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PHYTOSTABILIZATION OF TECHNOSOLS CONTAMINATED WITH ARSENIC AND LEAD

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Abstract

Seeds of Douglas fir were sown and cultivated in greenhouse for 3 months on two Technosols from former mines located in central France (La Petite Faye LPF and Pontgibaud PGB) and contaminated with As and Pb. Plantlets grown on PGB soil had a high concentration of Pb in their roots, underwent an oxidative stress and their total biomass was reduced by a factor two while plantlets grown on LPF were less altered but accumulated As in their aerial organs. In order to improve growth and reduce metal (loid) concentration in plantlets, Technosols were amended with composted sewage sludge (CSS). The results showed that CSS significantly stimulated growth of plantlets grown on PGB soil with a biomass close to control plantlets. Moreover, plantlets grown of LPF exhibited a lower As concentration in their aboveground organs, indicating that CSS is an efficient amendment to phytostabilize the contaminated soils. We then cultivated herbaceous species (common vetch, white lupin, buckwheat) for 3 weeks on PGB soil. The aim is to increase the future vegetation cover with a co-culture of trees and herbaceous. Without amendment, the growth of common vetch and buckwheat was strongly reduced but not for white lupin. All herbaceous species exhibited low As concentration in whole organs but high Pb concentration in roots, indicating that CSS should be added as for Douglas fir for a better phytostabilization.

Keywords : *Fagopyrum esculentum*, *Lupinus albus*, Phytoremediation, *Pseudotsuga menziesii*, *Vicia sativa*

PHYTOSTABILISATION DE TECHNOSOLS CONTAMINÉS PAR DE L'ARSENIC ET DU PLOMB

Résumé

Des graines de Douglas ont été semées et cultivées en serre pendant 3 mois sur 2 Technosols d’anciennes mines localisées au centre de la France (La Petite Faye LPF et Pontgibaud PGB) et contaminés en As et Pb. Les plantules sur le sol PGB étaient plus concentrées en Pb, ont subi un stress oxydant et leur biomasse totale a été réduite par deux alors que les plantules sur le sol LPF ont été moins altérées mais ont accumulé de l’As dans leurs parties aériennes. Afin d’améliorer la croissance et de réduire la concentration en métal (loïde) dans les plantules, les Technosols ont été amendés avec des boues d’épuration compostées (BEC). Les résultats ont montré que les BEC ont stimulé de manière significative la croissance des plantules sur le sol PGB avec une biomasse proche des plantules témoins. De plus, les plantules sur le sol LPF ont présenté une plus faible concentration en As dans les parties aériennes, indiquant que les BEC sont un amendement efficace pour phytostabiliser les sols contaminés. Nous avons ensuite cultivé des espèces herbacées (vesce cultivée, lupin blanc, sarrasin) pendant 3 semaines sur le sol PGB. L’objectif est d’augmenter la future couverture végétale avec une co-culture d’arbres et d’herbacées. Sans amendement, la croissance de la vesce et du sarrasin a été fortement réduite mais pas pour le lupin blanc. Toutes les herbacées avaient une faible concentration en As dans tous les organes mais une forte concentration en Pb dans les racines, indiquant que les BEC devraient être ajoutées de la même manière que pour le Douglas pour une meilleure phytostabilisation.

Mots clés: : *Fagopyrum esculentum*, *Lupinus albus*, Phytoremediation, *Pseudotsuga menziesii*, *Vicia sativa*

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INTRODUCTION

Mining activity generates waste often containing high concentrations of metal(loid)s that can be leached and thus contaminate surrounding ecosystems. Soils can be cleaned by excavation with subsequent treatment outside the site. However, excavation is expensive and sterilizes soils thus the rehabilitation of the site has a supplemental and not negligible cost. Phytoextraction is an alternative process that is cheaper and more ecological [1]. However, it can't be used on highly contaminated soils as this process would take hundreds of years. As there is still no efficient method for highly contaminated sites, the strategy is often to stabilize soils to avoid leaching. The most eco-friendly process is phytostabilization that consists in establishing a vegetation cover with plants that accumulate no of few meta l (loid)s [2]. The selection of plant species depends on soil characteristics: pH, organic matter, concentration of nutrients and pollutants… As highly contaminated soils are often acid and poor in nutrients and/or organic matter, amendments can be added to improve soil fertility taking care to not solubilize metal (loid)s adsorbed on soil particles. This process is called assisted phytostabilization [3]. In order to develop an ecological and cheap process, it is suitable to use amendments that could valorize organic waste such as compost, sludge, biochar...

The aim of our work was to: *(i)* study if plants can grow on contaminated Technosols with an acceptable biomass production. We used a tree species (Douglas fir) and 3 herbaceous species (common vetch, buckwheat and white lupin) to compare their growth and their tolerance to metal (loid)s., *(ii)* determine if plants store or not metal(loid)s in aboveground and/or underground organs. We determined the concentration of As and Pb in aerial organs and in roots for each plant species we used and we calculated the factors of translocation (TF) and bioconcentration (BCF). TF and BCF are the ratios of concentrations of a metal (loid) between aerial organs and roots and aerial organs and soil, respectively. For phytostabilization, TF and BCF have to be lower than 1 [4], *(iii)* evaluate if amendments can stimulate growth, limit metal (loid)s uptake and thus improve phytostabilization. We tested several amendments and the most efficient is presented in this publication.

MATERIALS AND METHODS

1. Soil samples, plant species and growth conditions

The first 20 cm of soils were collected on a former mine of Pb and Ag at Pontgibaud (PGB: 2° 49′ 39.39″ E, 45° 47′ 28.18″ N) [5], and on a former mine of Au at La Petite Faye (LPF: 1° 34′ 25.3″ E, 46° 08′ 37.0″ N) [6]. Soils were air-dried and sieve to 2 mm. In parallel, a control soil without metal(loid)s was prepared with 75% compost and 25% sand. The pH, organic matter (OM), cation-exchange capacity (CEC), total and phytoavailable As and Pb in soils were recorded [7].

Seeds of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) were purchased from "Semences Du Puy SARL" (Le Puy-En-Velay, France) and stored for 2 months at 4 °C in plastic bags containing sterile moist sand for stratification [8]. They were then transferred for 3 months in greenhouse in pots of 3.5 L containing control, LPF and PGB soils with or without amendment. Amendment was composted sewage sludge applied at 5 % (v/v) [9]. Seeds of common vetch (*Vicia sativa* L.), white lupin (*Lupinus albus* L.) and buckwheat (*Fagopyrum esculentum* Moench) were purchased from "Graines Baumaux" (Mirecourt, France) and directly grown in greenhouse for 3 weeks on control and PGB soils without amendment.

2. Growth parameters and biochemical analyses

Plants were harvested, and roots were incubated for 1 h in 20 mM EDTA to remove adsorbed metal (loid)s and then rinsed with demineralized water. Stem height and main root length were measured and fresh weight was recorded for roots, stems and needles. Organs were dried at 80°C for 2 days to obtain dry weight (DW) and samples for metal (loid) quantification. A part of fresh organs was stored at - 20 °C until the following analyses: concentration of photosynthetic pigments, soluble carbohydrates, starch, soluble proteins, total amino acids, proline, malondialdehyde, non-protein thiols, and metal(loid)s (As and Pb) [8]. We quantified only photosynthetic pigments and metal(loid)s (As and Pb) for Douglas fir grown on no amended soils [7] and for herbaceous species grown on PGB.

3. Statistical analysis

Results were the mean of 3 independent experiments. For plant growth parameters, 30 plants were used per experiment and per condition. Two-way ANOVA tests were performed with the PAST software (version 2.17) to compare each set of plants with respect to the measured parameter. When significant difference was found ($p < 0.05$), Tukey's test was carried out.

RESULTS AND DISCUSSION

1. Soil characteristics without and with amendment and Douglas fir growth parameters

The results showed that LPF soil is poor in OM but had a pH close to control soil and an acceptable CEC. In this soil, the concentration of total Pb and As was very high but Pb was not phytoavailable whereas As was lowly available with a concentration of 17 mg. kg^{-1} (Table 1). The amendment with CSS increased OM and did not modify metal(loid)s phytoavailability. The PGB soil was very acid (pH 4.2), very poor in OM, had a low CEC and the concentration of As and Pb was very high, particularly for Pb. Moreover, Pb was strongly bioavailable with a concentration of 480 mg. kg^{-1}.

Table 1. Characteristics of soils without and with amendment with composted sewage sludge

	Control		LPF			PGB	
	NA	+ CSS	NA	+ CSS		NA	+ CSS
[As]$_{total}$ (mg. kg^{-1})	2	4	1695	1514		1064	987
[Pb]$_{total}$ (mg. kg^{-1})	7	10	374	345		12360	11150
[As]$_{available}$ (mg. kg^{-1})	0	0	17	20		0	0.4
[Pb]$_{available}$ (mg. kg^{-1})	0	0	0	0		480	12*
pH	5.8	5.8	5.8	5.9		4.2	5.6*
OM (% w/w)	26.4	33.5*	6.3	12.8*	*	3.8	6.1*
CEC (cmol+. kg^{-1})	29.1	31.8	19	21.9		3.8	8.9*

NA: non amended soil ; + CSS : soil amended with composted sewage sludge.
*: significant difference between + CSS and NA conditions.

The growth of Douglas fir grown on LPF was reduced compared to the control for the aboveground organs but the root system was stimulated (Table 2). It can be a strategy of defense to reach better conditions. Indeed, it was reported that plants can develop their roots in soils contaminated with metal(loid)s or with a deficiency in essential minerals to avoid the stress [10]. On PGB, the growth was much more reduced for all organs (by 50 - 60 %) indicating that the stress is stronger. It can be explained by the low pH and MO content as well as the high concentration of phytoavalailable Pb that can be toxic.

Table 2. Dry weights and sizes (stem height and root length) of Douglas firs grown on soil without and with amendment with composted sewage sludge.

	Needles			Stem			Roots		
	Control	LPF	PGB	Control	LPF	PGB	Control	LPF	PGB
DW NA (mg)	23.45	16.89	8.50	8.81	5.13	3.84	5.82	6.12	2.91
DW +CSS (mg)	29.28*	17.29	22.39*	10.83*	7.44*	8.08*	6.71	4.31	4.33*
size NA (cm)				7.22	5.46	3.47	7.92	11.12	2.85
size + CSS (cm)				7.81	6.70*	7.03*	8.45	8.81*	6.73*

NA: non amended soil ; + CSS : soil amended with composted sewage sludge.
*: significant difference for Douglas firs grown on + CSS compared to NA.

When CSS was added, the growth of plantlets on LPF soil was slightly stimulated for aerial organs and reduced for roots. As the amendment seemed to alleviate the stress, plantlets did not need to elongate their root system to avoid the contamination. For plantlets grown on PGB with CSS, the growth was strongly stimulated and the dry weight was close to control plantlets. It is probably due to the increase of pH (from 4.2 to 5.6) and the lower phytoavailability of Pb.

2. Physiological responses of Douglas fir and metal(loid) accumulation

Without amendment, photosynthetic pigment (Fig. 1A) and starch (Fig. 1C) contents were not modified in Douglas firs grown on LPF soil but the soluble carbohydrates accumulated in needles (Fig. 1B). This increase might be due to a lower carbohydrate translocation or a higher requirement of soluble carbohydrates to protect membrane and more especially chloroplasts [11]. Plantlets did not accumulate malondialdehyde (MDA) (Fig. 1D) thus there was no real oxidative stress. As a consequence, proline (Fig. 1E) and non-protein thiols (NPT) (Fig. 1F) did not accumulated. Total amino acid content (Fig. 1G) was not modified but we recorded a decrease in protein content in roots (Fig. 1H) suggesting that plantlets may mobilize nitrogen for other syntheses. For example, it known that nitrogenous compounds such as polyamines can be involved in defense again metal (loid)s [12].

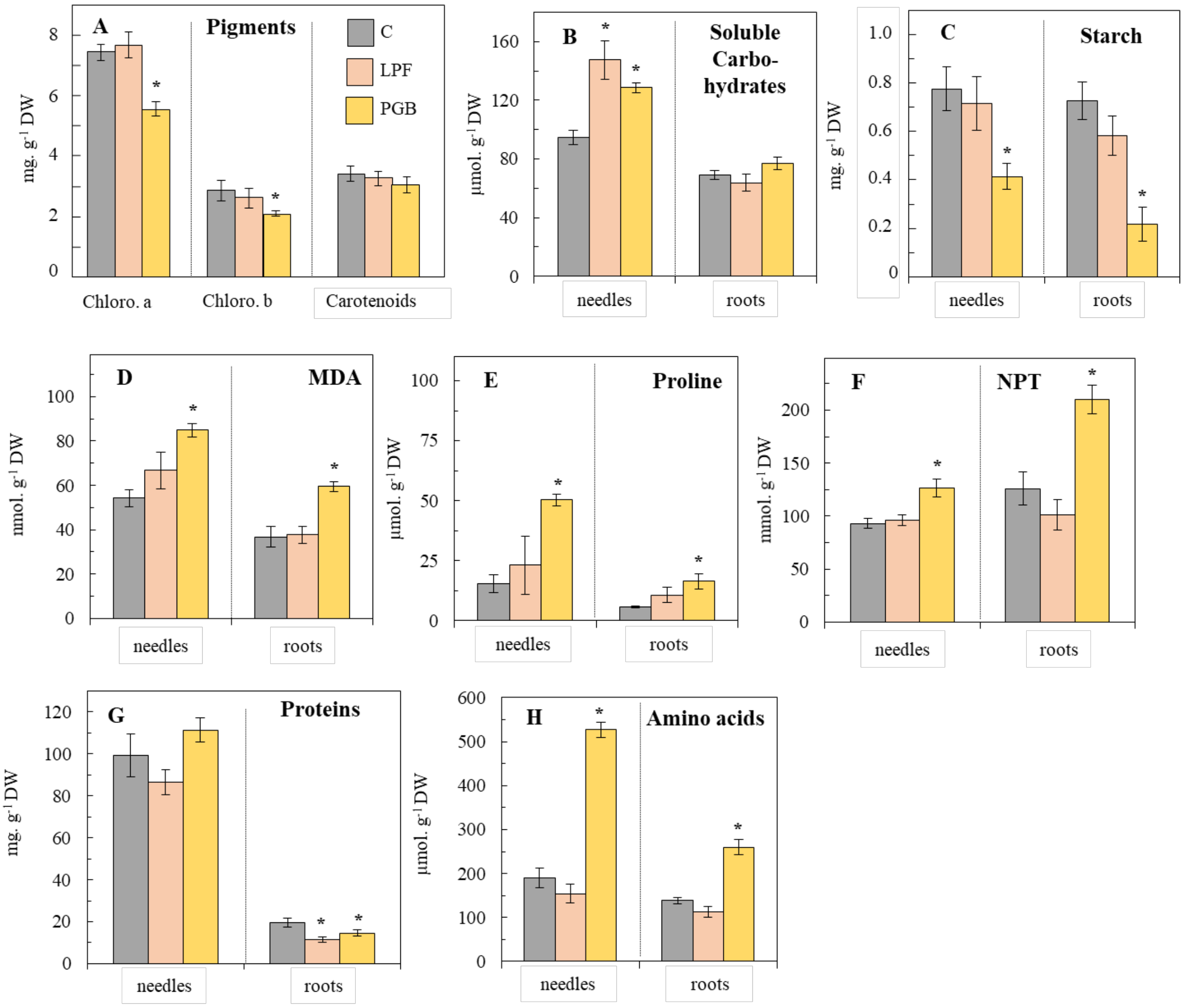

Figure 1. Physiological responses of Douglas fir grown for three months on control and contaminated soils. Photosynthetic pigment concentration (A) was determined in needle while the contents of soluble carbohydrates (B), starch (C), malondialdehyde (MDA) (D), proline (E), non-protein thiols (NPT) (F), total soluble proteins (G), and total free amino acids (H) were assayed in both needles and roots. *: significant difference compared to control.

For plantlets grown on PGB soil, the increase in carbohydrate content is probably due to the decrease in pigment content and the hydrolysis of starch. The alteration of photosynthesis can be explained by an oxidative stress as MDA content increased. Plantlets increased their content in proline and NPT that are involved in scavenging reactive oxygen species and/or metal(loid)s but these defenses were not enough efficient. Moreover, as the content of amino acid (including proline) strongly increased, the decrease in root protein content can be explained by a hydrolysis of protein or a disturbing in protein synthesis.

Plantlets grown on LPF soil accumulated a high concentration of As in aerial organs and a slightly lower concentration in roots leading to a TF of 1.83 (Table 3). For phytostabilization, this factor as well as BCF should be lower than 1 what was not the case on our experiment. However, with CSS the As concentration was strongly reduced in aerial organs thus TF and BCF were lower than 1 (0.63 and 0.19, respectively). Moreover, the phytostabilization of Pb is possible on this soil as the concentration was low in the plantlets.

Table 3. Concentration of As and Pb in Douglas firs grown for three months on soils with and without amendment.

	As				**Pb**			
	LPF		PGB		LPF		PGB	
	NA	+ CSS	NA	+ CSS	NA	+ CSS	NA	+ CSS
Aerial organs	1067	319*	53	59	1.9	2	418	148*
Roots	605	518	195	116*	10	6	8549	548*
Soil	1695		1064		374		12360	
TF	1.83	0.63*	0.28	0.51	0.19	0.32	0.05	0.29
BCF	0.63	0.19*	0.05	0.05	0.005	0.005	0.03	0.01

Concentrations of As and Pb in plant organs and soils were expressed in mg. kg^{-1} dry material.
TF: translocation factor ; BCF: bioconcentration factor.

Plantlets grown on PGB soil were not highly concentrated in As and TF and BCF remained lower than 1 with or without amendment. These factors were also lower than 1 for Pb but plantlets accumulated a high concentration of Pb in root on non-amended soil, confirming the toxicity of this soils and the subsequent strong reduction of plantlet growth. However, the amendment considerably decreased the Pb phytoavailability thus the concentration in aerial organs and in roots was strongly reduced. It was showed that several tree species can be used for As phytostabilization according to the type of soil and climate such as *Eucalyptus* species for dry climates [13], *Coronopus erectus* for tropical and sub-tropical areas [14], and *Populus deltoides* [14] for temperate or colder climates. Other tree species are good candidates for Pb phytostabilization under dry or temperate climates [15, 16]. We showed here that Douglas fir under temperate to cold climate could be a tree species for phytostabilization of both metal (loid)s As and Pb.

3. Growth parameters and metal (loid) accumulation of herbaceous species grown on PBG soil

In order to improve the phytostabilization, it could be interesting to add other plant species for a better vegetation cover, particularly herbaceous species in association with Douglas fir. We thus tested plantlets able to grow on quite acid soil as conifers are known to progressively acidify the soil. The results showed that the growth of common vetch and buckwheat was reduced on PGB soil while white lupin had a DW close to the control, excepted a slight reduction of stem (Fig. 2).

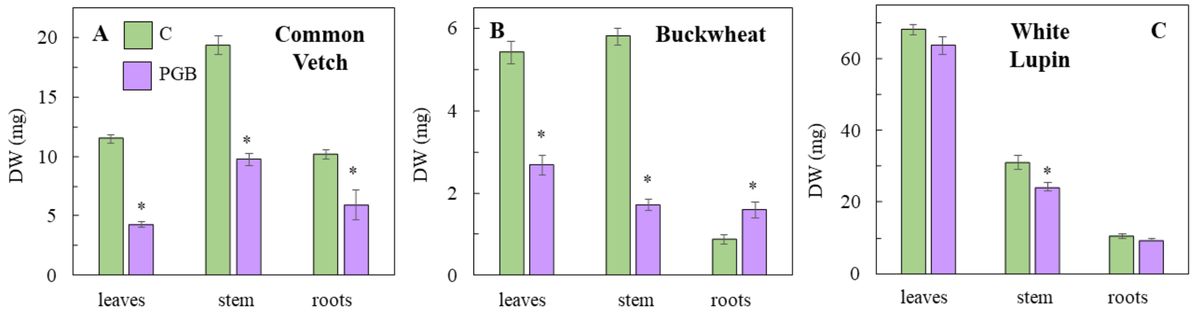

Figure 2. Dry weight of herbaceous species grown for three weeks on control and PGB soils.
*: significant difference compared to control.

The As concentration in these species was low as well as TF and BCF, suggesting that As phytostabilization is possible (Table 4). Pb did not accumulate in aerial organs thus TF and BCF were very low but the concentration in roots was high thus it could be toxic after a longer time of exposure. It is however interesting to note that without amendment and despite the high Pb concentration in roots, the growth of white lupin was not drastically reduced. This species was already showed to be a good candidate for phytostabilization of As in acid soils [17]. Moreover, leguminous herbaceous such as *Vicia* sp. or *Trifolium* sp. were shown to be interesting for Pb phytostabilization when organic amendments were added as Pb was less available [18].

Table 4. Concentration of As and Pb in herbaceous species grown for three weeks on PGB soil.

	As			**Pb**		
Species	Common Vetch	Buckwheat	White Lupin	Common Vetch	Buckwheat	White Lupin
Aerial organs	0.002	0.0004	0.02	0.02	0.04	8.68
Roots	1.089	0.1717	171	393	9857	2330
Soil	1064			12360		
TF	0.002	0.002	0.0001	6.10^{-5}	4.10^{-6}	0.003
BCF	2.10^{-6}	4.10^{-7}	2.10^{-5}	2.10^{-6}	3.10^{-6}	7.10^{-4}

Concentrations of As and Pb in plant organs were expressed in mg. kg^{-1} dry weight.

CONCLUSIONS

Our results showed that Douglas fir may be used for phytostabilization of As and Pb on the sites we studied if amendment with CSS is applied. Indeed, CSS stimulated the growth of plantlets and contributed to strongly decrease the phytoavailability of Pb on PGB.

Among the tested herbaceous tested, the white lupin was more resistant on PGB soil but strongly accumulated Pb in roots. It could thus be interesting to study if the application of CSS could reduce Pb concentration in the white lupin and if it could stimulate the growth and the resistance of the two other plant species (common vetch and buckwheat).

These experiments were performed in greenhouse thus the next step would be to establish a co-culture of Douglas fir and herbaceous species directly on the contaminated soils after the application of amendment such as CSS.

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