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

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20 **Abstract**

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

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

44 **1. Introduction**

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

65 The suitability of a certain plant for PTE remediation is determined by various plant
66 properties, such as: (i) heavy metal tolerance; (ii) size, growth rate and rooting depth; (iii)
67 heavy metal accumulation in above-ground plant parts; and (iv) climatic adaptation and pest
68 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;

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

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

105 2. Materials and methods

106 2.1. Site description and soil preparation before plantation

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

110 The experimental site of Thann is located in the valley of the Thur in Alsace-France
111 (47°47'48.4"N 7°08'20.8"E) near the border with Germany and Switzerland, on a 15 ha-
112 chemistry-based industrial complex. The Thann plant owned by the Cristal company is one of
113 the most important sites for TiO₂ production in Europe and is the second-largest production
114 facility in the world (Assad et al., 2017). The Ti extraction process generates tailings made of
115 red gypsum from the neutralization and precipitation of effluents containing sulfuric acid,
116 which is used to dissolve the PTE contained in the ore. Red gypsum, a solid residue enriched
117 with Fe, S and Mn, is stored on the Ochsenfeld landfill (Alsace, France), located on an 80-ha
118 landfill 3 km away from the industrial complex. The landfill site was prepared before

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

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

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

141 **2.2. Experimental design**

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

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

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

169 **2.3. Soil and tree analyses**

170 **2.3.1 Soil physico-chemical characterization**

171 At site implementation, 10 top-soil (0–20 cm) samples were gathered from each plot
172 with a hand auger after site preparation and mixed together to create composite samples. The
173 24 soil composite samples (3 sites x 8 composite samples) were analyzed for physico-
174 chemical characterization. The soil samples were dried at 40 °C in a forced air oven to a
175 constant weight and ground with a grinder (agate mortar, Retsch RM100). The samples were
176 sent to a certified laboratory (SADEF, France) for the following analyses: particle size
177 distribution, exchangeable Ca (CaO), Mg (MgO), K (K₂O) and Na (Na₂O), cation exchange
178 capacity (CEC) (NF X 31-130), organic matter (OM) (NF ISO 14235), total CaCO₃ (NF ISO
179 10693), total nitrogen (N_t) (NF ISO 13878), total phosphorus (Joret-Hebert method) (NF X
180 31-161) and soluble boron (B) (NF X 31-122).

181 **2.3.2 Soil and plant trace element analyses**

182 The total element concentrations (Al, Cd, Fe, Mn, Pb, S, Zn) in the soil at the start
183 time were determined using inductively coupled plasma atomic emission spectrometry (ICP-
184 AES, Thermo Fischer Scientific, Inc., Pittsburgh, USA) following the acid digestion of 500
185 mg of sample (DigiPREP system, SCP Sciences, Courtaboeuf, France) using a mix of 2 mL of
186 67% nitric acid, 6 mL of 34% hydrochloric acid and 2 mL of 48% hydrofluoric acid. The
187 available element concentrations in the soil were assessed using a 0.01 M CaCl₂-extraction
188 with a sample extraction ratio of 1:10 (Houba et al., 1996) via ICP-AES. The total and
189 available elemental concentrations in the soils are reported in Table 3.

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

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

199 **2.3.3 Tree survival rate and growth estimation**

200 The survival rates of plants were estimated in 2015. To avoid destructive sampling at this
201 stage, after two years of growth, tree development was estimated by measuring the tree
202 heights (Fig. 1) of all trees at the three sites in October 2015 and compared to the nursery
203 standard. The nursery trees were grown in soil for the same length of time, without any
204 fertilizers nor irrigation. However, they did not experience the transplantation event. The
205 height of each tree was estimated in the following ranges expressed in cm: 40/60; 60/80;
206 80/100; 100/120; 120/150; 150/175; 175/200; 200/250. The initial height of the planted trees
207 was 40/60. Once the growth report was completed, an average value was assigned to each
208 tree.

209 **2.4. Statistical analyses**

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

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

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

224 **3. Results**

225 **3.1. Soil characteristics**

226 The main characteristics of the three studied soils are reported in Table 2. The average
227 pH values of the three sites were close to neutrality or slightly acidic (7.4, 7.2 and 6.5 for
228 Thann, Carrières-sous-Poissy and Leforest, respectively). The soil texture was sandy-silt,
229 sandy and silty-loam for Thann, Carrières-sous-Poissy and Leforest, respectively. The organic
230 status reported in Table 2 demonstrated that Carrières-sous-Poissy presented much higher
231 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
232 the other two sites, especially the Thann site. In general, for the exchangeable oligos, higher
233 values were observed for Carrières-sous-Poissy and Leforest, except for Na_2O_{ex} at Carrières-
234 sous-Poissy.

235 Although a set of elements in the soil was analyzed, only the most abundant and the
236 major contaminants are shown for this study (Table 3). The results showed high total
237 concentrations in soil for all of the elements reported in Table 3, except for Cd at Thann.
238 According to these values, Thann presented much higher contents of Fe, Mn and S, whereas
239 Carrières-sous-Poissy and Leforest exhibited higher values of Cd, Pb and Zn and were similar
240 to those obtained in previous studies of metal-contaminated sites in France (Assad et al.,
241 2017, Foulon et al., 2016a, b; Douay et al., 2013; Lamy et al., 2006; Pourrut et al., 2011). The
242 $CaCl_2$ extractable fractions also differed between sites and followed the same trends of the
243 total concentrations, except for Mn at Thann. Indeed, the Mn $CaCl_2$ extractable fractions at

244 Leforest accounted for approximately 5% of the total Mn, whereas at Thann it accounted for
245 less than 0.03%. Pb was not detected in the CaCl₂ extractable fractions for any of the sites.
246 Despite its elevated total concentrations of most elements, the Thann soil was less appropriate
247 for plant growth, as the availability of its nutrients was rather low.

248 **3.2. Tree development at the three experimental sites**

249 For each species and each site, the survival rates are given for the 8 plots (Table S4).
250 The data were compared with the average rate of survival seen in nurseries (an average of
251 90% after 2 years, under favorable soil and crop conditions). Due to the high mortality after
252 the first year for unknown reasons, the *Betula* species needed to be replanted in January 2016.
253 After 2 years of growth, the average survival rates at the Thann, Leforest and Carrières-sous-
254 Poissy sites were 73%, 77%, and 85%, respectively, which seems acceptable considering the
255 soil constraints (high PTE loads) of the sites and the drastic weather conditions during
256 summer 2015, which were marked by a strong drought.

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

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

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

283 **3.3. Elements in trees (leaves and stems)**

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

293 The complete panel of analyzed elements (Fig. 2) was investigated using a
294 multivariate analysis (PCA). Based on the PCA, the *Salix*, *Populus* and *Betula* species for the
295 three sites and, additionally, the 3 *Quercus* species for Thann site were closely correlated with
296 the major PTE contents (Cd, Pb, Zn and Mn). The strongest correlations were observed for *S.*
297 *aquatica grandis*, one of the three common species in all the sites, for Cd, Zn, Ni and Co (Fig.
298 2, a-f), and for *B. verrucosa* with Mn at the Thann site (Fig. 2, a and b). In more detail, the
299 *Salix*, *Populus* and *Betula* species showed the highest accumulation capacities for Cd and Zn
300 in their leaves, with especially very high values for the *Salicaceous* species growing at
301 Leforest (Table S2). In particular, *S. aquatica grandis* demonstrated the highest capacity to
302 accumulate Cd (>100 µg/g DM) and Zn (>2500 µg/g DM). *P. stenoptera* at the Leforest site
303 was the only species never tested before in phytomanagement, which demonstrated an
304 accumulation capacity for Zn in leaves but at lower levels than those observed for the
305 *Salicaceous* species. Concerning Mn, only species grown at the Thann site accumulated
306 higher Mn contents compared with the range considered to be “physiological” in tree leaves.
307 Species of the genera *Salix*, *Betula* and *Quercus* showed high Mn uptake capacities. At the
308 same time, they were also able to accumulate Fe and S (the other principal elements at the
309 Thann site) in leaves above the range considered physiological in trees, reaching values ≈
310 400 µg/g Mn for *Q. rysophylla* and *B. papyrifera* and >300 µg/g Mn for *S. aquatica grandis*
311 (Table S2). For the three sites, all the other elements analyzed were in the background range
312 for PTE in plants (Table S2) according to Kabata-Pendias and Pendias (1992) and for Fe
313 according to Bowen (1979).

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

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

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

330 **4. Discussion**

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

334 The first group includes species that grew rather well and did not accumulate PTEs.
335 Our results based on growth data (Fig. 1 and Table S4) and PCA analysis (Fig. 2) showed that
336 *O. carpinifolia* performed very well on the three sites compared to the nursery standard and
337 accumulated PTE in the leaf tissues within normal physiological levels (Table S2) (Kabata-
338 Pendias and Pendias, 2001; Bowen, 1979). To our knowledge, this tree species has never
339 before been tested in a phytomanagement context. Similarly, *P. orientalis* showed good
340 growth capabilities at two of the three sites (Thann and Leforest). In addition, 2 *Fraxinus* and
341 2 *Syringa* species, the *Acer*, *Alnus*, *Carpinus*, *Hippophae*, *Ulmus* and *Zelkova* species,
342 performed rather well on at least one experimental site, showing no accumulation of PTE. The

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

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

368 example, *S. aquatica grandis* was able to accumulate the highest concentrations of Cd and Zn
369 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
370 in leaves (Table S2). The Cd accumulation rate in leaves was somehow linked to the CaCl_2
371 extractable Cd fraction. At the Leforest site, the leaf Cd and the CaCl_2 extractable contents
372 were 4- and 5-fold higher than those at the Carrières-sous-Poissy site, respectively. The
373 lowest Cd content in *S. aquatica grandis* leaves ($< 2.5 \mu\text{g/g D Wt}$) was recorded at the Thann
374 site, where the CaCl_2 extractable Cd fraction was lower than the detection limit. Zn
375 concentrations, in leaf and soil samples were in the same order of magnitude at the two
376 Carrières-sous-Poissy and Leforest sites. In this context, among the trees used in
377 phytomanagement, the species able to tolerate and accumulate high concentrations of PTE
378 (Ali, 2013) in their tissues without visible toxicity symptoms are considered
379 hyperaccumulators. *S. aquatica grandis* may possibly be considered as a Cd and Zn
380 hyperaccumulator in our study (Leforest site). It remains to be determined whether its growth
381 will be affected in the long term by the high availability of Cd and Zn at the Leforest site.

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

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

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

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

414 Concerning Pb, previous studies (Baker, 1981; Borishev et al., 2009; Stoltz and Greger,
415 2002) observed restricted translocation from roots to stems and leaves due to a PTE exclusion
416 mechanism in roots by which plants avoid damages in their photosynthetic processes.
417 Moreover, Pb is considered to have low solubility and therefore a low availability to plants

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

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

436 **5. Conclusions**

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

443 Asad et al., 2017, Bert et al., 2017), using this non-accumulating biomass would allow
444 researchers to by-pass this additional step. In contrast, *S. aquatica grandis*, *P. oxford*,
445 *Q. rysophylla* and *B. papyrifera* trees were able to take up high quantities of one or more of
446 the analyzed PTE without presenting toxic symptoms. For this reason, they could be classified
447 as phytoextracting species and used with regard to the restoration objectives and a PTE
448 management strategy. Indeed, these species with TE-enriched biomass could lead to the
449 marketing of a truly eco-innovating production chain that could meet the needs of an
450 emerging, high-prospect market within the bio-economy, such as eco-catalysis (Grison,
451 2015). These innovative eco-solutions for the treatment of polluted soils respond perfectly to
452 the needs of sustainable development.

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

461

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