaud soil.

Free proline and TFAA also participate in osmotic adjustment (Gosh et al. 2021). The TFAA level did not vary in white lupin roots and slightly decreased in leaves. However, free proline increased significantly and could be added to TSC to maintain osmolarity. Common vetch also accumulated free proline in leaves and roots as well as TFAA (but to a lesser extent) unlike buckwheat which only showed an accumulation of TFAA in roots on Pontgibaud soil. It should be noted however that the accumulation of amino acids is at the expense of protein synthesis which dropped in the three species that grew on contaminated soils.

All plant species were subjected to an oxidative stress

Regardless of the species the results showed that H_2O_2 accumulated strongly in plants grown on Pontgibaud soil but at a lower level on Vaulry soil. On Pontgibaud soil white lupin had the lowest content in the leaves but the highest in the roots compared to the other two species. The increase of reactive oxygen species (ROS) is often observed in plants subjected to metal(loid) contamination (Pardo-Hernández et al. 2021; Ghuge et al. 2023). To mitigate oxidation plants can enhance antioxidant responses and / or strategies of metal(loid) scavenging. Among antioxidant compounds it has been shown that TPC and proline can participate in ROS reduction (Michalak 2006; Sharma and Dietz 2006; Ghuge et al. 2023; Narayanan and Ma 2023). Our results showed that TPC content increased in the three plant species and on both contaminated soils with a higher level in buckwheat. This increase probably allowed buckwheat to limit the accumulation of H_2O_2 in the roots compared to the two other species but not at the foliar level on Pontgibaud soil. Moreover, among NPT glutathione is known to play a role against oxidation (Hasanuzzaman et al. 2017; Zulfiqar and Ashraf 2022). It is possible that the high increase of NPT content in buckwheat also partially contributed to limit oxidation. The NPT (mainly phytochelatins and glutathione) and proline are among the molecules that can bind metals and thus reduce oxidation (Sharma and Dietz 2006; Ghuge et al. 2023; Narayanan and Ma 2023). Our results showed different strategies between the tested plants: white lupin and common vetch favored proline accumulation while buckwheat had the highest NPT level. Despite the increase in antioxidant molecules and metal scavenging TBARS content remained significantly higher in plants grown on contaminated soils regardless of species, indicating a sensitivity of plants to the metal(loid)s and / or to soil characteristics such as acidic pH and / or low CEC.

Metal(loid) uptake was lower in white lupin

On Pontgibaud soil As, Sb, and Sn are not strongly bioavailable unlike Pb. When grown on this soil white lupin had the lowest Pb concentrations compared to common vetch and buckwheat with 5 to 25 times less Pb in the roots and 8 to 21 times less in the leaves, respectively. This species is therefore the most interesting for Pb phytostabilization. It was shown by Garau et al. (2021) that on a highly contaminated soil at 15 g Pb kg^{-1} (thus close to Pontgibaud soil) white lupin stored Pb in the roots with a concentration around 2 g Pb kg^{-1} but the concentration in the aerial parts was low with a translocation factor of 0.08. Martínez-Alcalá et al. (2010) showed that Pb concentration remained much higher in roots than in shoots with a translocation factor around 0.2 confirming the interest to use white lupin for Pb phytostabilization. In our conditions Pb concentration in roots of white lupin was lower than 0.4 g kg^{-1} with a translocation factor from roots to leaves around 0.3. Buckwheat was reported by Tamura et al. (2005) to be tolerant to high Pb concentrations but the growth conditions described by the authors were more optimal (soil had higher pH and CEC) and our soil contained other contaminants such as As. Domańska et al. (2021) proposed buckwheat for Pb phytostabilization and showed that plants had biomass closed to control plants but in their experiments the soil had a higher pH and / or contained more organic matter than in ours. A recent review reported vetch tolerance to Cd, Cu, and Zn, as well as sensitivity to Ni, but the literature does not mention any studies of vetch plants exposed to Pb (Ramírez-Parra and De La Rosa 2023).

Despite the low As bioavailability in Pontgibaud soil our results showed a high concentration in the roots of the 3 plant species (and in the leaves of buckwheat) which suggests that these plants may have secreted exudates that enhanced As solubilization. It was reported that white lupin and buckwheat grown on phosphate-deficient soils may stimulate the secretion of exudates to improve phosphate uptake (Possinger et

al. 2013; Fresno et al. 2017; Ma et al. 2022). As arsenate (generally considered to be the main species of As in aerobic soils) and phosphate are uptaken by similar root transporters thus the strategy to solubilize phosphate may have increased As solubilization and its subsequent uptake (Zhao et al. 2009). The same hypothesis can be formulated for the Vaulry soil as the roots of the 3 species exhibited a non-negligible As content in roots even if it was much lower than for the plants grown on Pontgibaud soil. White lupin was proposed as an As-excluder but the authors stated that As concentration in roots remained significant (Vàsquez et al. 2006). Common vetch was also reported as a species accumulating more As in roots than in shoots confirming our results (Aslan et al. 2021). It was reported that As accumulation in roots may be due to scavenging with compounds such as phytochelatins or damages on transporters that lead to a reduction of As translocation to shoots (Yañez et al. 2019, Aslan et al. 2021).

Conclusion

Of the 3 plant species tested on metal(loid)-contaminated soils white lupin was able to maintain a growth rate close to the control and had a low rate of metal translocation from roots to leaves. These characteristics suggest that white lupin could be a species of choice for phytostabilization. However, our results showed an accumulation of metal(loid)s in the roots that could have a long-term toxicity effect as the level of antioxidant defenses and metal(loid) scavenging molecules was not enough efficient to avoid lipid peroxidation. In a previous study on Pontgibaud soil we showed that the addition of amendment and more specifically composted sewage sludge strongly reduced Pb bioavailability in the soil and the accumulation of Pb and as in Douglas-fir seedlings (Hbaieb et al. 2018). Therefore, it would be interesting to test this type of amendment with white lupin to determine if phytostabilization could be improved. The choice of amendment can be tricky because Garau et al. (2021) showed in white lupin that an addition of compost decreased root accumulation of Cd and Zn but increased accumulation of As, Cu, Pb, and Sb. In another study, an amendment with 5% biochar significantly reduced the accumulation of Cd in roots of white lupin but had no effect on the concentrations of Pb and Zn (Houben and Sonnet 2015). Phytostabilization with white lupin therefore requires to compare the effectiveness of several amendments to select the one that best limits the metal(loid) uptake by the plants.

Declarations

Ethical Approval

Not Applicable.

Consent to Participate

Not Applicable.

Consent to Publish

All authors participated to this manuscript and thus consent to publish all the data.

Authors Contributions

G. Saladin conducted the experimental culture, plant harvest, plant assays and statistical analysis. M. Soubrand analyzed all parameters related to soils and metal(loid)s. E. Joussein made the soil samplings. Ihlam Benjelloun participated to plant culture and assays, and soil analyses for the plants grown on Pontgibaud soil. G. Saladin wrote the manuscript with the help of M. Soubrand and E. Joussein for the physicochemical properties of soils and the bioavailability of metal(loid)s.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Tables

Table 1 Characteristics and metal(loid) concentrations in control and contaminated soils (Pontgibaud and Vaulry)

	Control	Pontgibaud	Vaulry
pH	7.6	4.2	4.7
Texture	-	Sandy loam	Sandy loam
Organic matter (% w/w)	26	4.3	3.5
CEC (cmol kg ⁻¹)	29	3.8	3.1
Available P (mg ⁻¹ kg)	7.561	0.422	0.385
As total / bioavailable (mg ⁻¹ kg)	< l.d.	1064 / < l.d.	772 / 0.26
Pb total / bioavailable (mg ⁻¹ kg)	< l.d.	12340 / 490	121 / 0.03
Sb total / bioavailable (mg ⁻¹ kg)	< l.d.	154 / 0.04	4.6 / 0.01
Sn total / bioavailable (mg ⁻¹ kg)	< l.d.	< l.d. / < l.d.	221 / < l.d.

< l.d. value lower than the limit of detection. The concentration of bioavailable metal(loid)s in control soil was not measured as the total concentrations were below the limit of detection.

Table 2 Concentration of Pb, As, Sb, and Sn in organs of white lupin, common vetch, and buckwheat after 3 weeks of growth on contaminated soils

Species	Organ	Pb		As		Sb		Sn
		Pontgibaud	Vaulry	Pontgibaud	Vaulry	Pontgibaud	Vaulry	Vaulry
White Lupin	Roots	392 ± 48 a	18 ± 1.3 a	1618 ± 288 a	144 ± 13.5 a	2.2 ± 0.27 a	0.51 ± 0.11 ab	1.80 ± 0.33 c
	Stem	124 ± 23 bc	11 ± 0.3 bc	25 ± 3 bc	3 ± 0.7 b	0.4 ± 0.06 b	0.07 ± 0.04 a	0.23 ± 0.03 d
	Leaves	128 ± 12 b	11 ± 0.1 de	23 ± 2 b	2 ± 0.6 be	0.4 ± 0.02 b	0.16 ± 0.01 b	0.18 ± 0.02 d
Common vetch	Roots	1936 ± 274 a	21 ± 1.7 a	1813 ± 195 a	466 ± 35.2 a	2.1 ± 0.20 c	0.30 ± 0.04 a	3.28 ± 0.23 b
	Stem	475 ± 139 b	12 ± 0.8 d	139 ± 8 c	11 ± 0.4 c	0.3 ± 0.01 a	0.11 ± 0.03 a	0.31 ± 0.03 de
	Leaves	1057 ± 92 b	18 ± 1.5 e	43 ± 11 c	5 ± 0.3 b	0.3 ± 0.01 a	0.07 ± 0.01 b	0.30 ± 0.06 de
Buckwheat	Roots	9857 ± 596 a	30 ± 4.0 a	1962 ± 352 d	428 ± 13.8 d	6.7 ± 0.43 d	0.26 ± 0.06 c	4.32 ± 0.30 a
	Stem	498 ± 71 c	14 ± 1.7 b	174 ± 94 bc	9 ± 1.1 e	0.8 ± 0.10 b	0.05 ± 0.01 d	0.49 ± 0.08 e
	Leaves	2726 ± 322 c	14 ± 1.0 c	557 ± 108 bc	15 ± 2.7 e	2.1 ± 0.09 b	0.13 ± 0.01 d	0.42 ± 0.02 e
Two-way ANOVA (p value)	Species S	4.74 10 ⁻¹⁶	9.10 10 ⁻⁵	0.056	1.74 10 ⁻¹⁰	3.16 10 ⁻¹⁶	0.0316	1.05 10 ⁻⁷
	Organ O	4.92 10 ⁻¹⁵	2.62 10 ⁻⁸	4.76 10 ⁻¹⁴	1.20 10 ⁻¹³	6.25 10 ⁻²⁰	4.67 10 ⁻⁸	2.28 10 ⁻²²
	S x O	1.29 10 ⁻¹⁴	0.0022	0.623	9.54 10 ⁻¹²	1.78 10 ⁻¹¹	0.0574	2.73 10 ⁻⁷

Results are the mean ± SE of 5 independent experiments for each condition and are expressed in mg metal(loid) kg⁻¹ DW. For each column, different letters between two means (± SE) correspond to significant differences ($p < 0.05$).

Table 3 Two-way ANOVA results (p values) with species and soil condition as variables

	Leaf DW	Stem DW	Root DW	Leaf water	Stem water	Root water	Stem length	Root length
Species Sp	$3.8 \cdot 10^{-63}$	$8.14 \cdot 10^{-57}$	$4.99 \cdot 10^{-69}$	$2.18 \cdot 10^{-15}$	$3.09 \cdot 10^{-22}$	$9.44 \cdot 10^{-14}$	$1.53 \cdot 10^{-186}$	$4.07 \cdot 10^{-80}$
Soil So	$6.80 \cdot 10^{-9}$	$5.53 \cdot 10^{-16}$	$2.82 \cdot 10^{-9}$	$7.11 \cdot 10^{-17}$	$3.24 \cdot 10^{-15}$	$2.64 \cdot 10^{-9}$	$3.18 \cdot 10^{-100}$	$4.32 \cdot 10^{-99}$
Sp x So	$6.63 \cdot 10^{-11}$	$1.48 \cdot 10^{-13}$	$9.97 \cdot 10^{-19}$	0.0327	$7.68 \cdot 10^{-5}$	$5.57 \cdot 10^{-8}$	$1.22 \cdot 10^{-83}$	$8.3 \cdot 10^{-68}$
	Chlorophyll a	Chlorophyll b	Carotenoids	Leaf TSC	Root TSC	Leaf starch	Root starch	
Species Sp	0.0487	0.1447	0.0029	0.0212	$9.35 \cdot 10^{-12}$	$1.49 \cdot 10^{-6}$	0.0002	
Soil So	$6.12 \cdot 10^{-5}$	$5.93 \cdot 10^{-10}$	$5.04 \cdot 10^{-9}$	$1.24 \cdot 10^{-12}$	$5.63 \cdot 10^{-13}$	$3.84 \cdot 10^{-10}$	$6.93 \cdot 10^{-12}$	
Sp x So	0.028	$3.56 \cdot 10^{-5}$	$5.72 \cdot 10^{-5}$	0.027	$1.1 \cdot 10^{-8}$	0.0959	0.1164	
	Leaf proteins	Root proteins	Leaf TFAA	Root TFAA	Leaf proline	Root proline	Leaf NPT	Root NPT
Species Sp	0.0207	$9.3 \cdot 10^{-12}$	$5.57 \cdot 10^{-22}$	$4.5 \cdot 10^{-18}$	$7.93 \cdot 10^{-24}$	$1.95 \cdot 10^{-11}$	$6.28 \cdot 10^{-17}$	$7.33 \cdot 10^{-17}$
Soil So	$9.94 \cdot 10^{-13}$	$2.14 \cdot 10^{-13}$	0.5051	0.0029	$9.81 \cdot 10^{-18}$	$3.96 \cdot 10^{-9}$	$2.08 \cdot 10^{-6}$	$3.89 \cdot 10^{-19}$
Sp x So	0.0409	$3.55 \cdot 10^{-8}$	$8.98 \cdot 10^{-7}$	0.0023	$2.48 \cdot 10^{-14}$	$1.88 \cdot 10^{-8}$	0.0045	$2.83 \cdot 10^{-14}$
	Leaf TPC	Root TPC	Leaf H ₂ O ₂	Root H ₂ O ₂	Leaf TBARS	Root TBARS		
Species Sp	$1.73 \cdot 10^{-19}$	$1.2 \cdot 10^{-22}$	$4.51 \cdot 10^{-7}$	$4.55 \cdot 10^{-18}$	$1.41 \cdot 10^{-8}$	0.0218		
Soil So	$2.48 \cdot 10^{-5}$	$1.6 \cdot 10^{-17}$	$3.57 \cdot 10^{-19}$	$1.24 \cdot 10^{-17}$	$8.25 \cdot 10^{-15}$	$1.02 \cdot 10^{-10}$		
Sp x So	$9.4 \cdot 10^{-13}$	$1.19 \cdot 10^{-12}$	0.0247	$3.77 \cdot 10^{-5}$	0.0008	$9.48 \cdot 10^{-6}$		

Figures

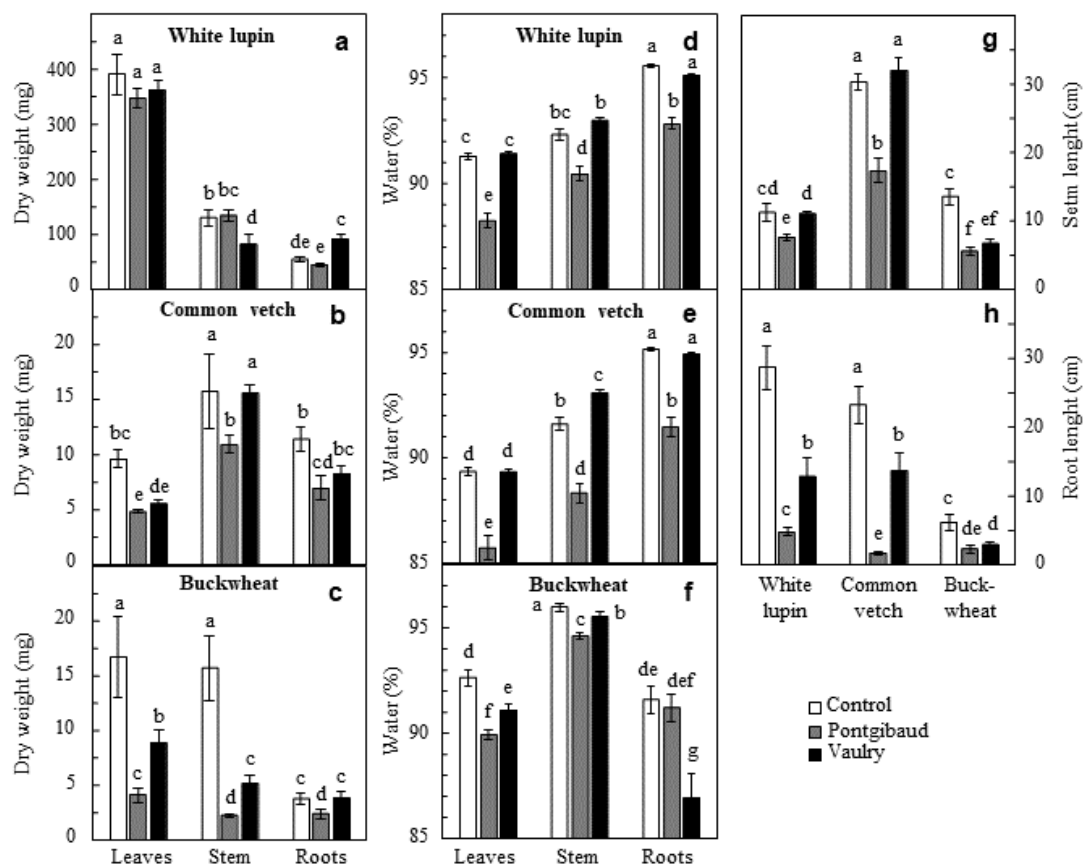


Fig. 1

Figure 1

Effect of contaminated soils on plant growth. Seeds were sown on control (white bars), Pontgibaud (grey bars), and Vaulry (black bars) soils and plants were harvested 3 weeks later to measure dry weight (DW) of leaves, stem, and roots in white lupin (A), common vetch (B), and Buckwheat (C), the height of the stem (D) and the length of the main root (E). Results are the mean $\pm$ standard error of 5 independent experiments with 5 to 10 plants per experiment. Different letters indicate significant difference

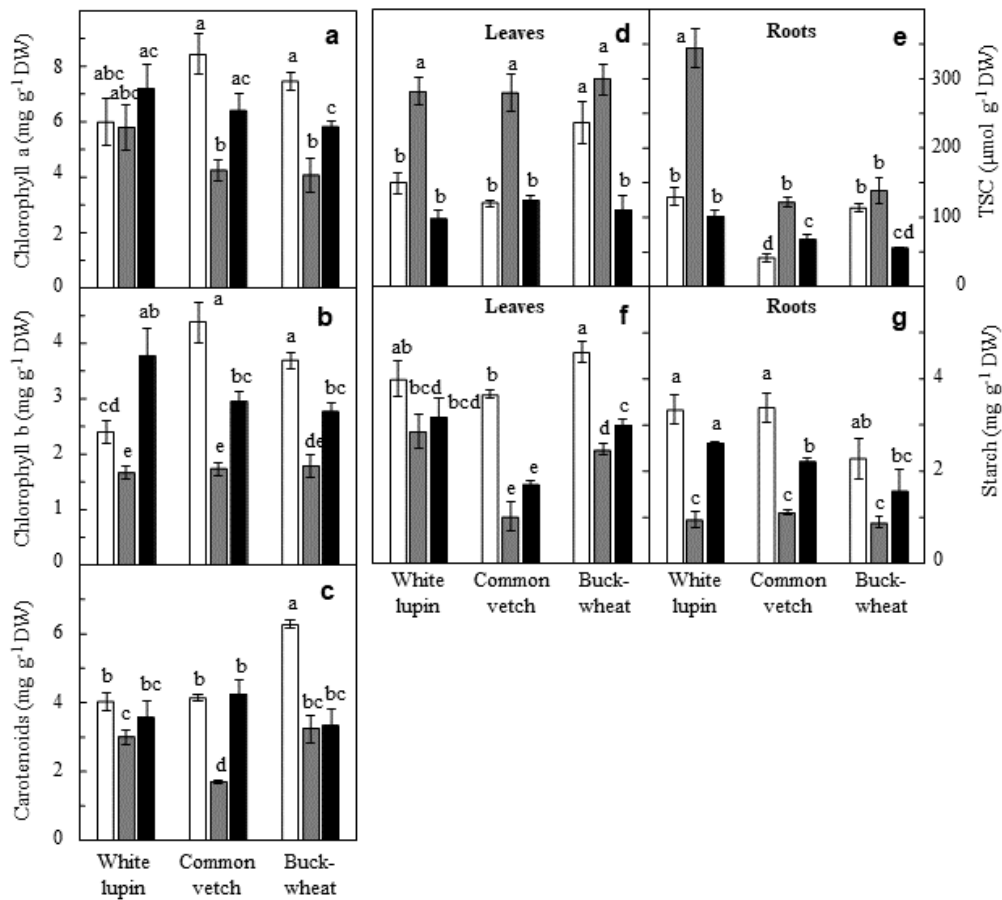


Fig. 2

Figure 2

Effect of contaminated soils on plant photosynthesis. Seeds were sown on control (white bars), Pontgibaud (grey bars), and Vaulry (black bars) soils and plants were harvested 3 weeks later to measure the content of chlorophyll a (A), chlorophyll b (B), carotenoids (C), leaf (D) and root (E) total soluble carbohydrates (TSC), and leaf (F) and roots (G) starch. Results are the mean $\pm$ standard error of 5 independent experiments with 5 to 10 plants per experiment. Different letters indicate significant difference

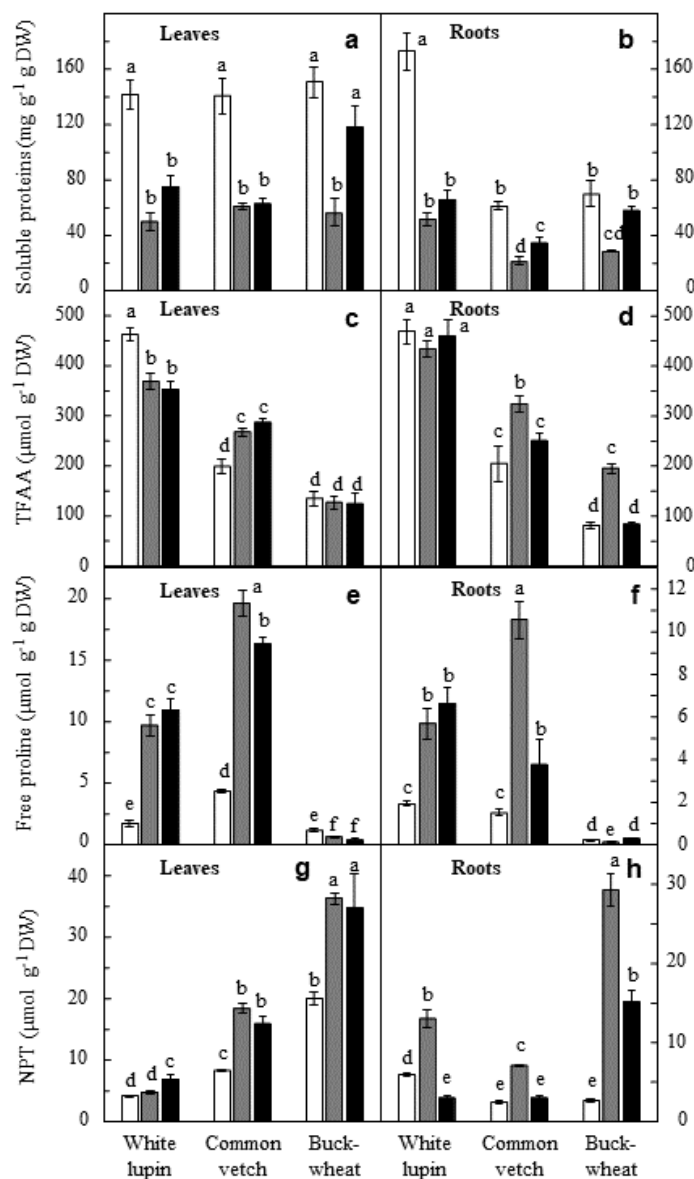


Fig. 3

Figure 3

Effect of contaminated soils on plant nitrogenous compounds. Seeds were sown on control (white bars), Pontgibaud (grey bars), and Vaulry (black bars) soils and plants were harvested 3 weeks later to measure the content of leaf (A) and root (B) total soluble proteins, leaf (C) and root (D) total free amino acids (TFAA), leaf (E) and roots (F) free proline, and leaf (G) and root (H) non-protein thiols (NPT). Results are the mean ±

standard error of 5 independent experiments with 5 to 10 plants per experiment. Different letters indicate significant difference

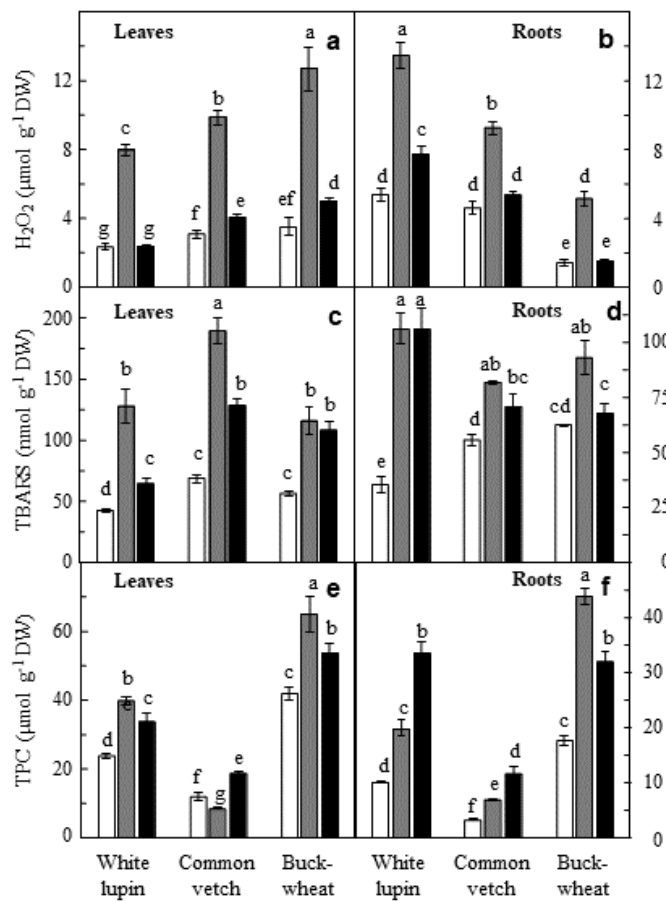


Fig. 4

Figure 4

Effect of contaminated soils on plant oxidation level. Seeds were sown on control (white bars), Pontgibaud (grey bars), and Vaulry (black bars) soils and plants were harvested 3 weeks later to measure the content of leaf (A) and root (B) H_2O_2 , leaf (C) and root (D) thiobarbituric acid reactive substances (TBARS), leaf (E) and

root (F) total soluble phenolic compounds. Results are the mean $\pm$ standard error of 5 independent experiments with 5 to 10 plants per experiment. Different letters indicate significant difference