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**Early screening of new accumulating *versus* non-accumulating tree species
for the phytomanagement of marginal lands**

**Lisa Ciadamidaro^a, J. Parelle^a, F. Tatin-Froux^a, C. Moyen^a, A. Durand^a, C. Zappelini^a,
N. Morin-Crini^a, D. Soupe^b, D. Blaudez^c and M. Chalot^{a,d}**

^aUniversité de Bourgogne Franche-Comté, UMR CNRS 6249 Laboratoire Chrono-
environnement, Pôle Universitaire du Pays de Montbéliard, 4 place Tharradin, BP 71427, F-
25211, Montbéliard, France.

^bPépinières Daniel SOUPE S.A.S., Les Lazares – Route de Thoissey, 01400 Châtillon sur
Chalaronne, France.

^cUniversité de Lorraine, CNRS, LIEC, F-54000 Nancy, France.

^dUniversité de Lorraine, F-54000 Nancy, France.

*email: lisa.ciadamidaro@univ-fcomte.fr; Telephone: +33 3 81 66 57 14

Abstract

The use of fast-growing trees producing a high quantity of biomass can bring significant practical and economic benefits to the reclamation of marginal lands. The present study aims to identify new shrub/tree species to offer a wider range of shrubs/trees useful for phytomanagement practices. We implemented three experimental sites in France of 1 ha each (Thann, Carrières-sous-Poissy and Leforest) contaminated by different potentially toxic elements (PTE) with a total of 38 different tree species. After two years of growth, the element concentrations in stem and leaf biomasses, tree survival rate and growth of plants were assessed. Although the three sites had elevated concentrations of total PTE and nutrients in their soil, the element contents in the trees strongly differed depending on the species. *Alnus subcordata*, *Platanus orientalis*, *Ulmus pumila*, *Ostrya carpinifolia* and the *Acer* species appeared to be well adapted to the drastic conditions of the sites and presented the lowest PTE concentrations in their aboveground biomass. Conversely, the *Salix*, *Populus*, *Betula* and *Quercus* species, especially *Salix aquatica grandis*, exhibited the highest accumulations of Cd, Zn and/or Mn at the three sites. Inoculation with the endomycorrhizal fungus *Rhizophagus irregularis* did not have a significant impact on the growth of the tree species at the three sites after two growing seasons. Overall, the present study offers two options for the phytomanagement of marginal lands. The first option is based on the production of clean biomass, which is useful for plant-based industrial processes (e.g., biomaterials, bioenergy) that could valorize this biomass. The second option is based on the production of TE-enriched biomass that could be better exploited in the eco-catalysis process.

Key words: Non-accumulating tree species, trace elements, phytomanagement, *Salix aquatica grandis*

1. Introduction

Pollution has been identified by the European Commission as a major threat to soils. In EU countries, the number of potentially contaminated sites adds up to nearly 3 million (Europe's environment - The fourth assessment). More than 250,000 polluted sites in the member states require urgent clean up. Due to several anthropogenic activities, such as mining, industries and the production of agricultural wastewater, the spread of PTE in the environment has become a major problem (Khan et al., 2010; Sheoran et al., 2010). Therefore, the remediation of polluted soils is crucial to ensure that soil remains a key natural resource for human society and to promote sustainable environment and economic development. As such, pollution treatment represents an economic need, which often remains unanswered by conventional civil engineering methods due to their inappropriateness, environmental impact and costs (notably for large sites). Currently, several phytotechnologies can be considered and applied to PTE polluted soils: (1) phytostabilization, which uses perennials able to sorb and immobilize potentially toxic PTE in the root zone, avoiding their transfer towards groundwater and aerial parts, preventing their bioaccumulation in the food chain, as well as their dispersion by natural agents (wind erosion, water); and (2) phytoextraction, which is based on root-to-shoot transfer and the storage of potentially toxic PTE in harvestable tree parts (Pilon-Smith, 2005). These eco-innovative phytotechnologies can remediate soil layers in contact with the roots and at the same time provide tree biomass (Robinson et al., 2009), which can be used to reach the targets for the use of renewable energy sources (Mench et al., 2009, Ciadamidaro et al., 2017).

The suitability of a certain plant for PTE remediation is determined by various plant properties, such as: (i) heavy metal tolerance; (ii) size, growth rate and rooting depth; (iii) heavy metal accumulation in above-ground plant parts; and (iv) climatic adaptation and pest resistance (Kirkham, 2006; Pulford and Watson, 2003). Over the years, several studies have

69 been carried out to optimize the method and to find the most suitable plants. Poplars and
70 willows have been extensively tested for the phytoremediation of land polluted by PTE
71 (Dickinson, 2006; French et al., 2006; Meers et al., 2007; Ciadamidaro et al., 2014). Their
72 potential resides in large biomass production, extensive root systems, considerable tolerance
73 to PTEs, and high accumulation of PTE in the biomass (Pulford and Watson, 2003). Other
74 studies (Mertens et al., 2007; Evangelou et al., 2012) have tested different trees, such as birch
75 (*Betula pendula*), oak (*Quercus robur*), ash (*Fraxinus excelsior*) and maple (*Acer*
76 *pseudoplatanus*) and demonstrated different behaviors and adaptation characteristics to PTE
77 contamination. These differences have had impacts on the final outcome of phytomanagement
78 strategies, and a careful selection of appropriate tree species/clones seems to be a prerequisite
79 for any field-based phytoremediation attempts (Pulford and Watson, 2003).

80 During the last two decades, we have witnessed the emergence of using different tree
81 species in combination with microbial biotechnologies (Mench et al., 2010). It is now widely
82 recognized that microorganisms are of utmost importance for the efficacious establishment of
83 phytomanagement strategies (Moreira et al., 2016). Indeed, it is now proved that mycorrhizal
84 fungus associations enhance plant growth at field levels (Ciadamidaro et al., 2017) and
85 vitality on TE-disturbed habitats and reduce metal toxicity, affecting plant metal uptake and
86 further accumulation in plant tissues (Phanthavongsa et al., 2017). Moreover, mycorrhizal
87 fungi enhances plant growth and their resistance to adverse conditions including toxicity
88 produced by TEs, affecting the phytoavailability of TEs in soil and the uptake of metals by
89 plants from soil and translocation from root to shoot (Yilmaz and Parlak, 2011, Meier et al.,
90 2012). Most importantly, the selection of adequate trees and microbes is a key feature in a
91 phytomanagement approach (Ciadamidaro et al., 2017). However, current knowledge about
92 the performance of trees and their associated microbes in phytomanagement strategies is
93 particularly limited to a few genera, such as *Populus* and *Salix* (Dominguez et al., 2008;

Ciadamidaro et al., 2014, Phanthavongsa et al., 2017).

~~We hypothesized that testing a large number of woody species on various marginal lands would increase the panel of trees relevant for phytomanagement.~~ The purpose of the present study was to identify a panel of woody species with phytoremediating capacities, either within a phytostabilization strategy (high productivity, low PTE accumulation), or within a phytoextraction strategy (high productivity, high PTE accumulation), which would allow greater efficiency and ensure the biodiversity needed to restore the ecosystem. We chose to work on a short time scale to be able to rapidly select species useful for phytomanagement. To better address the question of the replicability of our data, we implemented three different sites with different contamination histories. We also aimed to test the impact of endomycorrhizal inoculation on the PTE uptake by various tree species.

2. Materials and methods

2.1. Site description and soil preparation before plantation

The experiment was carried out at three different sites, i.e., Thann, Carrières-sous-Poissy and Leforest, where phytomanagement has been identified as a relevant strategy to remediate soil pollution.

The experimental site of Thann is located in the valley of the Thur in Alsace-France (47°47'48.4"N 7°08'20.8"E) near the border with Germany and Switzerland, on a 15 ha-chemistry-based industrial complex. The Thann plant owned by the Cristal company is one of the most important sites for TiO₂ production in Europe and is the second-largest production facility in the world (Assad et al., 2017). The Ti extraction process generates tailings made of red gypsum from the neutralization and precipitation of effluents containing sulfuric acid, which is used to dissolve the PTE contained in the ore. Red gypsum, a solid residue enriched with Fe, S and Mn, is stored on the Ochsenfeld landfill (Alsace, France), located on an 80-ha landfill 3 km away from the industrial complex. The landfill site was prepared before

plantation by adding a 30-cm layer of soil obtained from a local source and incorporated into the red gypsum layer by plowing to a depth of 50 cm. Previous assays have indeed demonstrated the need for reconstructing a soil layer to allow for tree growth prior to plantation. Direct implementation on the red gypsum substrate would result in poor growth and high mortality at this site.

The experimental site at Carrières-sous-Poissy (48°57'39.7"N 2°02'09.6"E) was watered with raw wastewater of the Paris conurbation from 1899 to 2002, hence becoming highly enriched in nutrients and organic matter (OM) and accelerating the production of market vegetables until the late 1990's (Lamy et al., 2006). However, PTE accumulation in the soil led to multi-pollution characterized by Pb, Zn and Cd concentrations 10 times higher than those in a non-irrigated reference soil. Before plantation, the soil was plowed to a depth of 30 cm.

The experimental site at Leforest is located close to Noyelles-Godault, in Northern France (50°25'40.5"N 3°02'40.5"E), in the proximity of the Metaleurop plant, which is one of the biggest Zn and Pb production units in Europe (Douay et al., 2009). It was built in 1894 and produced approximately 170,000 tons of Pb and 105,000 tons of Zn per year until it was closed in 2003. This plant produced a significant quantity of dust, and its fallout contaminated the adjacent area to a distance of several km, with high amounts of Cd, Zn and Pb. Studies have been undertaken to investigate the nature of the contamination (Sterckeman et al., 1996) and the transfer of contaminants to local vegetation (Azimi et al., 2003; Migeon et al., 2009; Pourrut et al., 2011) and macrofauna (Pauget et al., 2013). Before plantation, the soil was plowed to a depth of 30 cm.

2.2. Experimental design

The three experimental sites were established in November 2013 (Carrières-sous-Poissy) and April 2014 (Thann and Leforest). Each site was divided into 8 different plots, in

half of them the rooted trees were inoculated (4 inoculated plots $\times$ tree species $\times$ site) with a commercial mycorrhizal inoculum and the other half was left non-inoculated (4 control plots $\times$ tree species $\times$ site). The fungal inoculum consisting of the AM *Rhizophagus irregularis* DAOM 197198 strain was provided by the Agronutrition company (Toulouse, France). The inoculation was performed at the nursery prior to plantation by soaking the roots into a mixture of propagules / peat (200000 propagules / 5 L of peat).

The experimental designs slightly differed from site to site as detailed hereafter. At the Thann site (8250 m²), each plot consisted of 18 different tree species (594 trees per plot, i.e., a total of 4752 trees) and each tree species was represented by 264 individuals distributed equally in each of the 8 plots (33 individuals per tree species per plot). The trees were planted in lines with a spacing of 0.60 m, resulting in a final density of 5760 trees/ha. At the Carrières-sous-Poissy (9840 m²) and Leforest (8706 m²) sites, each plot consisted of 16 different tree species (496 trees per plot, i.e., a total of 3968 trees), and each tree species was represented by 248 individuals distributed equally in each of the 8 plots (31 individuals per tree species per plot). The trees were planted in lines with a spacing of 0.70 m, resulting in final densities of 4033 and 4558 trees/ha for Carrières-sous-Poissy and Leforest, respectively. The criteria for the selection of tree species at each site were as follows: recognized environmental adaptations to local soil conditions, endogenous characteristics (spontaneous presence at the site), level of production at the nursery, and, in some cases, the lack of data in the phytomanagement literature. The three sites contained three tree species in common (*Ostrya carpinifolia*, *Pterocarya stenoptera* and *Salix aquatica grandis*). The Carrières-sous-Poissy and Leforest sites had 4 additional tree species in common (*Populus oxford*, *Quercus petraea*, *Quercus cerris* and *Quercus pedunculata*). The Thann and Leforest sites had 2 additional tree species in common (*Platanus orientalis* and *Salix alba*). A detailed list of the tree species implemented at each site is reported in Table 1.

2.3. Soil and tree analyses

2.3.1 Soil physico-chemical characterization

At site implementation, 10 top-soil (0–20 cm) samples were gathered from each plot with a hand auger after site preparation and mixed together to create composite samples. The 24 soil composite samples (3 sites x 8 composite samples) were analyzed for physico-chemical characterization. The soil samples were dried at 40 °C in a forced air oven to a constant weight and ground with a grinder (agate mortar, Retsch RM100). The samples were sent to a certified laboratory (SADEF, France) for the following analyses: particle size distribution, exchangeable Ca (CaO), Mg (MgO), K (K₂O) and Na (Na₂O), cation exchange capacity (CEC) (NF X 31-130), organic matter (OM) (NF ISO 14235), total CaCO₃ (NF ISO 10693), total nitrogen (N_t) (NF ISO 13878), total phosphorus (Joret-Hebert method) (NF X 31-161) and soluble boron (B) (NF X 31-122).

2.3.2 Soil and plant trace element analyses

The total element concentrations (Al, Cd, Fe, Mn, Pb, S, Zn) in the soil at the start time were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, Thermo Fischer Scientific, Inc., Pittsburgh, USA) following the acid digestion of 500 mg of sample (DigiPREP system, SCP Sciences, Courtaboeuf, France) using a mix of 2 mL of 67% nitric acid, 6 mL of 34% hydrochloric acid and 2 mL of 48% hydrofluoric acid. The available element concentrations in the soil were assessed using a 0.01 M CaCl₂-extraction with a sample extraction ratio of 1:10 (Houba et al., 1996) via ICP-AES. The total and available elemental concentrations in the soils are reported in Table 3.

At each sampling site, leaves and stems from 6 trees per plant species per plot were sampled in autumn 2015, for a total of 2400 analyses. Leaf and stem samples were washed with distilled water, dried at 70°C for at least 48 h, and ground using a stainless-steel mill. The studied elements (Al, Cd, Fe, Mn, Pb, S and Zn) were determined by ICP-AES following

the acid digestion with concentrated HNO₃ (DigiPREP system). All samples were run together with certified reference materials, including oriental basma tobacco leaves (INCT-OBTL-5, LGC Promochem, Molsheim, France) and a loamy clay soil (CRM052, LGC Promochem, Molsheim, France). The percentage of recovery of the studied elements ranged between 77 and 110%.

2.3.3 Tree survival rate and growth estimation

The survival rates of plants were estimated in 2015. To avoid destructive sampling at this stage, after two years of growth, tree development was estimated by measuring the tree heights (Fig. 1) of all trees at the three sites in October 2015 and compared to the nursery standard. The nursery trees were grown in soil for the same length of time, without any fertilizers nor irrigation. However, they did not experience the transplantation event. The height of each tree was estimated in the following ranges expressed in cm: 40/60; 60/80; 80/100; 100/120; 120/150; 150/175; 175/200; 200/250. The initial height of the planted trees was 40/60. Once the growth report was completed, an average value was assigned to each tree.

2.4. Statistical analyses

Statistical analyses were carried out using R 3.2.0 software (R Development Core Team, 2015) for Windows, and the results are expressed as mean values with standard deviations. All tests were considered significant when $p < 0.05$. Normality of variable or residual values were tested with a Shapiro-Wilk test and homoscedasticity with a Bartlett test.

The mean comparison between treatments (Control and Inoculated) for the results of nutrients and trace elements in soil was performed using post hoc tests following a one-factor analysis of variance (ANOVA) considering the sites separately and each site as the independent variable. To test the effects of the inoculation and tree species on PTE uptake in trees, a multifactor ANOVA (MANOVA) was performed to obtain the F values and

significance ($P \leq 0.05$) of each studied variable. Significant differences in soil variables between treatments were established by the Tukey test at $p < 0.05$. PCA analyses were performed using the FactoMinR package: data below the limit of quantification were considered to be null for the calculation of the principal components, and ellipses were drawn at the 95% confidence interval of the barycenter of each species.

3. Results

3.1. Soil characteristics

The main characteristics of the three studied soils are reported in Table 2. The average pH values of the three sites were close to neutrality or slightly acidic (7.4, 7.2 and 6.5 for Thann, Carrières-sous-Poissy and Leforest, respectively). The soil texture was sandy-silt, sandy and silty-loam for Thann, Carrières-sous-Poissy and Leforest, respectively. The organic status reported in Table 2 demonstrated that Carrières-sous-Poissy presented much higher values of C and N (39.6 g/kg of C_{org} , 68.5 g/kg of OM and 3.78 of N_{tot}) in comparison with the other two sites, especially the Thann site. In general, for the exchangeable oligos, higher values were observed for Carrières-sous-Poissy and Leforest, except for Na_2O_{ex} at Carrières-sous-Poissy.

Although a set of elements in the soil was analyzed, only the most abundant and the major contaminants are shown for this study (Table 3). The results showed high total concentrations in soil for all of the elements reported in Table 3, except for Cd at Thann. According to these values, Thann presented much higher contents of Fe, Mn and S, whereas Carrières-sous-Poissy and Leforest exhibited higher values of Cd, Pb and Zn and were similar to those obtained in previous studies of metal-contaminated sites in France (Assad et al., 2017, Foulon et al., 2016a, b; Douay et al., 2013; Lamy et al., 2006; Pourrut et al., 2011). The $CaCl_2$ extractable fractions also differed between sites and followed the same trends of the total concentrations, except for Mn at Thann. Indeed, the Mn $CaCl_2$ extractable fractions at

Leforest accounted for approximately 5% of the total Mn, whereas at Thann it accounted for less than 0.03%. Pb was not detected in the CaCl_2 extractable fractions for any of the sites. Despite its elevated total concentrations of most elements, the Thann soil was less appropriate for plant growth, as the availability of its nutrients was rather low.

3.2. Tree development at the three experimental sites

For each species and each site, the survival rates are given for the 8 plots (Table S4). The data were compared with the average rate of survival seen in nurseries (an average of 90% after 2 years, under favorable soil and crop conditions). Due to the high mortality after the first year for unknown reasons, the *Betula* species needed to be replanted in January 2016. After 2 years of growth, the average survival rates at the Thann, Leforest and Carrières-sous-Poissy sites were 73%, 77%, and 85%, respectively, which seems acceptable considering the soil constraints (high PTE loads) of the sites and the drastic weather conditions during summer 2015, which were marked by a strong drought.

During the year 2015, non-destructive plant measurements were undertaken, as described in the “materials and methods” section. The levels of tree growth at the Thann and Leforest sites were 42% and 20% lower than the nursery standards, respectively (Fig. 1). This reduced growth could be attributed to different factors, such as: i) site-specific soil-climatic conditions; ii) less follow-up and care than in nurseries; iii) an exceptional level of drought in the summer of 2015; iv) animal (rabbits and wild boar) grazing. The nursery data are provided in our manuscript as indicators of the optimal nursery performance for commercial purposes, but they cannot be considered as experimental controls. As is often the case in field experimental design, it was not possible to have field controls, as the studied sites lacked non-contaminated areas. Climatic conditions during the experimental period are reported in Table S1. The 2013 to 2015 data were in the range of the overall weather data at the three sites and

comparable. The 2015 summer (June or July) was however remarkably drier and warmer than the previous years, at the three sites.

Contrasting differences were reported for the following tree species at the Thann site: i) *Z. serrata*, with a growth rate 34% higher than that of the standards; ii) the *Salix*, *Platanus*, *Ulmus* and *Ostrya* species slightly grew less than the nursery standards; and iii) and a clear difference observed for the other species, for which tree growth was reduced by 61% on average and by up to 75% for the three *Quercus* species (Fig. 1). At the Carrières-sous-Poissy site, tree growth was overall only 14% lower than that of the nursery standards (Fig. 1). Most of the tree species performed quite well, with the *Acer*, *Eleagnus*, *Fraxinus*, *Ostrya* and *Syringa* species exhibiting heights equaling or exceeding the nursery standards. The *Paulownia* species showed the lowest growth rate. At the Leforest site, the *Alnus*, *Platanus*, *Populus* and *Salix* (3 species) showed worthy performances compared to the nursery standards. The growth of the two *Quercus* species was significantly lower than that of the standards (Fig. 1). Moreover, *P. stenoptera* as well as *S. aquatica grandis* and *S. caprea* appeared to be particularly impacted by the site conditions.

3.3. Elements in trees (leaves and stems)

The element concentrations of leaves and stems are detailed in Tables S2 and S3. For all the studied elements in leaves, a MANOVA was performed (Table S5) in order to highlight the effects of treatments (*I*), tree species (*S*) and interaction (*I* $\times$ *S*) between these two factors. The species (*S*) is the dominant factor affecting PTE accumulation in leaves at all sites. In general, inoculation (*I*) had no significant effect on PTE accumulation at the 3 sites (Table S5). Only at the Thann site, an interaction effect between treatment and species (*I* $\times$ *S*) slightly influenced the Al, Fe and Pb concentrations in leaves and that of Al at the Leforest site (Table S5). Therefore, according to these MANOVA results, data from the control and inoculated trees were pooled in all the following analyses.

The complete panel of analyzed elements (Fig. 2) was investigated using a multivariate analysis (PCA). Based on the PCA, the *Salix*, *Populus* and *Betula* species for the three sites and, additionally, the 3 *Quercus* species for Thann site were closely correlated with the major PTE contents (Cd, Pb, Zn and Mn). The strongest correlations were observed for *S. aquatica grandis*, one of the three common species in all the sites, for Cd, Zn, Ni and Co (Fig. 2, a-f), and for *B. verrucosa* with Mn at the Thann site (Fig. 2, a and b). In more detail, the *Salix*, *Populus* and *Betula* species showed the highest accumulation capacities for Cd and Zn in their leaves, with especially very high values for the *Salicaceous* species growing at Leforest (Table S2). In particular, *S. aquatica grandis* demonstrated the highest capacity to accumulate Cd (>100 µg/g DM) and Zn (>2500 µg/g DM). *P. stenoptera* at the Leforest site was the only species never tested before in phytomanagement, which demonstrated an accumulation capacity for Zn in leaves but at lower levels than those observed for the *Salicaceous* species. Concerning Mn, only species grown at the Thann site accumulated higher Mn contents compared with the range considered to be “physiological” in tree leaves. Species of the genera *Salix*, *Betula* and *Quercus* showed high Mn uptake capacities. At the same time, they were also able to accumulate Fe and S (the other principal elements at the Thann site) in leaves above the range considered physiological in trees, reaching values ≈ 400 µg/g Mn for *Q. rysophylla* and *B. papyrifera* and >300 µg/g Mn for *S. aquatica grandis* (Table S2). For the three sites, all the other elements analyzed were in the background range for PTE in plants (Table S2) according to Kabata-Pendias and Pendias (1992) and for Fe according to Bowen (1979).

In contrast, the PTE contents and PCA analysis revealed that *P. orientalis*, *U. pumila*, *O. carpinifolia* and the 3 studied *Acer* species showed poor or no correlation at all with any of the elements measured in the leaves at the three sites. In this work, species of the genera *Acer*, *Alnus*, *Eleagnus*, *Fraxinus*, *Hippophae*, *Quercus*, *Ostrya*, and *Paulownia* (which have only

seldom been tested in phytomanagement studies), did not show any accumulative trait for the elements measured.

We further analyzed the elemental concentrations in stems (Table S3). However, this analysis was restricted to those species that accumulated PTE in their leaves, since the stem element contents are usually lower than the leaf contents. For a general interpretation of the PTE concentrations in stems depending on the selected tree species, the data are presented as sunray plots, and the values of each tree species were transformed to their corresponding logarithms in order to fit them into the graphs (Fig. 3). In general, the PTE concentrations in stems were 5-10 times lower than the PTE concentrations in leaves (Tables S2 and S3). Nevertheless, the results confirmed the trends observed in leaves for all tree species, i.e., the higher accumulation of Cd and Zn at the Leforest site for *S. aquatica grandis* and that of Mn at the Thann site for *Q. cerris*, *Q. pedunculata* and *B. papyrifera*.

4. Discussion

Overall, in the present study, we tested the growth performance and element accumulation of 38 tree species at three different marginal lands. This set of data allowed us to identify two groups of trees that could be useful for phytomanagement strategies.

The first group includes species that grew rather well and did not accumulate PTEs. Our results based on growth data (Fig. 1 and Table S4) and PCA analysis (Fig. 2) showed that *O. carpinifolia* performed very well on the three sites compared to the nursery standard and accumulated PTE in the leaf tissues within normal physiological levels (Table S2) (Kabata-Pendias and Pendias, 2001; Bowen, 1979). To our knowledge, this tree species has never before been tested in a phytomanagement context. Similarly, *P. orientalis* showed good growth capabilities at two of the three sites (Thann and Leforest). In addition, 2 *Fraxinus* and 2 *Syringa* species, the *Acer*, *Alnus*, *Carpinus*, *Hippophae*, *Ulmus* and *Zelkova* species, performed rather well on at least one experimental site, showing no accumulation of PTE. The

satisfying potential growth of these species makes them good candidates for the restoration of marginal lands at no risk, since the produced biomass will be clean enough to further limit the recycling of contaminants through leaf fall. There is almost no data on the phytomanagement capacity of *P. orientalis*, *U. pumila*, *O. carpinifolia*, *A. saccharinum* and *Z. serrata* trees. Similar results were only found by Mertens et al. (2007), in which *A. pseudoplatanus* was able to reduce the mobilization of PTE in a contaminated site. According to our findings, these tree species could be considered relevant for phytomanagement strategies due to their capacity of reducing PTE uptake, exploiting tree transpiration and root growth in order to decrease leaching and control erosion (Robinson et al., 2003). Indeed, roots can help prevent the formation of reduced PTE species, which have often been reported to be more toxic and more mobile than oxidized species. The roots can also add organic matter to the substrate that binds the PTE (Robinson et al., 2009). Two of these tree species (*O. carpinifolia* and *F. ornus*) have been proposed for the stabilization of a mining area after soil reconstruction in NW Greece (Zagas et al., 2010).

The second group of relevant tree species includes those that grew well and accumulated PTE. During the screening and analysis of data, our results indeed showed that of the 38 total studied trees species from the three sites, 4 genera exhibited a clear capacity to accumulate PTE in their aboveground biomass. Generally, the *Betula*, *Populus*, *Quercus* and *Salix* species accumulated significantly more PTE compared with the other tree species. The accumulation of Zn and Cd by members of the *Salicaceae* family is now well established (Ciadamidaro et al., 2014; Madejon et al., 2013; Robinson et al., 2006). In more detail, *S. aquatica grandis*, *P. oxford* and *B. pubescens* accumulated Cd, Pb, and Zn at the Carrières-sous-Poissy and Leforest sites, whereas Fe, Mn and S were accumulated by *S. aquatica grandis*, *Q. rysophylla*, and *B. papyrifera* at the Thann site. However, in some cases, the rate of PTE accumulation was significantly different between the studied sites. In our work, for

example, *S. aquatica grandis* was able to accumulate the highest concentrations of Cd and Zn in all sites, reaching maximum values of $\approx 128 \mu\text{g/g D Wt}$ for Cd and $\approx 2800 \mu\text{g/g D Wt}$ for Zn in leaves (Table S2). The Cd accumulation rate in leaves was somehow linked to the CaCl_2 extractable Cd fraction. At the Leforest site, the leaf Cd and the CaCl_2 extractable contents were 4- and 5-fold higher than those at the Carrières-sous-Poissy site, respectively. The lowest Cd content in *S. aquatica grandis* leaves ($< 2.5 \mu\text{g/g D Wt}$) was recorded at the Thann site, where the CaCl_2 extractable Cd fraction was lower than the detection limit. Zn concentrations, in leaf and soil samples were in the same order of magnitude at the two Carrières-sous-Poissy and Leforest sites. In this context, among the trees used in phytomanagement, the species able to tolerate and accumulate high concentrations of PTE (Ali, 2013) in their tissues without visible toxicity symptoms are considered hyperaccumulators. *S. aquatica grandis* may possibly be considered as a Cd and Zn hyperaccumulator in our study (Leforest site). It remains to be determined whether its growth will be affected in the long term by the high availability of Cd and Zn at the Leforest site.

At the Thann site, *Q. rysophylla* and *B. papyrifera* showed the best accumulation capacities for Mn but it seems that there was no relation between the Mn in leaf tissues and that in the CaCl_2 extractable fraction. For the two *Quercus petraea* and *Q. pedunculata* species present at both the Leforest and Carrières-sous-Poissy sites, the leaf Mn levels were similar, whereas the Mn contents in the soil at the Leforest site were 6 times as high as those observed at Carrières-sous-Poissy. These results could support the hypothesis that *Quercus* genera presents accumulation capacities for Mn independently of soil physic-chemical characteristics and Mn concentrations in soil.

It is worth highlighting that the trees used for the phytoextraction of PTE from the soil must be efficient in accumulating PTE mostly in leaves and stems (Gomes et al., 2012; Rascio and Navari- Izzo, 2011). In the present study, although the highest PTE concentrations were

found in leaves compared to stems, the correlation coefficient between PTE in leaves and stems showed significant positive correlations for all elements, except for the Pb and S concentrations in the Carrières-sous-Poissy site and for Fe in the Thann and Leforest sites (Table 4). In general, the strongest correlations in all three sites were observed for Zn and Cd, especially considering that Zn and Cd seem to have similar geochemical and environmental properties, and they can interact during plant uptake, transport and accumulation in leaves and stems (Das et al., 1997, Han et al., 2010). In the case of Cd, Pulford and Watson (2003) determined that the compartmentalization of PTE within trees is considered to vary greatly by species and clones. Yang et al. (2015) showed that in the aboveground tissues of willows, Cd is usually sequestered in vacuoles, by which the toxic effects of Cd can be greatly minimized. Moreover, Salt et al. (1995) and Vassilev et al. (2005) concluded that the distribution and translocation of Cd to aboveground tissues were primarily driven by the transpiration stream. In the case of Zn, previous studies (Maxted et al., 2007; Migeon et al., 2009) agreed with our results and found significantly more Zn in the leaves than in the stems of *Salicaceous* species.

For Mn, significant correlations were obtained, especially at the Thann site, between leaf and stem contents (Table 4). This could be related to the ability of Mn to be rapidly taken up and translocated within plants (Kabata-Pendias and Pendias, 2001). It is worth noting that Mn was efficiently taken up in the *Betula* and *Quercus* species, despite its very low CaCl₂ availability at the Thann site (Table 3). This may be due to either intrinsic capabilities or to a specialized microbial community that may reduce the Mn mostly present in the soil as Mn oxides (Zappelini et al., in preparation).

Concerning Pb, previous studies (Baker, 1981; Borishev et al., 2009; Stoltz and Greger, 2002) observed restricted translocation from roots to stems and leaves due to a PTE exclusion mechanism in roots by which plants avoid damages in their photosynthetic processes. Moreover, Pb is considered to have low solubility and therefore a low availability to plants

because it precipitates as phosphates and sulfates found in the rhizosphere of plants, as exemplified by Blaylock and Huang (2000). Indeed, we could not recover the Pb in the CaCl₂ fraction, despite its elevated total concentration, especially at the Carrières-sous-Poissy site.

Although many studies have evinced that mycorrhizal fungus association could enhance tree growth and vitality on TE-disturbed habitats and reduce PTE toxicity, affecting tree PTE uptake and accumulation in plant tissues (Ciccatelli et al., 2010; Liu et al., 2014), no significant effect of mycorrhizal inoculation on tree development or PTE accumulation in trees was observed in our experimental conditions. It is well known that the effect of AM fungi on plant parameters depends on many factors, including tree and fungus species, the growth conditions of the tree, tree age, soil properties, and the type and concentration of PTE in the soil (Arriagada et al., 2005; Mrnka et al., 2012). The lack of beneficial effect, in contrast to those obtained in our previous study (Ciadamidaro et al. 2017), is probably due to the fact that a single endomycorrhizal fungal species was used here and that some of the tested trees are not as perceptive as poplars to endomycorrhizal colonization in their early growth stage. Despite differences in the characteristics of the three soils and of their original contamination, the behavior in growth and PTE uptake of the different tree species was quite similar at all sites, indicating that the overall effect species is more important than the effect of the inoculation, as stated by the MANOVA analysis.

5. Conclusions

Among the 38 total studied species, 4 species (*A. subcordata*, *P. orientalis*, *U. pumila*, *O. carpinifolia* and the 3 studied *Acer*), only seldom used before in a phytomanagement strategy, showed the highest growth capacity. Moreover, these tree species that produced a clean biomass could be considered as good candidates for the phytomanagement of marginal lands, for their valuation in various industrial sectors. Indeed, while it is necessary to treat the by-product fractions enriched in PTE when using contaminated biomass (Chalot et al., 2012,

Asad et al., 2017, Bert et al., 2017), using this non-accumulating biomass would allow researchers to by-pass this additional step. In contrast, *S. aquatica grandis*, *P. oxford*, *Q. rysophylla* and *B. papyrifera* trees were able to take up high quantities of one or more of the analyzed PTE without presenting toxic symptoms. For this reason, they could be classified as phytoextracting species and used with regard to the restoration objectives and a PTE management strategy. Indeed, these species with TE-enriched biomass could lead to the marketing of a truly eco-innovating production chain that could meet the needs of an emerging, high-prospect market within the bio-economy, such as eco-catalysis (Grisson, 2015). These innovative eco-solutions for the treatment of polluted soils respond perfectly to the needs of sustainable development.

Moreover, the most promising tree species could be retained, and a model stabilizer and accumulator/hyperaccumulator tree could be used as a control to study fundamental processes. This will permit (i) the recovery of PTE-contaminated sites from belowground to aboveground biomass and (ii) the production of valuable materials and fuels, focusing on the catalysis of industrial chemicals, performance and high-tech products, e.g., pharmaceutical, cosmetics, and flavors. A future comparative analysis of catalytic systems derived from hyperaccumulating and accumulating trees will be useful for a better understanding of phytomanagement processes.

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