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## Effect of mycorrhizal inoculation on metal accumulation by poplar leaves at phytomanaged sites

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### Highlights

- Cd and Zn concentrations in leaves exceeded physiological values
- PTE accumulation in poplar leaves was enhanced in more fertile soils

- Mycorrhizal inoculation limited Zn, Cu, Pb and Cr accumulation in Skado leaves
- The inoculum was effective in highly contaminated soils

## Abstract

Phytotechnologies for the management of lands contaminated with potentially toxic elements (PTEs) are considered as gentle alternatives to conventional remediation techniques. During the last few years, phytotechnologies have progressively shifted to phytomanagement, a concept that includes the valorization of the plant biomass produced on the contaminated site. This study aimed at evaluating the mid-term effect of ecto- and endomycorrhizal inoculation on the reduction of PTE mobility in soils and foliar accumulation by two poplar clones, Skado (*Populus trichocarpa* x *P. maximowiczii*) and I-214 (*P. deltoides* x *P. nigra*), dedicated to bioenergy purposes. The effects of inoculation were investigated in two large scale trials of 1 ha each. Poplars grown on highly contaminated soils accumulated excessive Cd and Zn in leaves compared with those planted on less contaminated soils, and the I-214 clone generally accumulated less PTEs than the Skado clone. Interestingly, the filtering capacity of mycorrhizal fungi was significant for Zn, Cu, Pb and Cr in Skado leaves only at the most contaminated areas after two growing seasons. These foliar concentrations were not correlated with  $\text{Ca}(\text{NO}_3)_2$ -extractable concentrations in soils, suggesting that mycorrhizal fungi limited PTE translocation from roots to leaves without impacting PTE mobility in soils. Therefore, the reduction of PTE accumulation in poplar leaves may be optimized by selecting appropriate combinations of cultivars and inocula at specific PTE levels and soil conditions. Because Cd and Zn may pose a risk after leaf abscission and wood harvest, further research is needed to efficiently reduce Cd and Zn concentrations in poplar tissues. Otherwise, the phytomanagement of metal contaminated sites with poplars should include options to safely manage both leaves and wood.

**Keywords:** mycorrhizal inoculation, phytostabilization, phytomanagement, Skado, I-214, trace elements

## 1. Introduction

Since the 1990s, phytotechnologies are considered as an alternative to conventional remediation techniques (Salt et al., 1995; Vangronsveld et al., 2009). They have been applied to manage sites contaminated by potentially toxic elements (PTEs), which may deeply alter the environment and human health through the food chain (Järup, 2003; Kabata-Pendias, 2004). The last few years, phytotechnologies have progressively shifted to phytomanagement, a concept which included the valorization of the plant biomass produced on contaminated sites, to generate economic revenue (Evangelou and Deram, 2014). Although some valorization options have been deeply investigated and provided opportunities for such biomass (Bert et al., 2017; Chalot et al., 2012; Delplanque et al., 2013) phytomanagement is still scarcely applied in European countries and suffer from lack of field experiment feedbacks (Cundy et al., 2013).

Among the different phytotechnologies that can be applied to large contaminated areas, aided phytostabilization aims at using plants and biological or chemical amendments to retain pollutants in soils via root uptake, chelation or reduction (Mench et al., 2010). Fast growing tree species such as willows and poplars have been suggested as suitable for phytostabilization due to their high biomass production and their wide-spreading root system which can protect soil surface from erosion (Pulford and Watson, 2003). In addition, this strategy is based on the selection of the less accumulating cultivars to avoid or limit the transfer of the contaminants in the food chain and in the end-products after wood conversion process (Bert et al., 2017). Short rotation coppice (SRC) of inoculated poplars for bioenergy production in metal contaminated lands has been proposed as a phytomanagement strategy that may control environmental impacts and increase economic opportunities (Chalot et al., 2012).

Biological amendments such as mycorrhizal fungi have been for long envisaged as relevant microorganisms to help for restoration or improvement of derelict soils. Indeed, when associated with endomycorrhizal fungi, poplars exhibit enhanced tolerance to biotic and abiotic stress, with better growth and higher biomass production (Cicatelli et al., 2014). When plants are introduced for site restoration, the *in-situ* success of these plants will likely be depending on the recruitment of appropriate rhizosphere microorganisms from their new environment (Bell et al., 2015). In a recent study, we demonstrated that the occurrence and persistence of ectomycorrhizal symbionts were greatly driven by site characteristics (Foulon et al., 2016). Mycorrhizal inoculation at plantation may be thus necessary, either to improve

mycorrhizal infection when the local symbionts are lacking, or to improve the competitiveness and tolerance of the mycorrhizal microbiome.

Most studies to date have proposed adequate tree/ecto- or endo-mycorrhizal combinations to either increase (Cicatelli et al., 2010; Sell et al., 2005) or decrease (Bojarczuk and Kieliszewska-Rokicka, 2010; Mrnka et al., 2012) PTE transfer from soil to aboveground plant tissues. Colpaert et al. (2011) pointed out the fact that most of these studies were based solely on pots/greenhouse experiments and field studies remained scarce. It is admitted that extrapolation from laboratory/greenhouse to field is not reliable, since soil–plant transfer of PTEs is a complex process governed by geochemical, climatic, biological and anthropogenic factors (Kabata-Pendias, 2004). These data nevertheless highlighted the filtering potential of some mycorrhizal fungi, in some specific conditions such as elevated concentrations of PTEs. This filter function is an interesting feature of mycorrhizal fungi, when the phytomanagement approach aims at valorizing the aboveground biomass to increase economic opportunities. As metal concentrations in wood are generally lower than those in leaves (Laureysens et al., 2004; Madejón et al., 2004; Robinson et al., 2000), the determination of leaf metal concentrations is commonly used as non-destructive method to assess poplar metal uptake.

In a recent paper, we demonstrated that ecto- and endomycorrhizal inoculation significantly increased poplar biomass production at phytomanaged sites (Ciadamidaro et al., 2017). In the present study, we hypothesized that the inoculation of poplars with these mycorrhizal fungi would reduce the transfer of PTEs from soil to the aboveground parts of the trees. The aim of this paper was to investigate, at field scale, the impact of mycorrhizal inoculation on the foliar accumulation of trace and major elements of two hybrid poplars Skado (*Populus trichocarpa* x *P. maximowiczii*) and I-214 (*P. deltoides* x *P. nigra*), grown at two different PTE-contaminated sites. The paper discusses the results from an applied point of view and provides recommendations for phytomanagement improvement.

## **2. Materials and Methods**

### ***2.1. Site description***

Two field sites of 1 hectare each located in Pierrelaye (Île-de-France, France, 49°01'47.4"N 2°10'29.2"E) and Fresnes-sur-Escaut (Hauts-de-France, France, 50°25'47.9"N 3°35'07.8"E) were set up in February and April 2011, respectively. The Pierrelaye site was formerly used for market gardening and was irrigated with raw wastewater in the end of the

19<sup>th</sup> century, for more than 100 years. These inputs led to an enrichment of PTEs such as Zn, Pb, Cd and Cu in top surface (Lamy et al., 2006). The Fresnes-sur-Escaut site (Fresnes further in the text) was established on a metal contaminated dredged sediment landfill site, operating between 1978 and 1989. The two sites were under the same temperate climatic conditions, with average annual temperatures of 10.8-10.9 °C and average annual rainfall ranging between 639-743 mm (Météofrance, 2016). The experimental designs of the two sites are presented in Fig. 1. Thirty-two plots of 12 m x 18 m each were planted with 1.5m length unrooted cuttings of the two poplar clones, Skado (*Populus trichocarpa* x *P. maximowiczii* section Tacahamaca) and I-214 (*P. deltoides* x *P. nigra* section Aigeiros), at a spacing of 1.8 m x 2.75 m with a plant density of 2,200 trees ha<sup>-1</sup> (Ciadamidaro et al. (2017). These two clones were selected for their high biomass production and reduced PTE accumulation in their harvestable parts according to the Phytopop project (Pottier et al., 2015). Half of the trees were inoculated with a mixture of endomycorrhizal (*Rhizophagus intraradices* BEG140, *Funelliformis mosseae* BEG95, *F. geosporum* BEG199, *Claroideoglossum claroideum* BEG96, *C. etunicatum* BEG92, *Glomus microaggregatum* BEG56, *Rhizophagus irregulare* DAOM1971989) and ectomycorrhizal (*Hebeloma mesophaeum*, *Amanita rubescens*, *Laccaria proxima*, *Paxillus involutus*, *Pisolithus arrhizus* and *Scleroderma citrinum*) strains. The inoculum was spread at the foot of the cuttings, at the moment of plantation. Each tree received 26,000 propagules of ectomycorrhizal and endomycorrhizal fungi, the latest being dominant in the mixture following the manufacturers' recommendations (Ciadamidaro et al., 2017). One additional plot in each site was left unplanted and used as control (P33 and F33).

## 2.2. Sampling and analysis of soil and sediment

At both sites, a sampling campaign was carried out during the establishment of the poplar plantation in 2011. We selected 8 plots per site corresponding to the least (F<sub>L</sub>, P<sub>L</sub>) and the most (F<sub>H</sub>, P<sub>H</sub>) metal polluted areas at each site (Fig. 1). In each plot, ten soil samples were collected with a hand auger at 20 cm from 10 planted poplars. In October 2012 (Fresnes) and September 2013 (Pierrelaye), 10 topsoil samples (0-20 cm) per plot were again collected at each site. Representative samples obtained by quartering following the French standard X31-100 were oven-dried at 40°C until constant weight and ground with a grinder (agate mortar, Retsch RM100).

For both sites, physico-chemical analyses were performed by an external laboratory (Galys, Blois, France) on the 8 soil and sediment samples. They included: particle size distribution, available P (P<sub>2</sub>O<sub>5</sub>) (NF ISO 11263), exchangeable Ca (CaO), Mg (MgO), K (K<sub>2</sub>O) and Na (Na<sub>2</sub>O), organic matter (OM) (NF ISO 14235), total nitrogen (N) (NF ISO 13878), available N (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) (ISO/TS 14256-1 / NF EN ISO 11732; NF EN ISO 13395), total CaCO<sub>3</sub> (NF ISO 10693), cation exchange capacity (CEC) (NF X 31-130) and soluble bore (B) (NF X 31-122).

Total concentrations of major and trace elements of soil and sediment samples were determined in 2011. Before analysis, 0.5 g samples were sieved to < 200 µm (Retsch AS 200 digit) then digested at 180°C for 20 min with 2 mL of nitric acid (67%), 6 mL of hydrochloric acid (34%) and 2 mL of hydrofluoric acid (48%). Extracts were neutralized by 20 mL of boric acid (4%) and filtered using 0.45 µm Whatman® filter paper. Total concentrations of Al, As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Na, Ni, Pb, Zn were measured using ICP-OES (Ultima 2, Jobin Yvon Horiba). Soil samples which presented element concentrations below the limit of quantification (LQ), i.e. As and Cd (LQ<sub>As</sub> < 5 mg kg<sup>-1</sup>, LQ<sub>Cd</sub> < 2 mg kg<sup>-1</sup>), were analyzed using ICP-MS (Agilent 7500). Two standard reference materials were included for analytical quality control (PACS-2, National Research Council of Canada; NCD 73310, Stream sediment). Recovery rates ranged between 92-110 % for Cd, 87-101 % for Cu, 83-92 % for Pb and 87-105 % for Zn. For the other elements, recovery rates ranged from 72 % to 112 %.

Ca(NO<sub>3</sub>)<sub>2</sub>-extractable fractions of PTE were determined in soil and sediment samples collected in 2011, 2012 and 2013. Extractions of As, Cd, Cr, Cu, Ni, Pb and Zn were performed with a 0.01 M Ca(NO<sub>3</sub>)<sub>2</sub> solution to estimate the plant-available fraction of PTE in the soil and sediment samples. Sixty grams of dried samples, sieved to 2 mm, were shaken

(Heidolph Reax 2) with 120 mL of  $\text{Ca}(\text{NO}_3)_2$  (0.01 M) for 48 hours. The resulting soil and sediment mixtures were filtered on filtration units at 0.45  $\mu\text{m}$  (Millipore). Eluates were acidified to a  $\text{pH} < 2$  for preservation and analyzed by ICP-MS (Agilent 7500).

Soil and sediment pH were measured following the NF ISO 10390 (2005) standard. Five grams of sieved sample were shaken (Heidolph Reax 2) with 25 mL of distilled water for 1 hr, then left to rest for 1 hr. The pH was measured under constant stirring.

### ***2.3. Sampling and analysis of poplar leaves***

Poplar leaves were collected in the 8 previously selected plots, in October 2012 (Fresnes site) and September 2013 (Pierrelaye site). On each studied plot, 10 samples of leaves were harvested at around 1.70 m height at the same time as the sediment or soil sampling. Leaves were carefully washed with tap and deionized water, then oven-dried at 40°C until constant weight. Dry leaves were ground and 0.5 g were digested at 180 °C for 20 min in 10 mL of nitric acid (67%) and 3 mL of ultra-pure water using a microwave digester (Mars Xpress, CEM). The digested samples were filtered to  $< 0.45 \mu\text{m}$  (Millipore) and acidified to a  $\text{pH} < 2$  for preservation. The analyses of Cd, Cr, Cu, Pb and Zn concentrations were performed by ICP-OES (Agilent 720ES) or ICP-MS (Agilent 7500). Two standard references of plant material (white cabbage “BCR-679”, Community Bureau of Reference and tobacco leaves “CTA-VTL-2”) were included for analytical quality control. Recovery rates ranged between 111-113 % for Cd, 75-78 % for Cr, 117-119 % for Cu, 126 % for Pb (in tobacco leaves), and 107-118 % for Zn.

### ***2.4. Statistical analyses***

Statistical analyses were performed using R software version 3.2.3 (R Core Team, 2015). All data distributions were checked for normality (Shapiro test). Concentrations of soil extractable and leaf PTEs were compared between plots planted with inoculated and non-inoculated poplars, among each site and clone using Kruskal-Wallis tests ( $p < 0.05$ ). Student's t-tests for a single sample were run to compare soil PTEs data between 2011 and 2012 or 2013. Paired samples t-tests were used to compare pH data between 2011 and 2012 or 2013, for the 8 selected plots in each site. A Principal Component Analysis (PCA) was performed to explore the distribution of foliar PTE concentrations using the R package “FactoMineR”. For values below the LQ, values were divided by 2 for the PCA. Correlations between PTEs data in poplars and soils were calculated using Pearson correlations tests ( $p < 0.01$ ; R package Hmisc).



### 3. Results and discussion

#### 3.1. Initial soil characterization

The soil properties of both sites are presented in Table 1. According to the French GEPPA triangle (1963), the soil from the Pierrelaye site had a sand texture whereas the dredged sediment from the Fresnes site was a silt loam. Higher levels of OM and clay were found in the Fresnes plots, resulting in a higher CEC and suggesting a strong buffering capacity (Baize, 2000). The average pH at both sites was alkaline ( $7.7 \pm 0.1$  and  $7.6 \pm 0.1$  for Pierrelaye and Fresnes, respectively). Available  $\text{NO}_3^-$  concentrations at the Fresnes site were almost 3-fold higher than those at the Pierrelaye site. Ammonium concentrations were much lower than  $\text{NO}_3^-$  concentrations but showed an opposite trend.

Concentrations of total major elements were contrasted between the two sites (Table 2). Total Al, Fe and K concentrations at the Pierrelaye site were about 30 times higher than those at the Fresnes site whereas the latter contained 2 to 5 times more Ca, Mg and Na. Regarding total PTE concentrations (Table 2), the Pierrelaye site showed high concentrations of Cd, Cu, Pb and Zn in the investigated plots compared with local usual agricultural concentrations (Lamy et al., 2006). At the Fresnes site, high concentrations of Cd, Cu, Pb and Zn were mostly found in the  $F_H$  area according to threshold values in dredged sediment (MEDD, 2006). The  $F_H$  area appeared to be the most heavily Cd and Zn contaminated area, although a high variability was noticed for Cd concentrations (from 0.93 to 67.1  $\text{mg kg}^{-1}$  DW). PTE concentrations in Pierrelaye plots ranged between those found at the  $F_L$  and  $F_H$  areas.

$\text{Ca}(\text{NO}_3)_2$ -extractable Cd, Cr, Cu, Ni and Pb concentrations in soil and sediment (Table 3) were extremely low as almost all values were below limits of quantification (LQ). Zn-extractable concentrations ranged from  $<0.06$  (LQ) to 1.56  $\text{mg kg}^{-1}$  DW and from  $<0.06$  to 0.77  $\text{mg kg}^{-1}$  DW in the Pierrelaye and the Fresnes sites, respectively. Thus, the maximal Zn extractable fractions represented 0.43 % of the total Zn concentrations at Pierrelaye and 0.03 % at Fresnes, showing a much higher Zn mobility at Pierrelaye. Regarding As, the maximal extractable fraction at Pierrelaye was higher (0.32 %) than that of Fresnes (0.25 %). In agreement with CEC values, higher extractable concentrations in soil were expected at Pierrelaye (Rieuwerts et al., 1998).

#### 3.2. Evolution of pH and PTE mobility

At Pierrelaye, a low but significant decrease of the average pH (from  $7.7 \pm 0.1$  to  $7.2 \pm 0.1$ ) was observed in the 8 selected plots after 2 years (see Supplemental Fig.1 for details). Soil extractable concentrations of PTEs increased significantly in most plots of the Pierrelaye site (Table 3). The increased extractable fractions of PTEs observed in the poplar planted plots at Pierrelaye between 2011 and 2013 can be linked to the decrease of the soil pH, which may have affected speciation of PTEs in soil, rendering PTEs more soluble (Kabata-Pendias, 2010a). Soil acidification has often been reported after afforestation (Jug et al., 1999; Makeshin, 1994), and may result from losses of base cations such as Ca and/or release of root acidic exudates involved in uptake mechanisms (Kabata-Pendias, 2004). However, the decrease in soil pH cannot be solely attributed to the presence of trees, as similar decreases were observed in the unplanted plots (see Supplemental Fig.1), in which increases of Cr, Cu, Ni, Pb and Zn extractable fractions were also noticed. However, since most of the concentrations were under the LQ for the 2011 sampling, these conclusions must be taken cautiously.

Conversely, the average pH at Fresnes increased significantly from  $7.6 \pm 0.1$  to  $8.1 \pm 0.1$ , between 2011 and 2012 (Supplemental Fig.1). At the same site, sediment extractable concentrations of As and Zn significantly increased in some plots of the most contaminated area ( $F_H$ ). The same trend occurred with extractable Cu (in all plots) and Pb (in  $F_H$ ). Overall, higher extractable concentrations of PTEs in soil were measured at Pierrelaye. At the Fresnes site, the limited increase of PTE-extractable concentrations between 2011 and 2012 may be explained by the alkalization of the sediment. Here, the changes in pH value may be related to the microbial activity and / or higher  $\text{CaCO}_3$  and Ca concentrations (van Hullebusch et al., 2005). Nevertheless, a longer-term monitoring of these field sites is needed to assess the evolution of pH and PTE mobility on these phytomanaged sites.

### ***3.3. Accumulation of major and trace elements in poplar leaves***

A principal component analysis (PCA) was first performed to characterize the accumulation of PTEs in leaves of both poplar clones grown at the two sites (Fig. 2). The first principal component (Dim1) opposed 4 variables (Fig. 2a), foliar Cu concentrations being negatively correlated to Cd, Zn and Cr foliar concentrations. These negative correlations revealed a well-known antagonistic relationship between Cu and Zn in leaves, when high levels of these micronutrients were reported (Läuchli and Bielecki, 1983). The second

principal component (Dim2) clustered poplar Skado clones from the Pierrelaye site, which accumulated the highest amounts of Pb (Fig. 2). Our analysis (Fig. 2b) clearly discriminated the two poplar clones, with the Skado clone accumulating more Cd, Cr, Pb, and Zn than the I-214 clone (except for I-214 at the F4 and F9 plots). Control of leaf PTE translocation might therefore be determined by genetic traits, as demonstrated in one of our previous study (Pottier et al., 2015) or other previous studies on Salicaceous trees (Castiglione et al., 2009; Konlechner et al., 2013; Laureysens et al., 2004).

In more details, both Skado and I-214 clones from the two sites exhibited excessive foliar concentrations of Cd and Zn compared to those usually measured in poplars grown on uncontaminated sites (Fig. 3). Indeed, Vandecasteele et al. (2003) reported baseline foliar concentrations for 2-year-old poplar clones grown in a tree-nursery of 2.0-4.4 mg Cd kg<sup>-1</sup> DW and 60-184 mg Zn kg<sup>-1</sup> DW whereas in our case, foliar Cd concentrations ranged from 1.10 to 18.9 mg kg<sup>-1</sup> DW and from 1.29 to 14.2 mg kg<sup>-1</sup> DW in I-214 and Skado, respectively. For Zn, foliar concentrations ranged from 101 to 748 mg kg<sup>-1</sup> DW and from 192 to 928 mg kg<sup>-1</sup> DW in I-214 and Skado, respectively. The highest Cd and Zn concentrations were measured in poplar leaves collected in the most heavily contaminated area F<sub>H</sub> at Fresnes (Fig. 3). No statistical differences were found between leaf Cd concentrations of I-214 and Skado at the Fresnes site, whereas Skado accumulated more Cd than I-214 at the Pierrelaye site. Skado accumulated more Zn than I-214 in most plots of the two sites. Similar concentration ranges of Cd and Zn in poplar leaves measured at the end of growing seasons were reported in several studies performed at moderately contaminated sites (Laureysens et al., 2004; Madejón et al., 2012; Mertens et al., 2004; Pottier et al., 2015). In our study, foliar Cd concentrations were strongly correlated with foliar Zn concentrations in both clones and sites ( $r$  ranged from 0.74 to 0.92,  $p < 0.01$ ) as reported in various studies on poplar (Lettens et al., 2011; Migeon et al., 2009; Pottier et al., 2015). Although Cd is not an essential element for plant nutrition, it may be transferred to plants with similar mechanisms to Zn, as it is chemically and physically similar to Zn (Kabata-Pendias, 2010b). In this regard, Pottier et al. (2015) pointed out a contribution of the NRAMP1 (Natural Resistance Associated Macrophage Protein 1) transporter for Cd and Zn accumulation by poplars. High concentrations of PTE in leaves can be the result of their bioavailability in the soil, high transpiration rate, and common transport pathways in plant tissues (Robinson et al., 2000).

Foliar Cu concentrations in both clones ranged within physiological values (Lettens et al., 2011) and did not vary between the two sites (Fig. 3). In contrast to I-214, foliar Pb

concentrations in Skado were close or above baseline values at both sites ( $1.5 \text{ mg Pb kg}^{-1} \text{ DW}$ ; Kalra, 1998). The highest concentrations of Cr in leaves were measured in I-214 and Skado clones at the Fresnes, these concentrations however remaining close to baseline values (about  $2 \text{ mg Cr kg}^{-1} \text{ DW}$ ; Giachetti and Sebastiani, 2006).

Adequate nutrient concentrations in tree growth are crucial when considering biomass production and foliar diagnosis can be a useful tool to determine nutritional status. Among the nutrients measured in poplar leaves at the Pierrelaye site, P, Ca, Mg and Mn concentrations (Fig. 4) fell within the range of “optimal” levels found in I-214 leaves (Garbaye et al., 1980) and other poplar leaves (Kalra, 1998). Conversely, leaf K and Fe concentrations were lower than expected, suggesting a potential deficiency. Skado clones were characterized by higher Ca and Fe leaf concentrations, whereas I-214 contained more Mg ( $p < 0.05$ ).

### 3.4. *Effects of mycorrhizal inoculation*

We evidenced a significant decrease in foliar Zn, Cu, Pb and Cr concentrations in inoculated Skado poplars compared with the non-inoculated ones at the Fresnes  $F_H$  area (Fig. 3). In the same area, there was a clear similar tendency for Zn concentrations measured in the I-214 leaves, and Cd concentrations in the I-214 and Skado leaves, although not statistically significant. Noticeably, the impact of inoculation was only evidenced for Skado clones at the Fresnes  $F_H$  area, which was characterized by the highest total PTE concentrations in soil (Table 2). This trend was confirmed by the PCA performed with PTE concentrations in leaves, which discriminated non-inoculated Skado (F-ni-F5) and I-214 (F-ni-F4) with higher concentrations of Zn and Cd in leaves, as compared with inoculated Skado (F-i-F12) and I-214 (F-i-F9), all from the Fresnes  $F_H$  area (Fig. 2b). Conversely, inoculation also induced an increase of foliar Cu and Zn concentrations in some plots of  $F_L$  and  $P_L$  areas.

It is interesting to note that the filter function of arbuscular mycorrhizal fungi (AMF), in the case of highly contaminated soils, dominates the literature, as for example in the works described by Meier et al. (2012b) and Garg and Aggarwal (2012), most of the studies being performed in pot experiments. Conversely, some authors demonstrated that the inoculation of plants by AMF such as *Glomus* species significantly enhanced PTE translocation to the aboveground parts of plants (Meier et al., 2012a). Levels of PTEs in soil may also affect the role of arbuscular mycorrhizal symbiosis (Audet and Charest, 2007), which can lead to an increase of plant metal uptake at low soil PTE concentrations as well as a reduction of PTE bioavailability via fungal metal-binding processes at high soil PTE levels. The mechanisms

underlying metal accumulation may involve metallothioneins, small proteins that bind metals in plant detoxification process, as demonstrated in a greenhouse experiment (Cicatelli et al., 2014). Garg and Aggarwal (2011) also reported that AMF induced the production of chelating agents (phytochelatins) and antioxidants (glutathione) to reduce PTE toxicity and translocation in plant shoots. By forming extraradical mycelium networks, AMF can also minimize the exposure of plants to excessive PTE levels by producing glomalins, proteins allowing PTE immobilization in hyphae (Cabral et al., 2015). The authors however often questioned the fact that investigations in field experiments are needed to demonstrate the applicability of their findings. Our work therefore corroborates this major conclusion. Compared with non-inoculated poplars, inoculation did not improve nutrient concentrations when considering P, K, Ca, Mg, Fe and Mn in leaves analyzed at the end of the growing season in 2013 (Fig. 4). Despite this, we measured significant increases in biomass production at both sites for the poplar Skado clone in early spring 2013 (Ciadamidaro et al., 2017). We might therefore conclude that mycorrhizal fungi may have increased the volume of soil explored by single trees, thus allowing for an improved production of biomass.

An increase in extractable PTE concentrations was significant in some plots (Table 3) planted with inoculated clones (on P31, P12, P23, F30, F31 and F9), suggesting an induction of PTE mobilization in soils by the inoculation. Mycorrhizal fungi can trigger root exudation of organic acids, and solubilize PTEs (Churchland and Grayston, 2014). Conversely, it is likely that the inoculation also decreased PTE mobility in other plots (P11, P23, F9 and F12), via metal-binding processes (Cicatelli et al., 2014). Moreover, the extractable PTE concentrations in soils was not correlated with the effect of inoculation on PTE accumulation by poplars (Fig. 2). As discussed in our previous paper (Ciadamidaro et al., 2017), mycorrhizal colonization rates did not differ statistically between the four areas (F<sub>L</sub>, F<sub>H</sub>, P<sub>L</sub> and P<sub>H</sub>), and therefore cannot explain the lowest accumulations of PTE in leaves of inoculated poplars, as compared with non-inoculated ones. As there are no means (and thereof no attempt) to definitely identify the mycorrhizal isolates that were added as biological amendments to the trees at the plantation, we could only speculate that the mycorrhizal fungi present in the inoculum would be more efficient than the endogenous mycorrhizal fungi in their filtering PTE capacity (by producing more glomalins for example).

### ***3.5. Influence of site conditions on PTE accumulation in poplar leaves***

Pearson correlation tests revealed a positive linear relationship between foliar Zn concentrations in both clones and extractable Zn concentrations in soil at the Fresnes site ( $r_{Zn}$

= 0.57 for I-214,  $r_{Zn} = 0.68$  for Skado;  $p < 0.01$ ). At the Pierrelaye site, Cd and Zn concentrations in Skado leaves were positively correlated with extractable concentrations in soil ( $r_{Cd} = 0.50$ ,  $r_{Zn} = 0.53$ ;  $p < 0.01$ ), as previously reported (Madejón et al., 2004; Pourrut et al., 2011). Despite similar concentrations of extractable PTEs between some plots of the two sites, poplars could accumulate more PTEs in their leaves at the Fresnes site, which implies other soil parameters in soil-plant transfers of PTEs. The addition of soil parameters in the PCA as illustrative variables highlighted potential correlations between leaf Cd and Zn concentrations and their total concentrations in soil,  $CaCO_3$ , N, OM, silt,  $P_2O_5$ , CEC, CaO,  $K_2O$ ,  $NO_3^-$  and clay (Supplemental Table 1). This analysis suggested that PTEs were more bioavailable in more fertile soils, which is indeed the case for the Fresnes soil. Besides, negative correlations were found between K in leaves and soil extractable concentrations of As, Cu, Ni (for I-214) and Cd (for Skado). As foliar K concentrations were below optimal values (Fig. 4), those negative correlations suggested an inhibition of K accumulation in leaf tissues by these PTEs.

Bioconcentration factors (BCFs) were calculated as the ratio of the PTE concentrations in leaves ( $mg\ kg^{-1}\ DW$ ) and the total PTE concentration in soil ( $mg\ kg^{-1}\ DW$ ). A BCF value above 1 indicates that the plant is a metal accumulator. Cd and Zn BCFs were highest in  $F_L$  and  $P_L$  (the least contaminated areas of Fresnes and Pierrelaye sites, respectively), where almost all values were above 1 for both clones (Table 4). At both sites, Cd BCF values were higher than Zn BCF values and Skado clones accumulated more Cd and Zn in their leaves than I-214. Conversely, poplars did not accumulate Zn in  $F_H$  ( $BCF < 1$ ), the most contaminated area of the Fresnes site. Such Cd and Zn BCF values were reported in other poplar species in field trials, which confirmed their abilities to take up and transfer these two PTEs in their leaves (Migeon et al., 2009; Vandecasteele et al., 2003). At the Fresnes site, BCF values calculated for inoculated poplars were higher than those of non-inoculated poplars in most cases, suggesting that mycorrhizal fungi did not act as an effective barrier against poplar PTE translocation in leaves, considering soil total element concentrations in soil. Besides, poplars did not accumulate Cr, Cu and Pb in their leaves ( $BCFs < 1$ ).

### ***3.6. Lessons from the field and recommendations***

In recent years, poplar SRC on uncontaminated soils have been widely studied under field conditions in Europe to promote their environmental (Tricker et al., 2009; Verlinden et al., 2013) and socio-economic advantages (Marron et al., 2012). On contaminated lands, the application of aided phytostabilization with bioenergy crops at field scale is a great challenge

that aims at controlling and reducing the environmental impact of those sites while developing economic opportunities. With regard to this purpose, feedback from field study is scarce.

Many authors highlighted the necessity to select appropriate combinations of plant genotypes and mycorrhizal strains for phytoremediation purposes (Lingua et al., 2008; Mrnka et al., 2012). In fact, specific root exudates released by trees trigger the expression of mycorrhization genes, resulting in symbiotic relationships between plants and mycorrhizal fungi (Churchland and Grayston, 2014). Depending on their type (endo- or ectomycorrhizas), mycorrhizal fungi may interact differently with plants, by transferring limited or larger amount of nutrients or PTEs to their hosts. The role of mycorrhizal fungi in poplar growth, tolerance, and metal accumulation in tissues has been largely demonstrated in pot experiments under controlled conditions, in uncontaminated or metal contaminated soils (Baum et al., 2002; Cicutelli et al., 2014; Mrnka et al., 2012) but rarely in field conditions. On a slightly contaminated site, Bissonnette et al. (2010) reported that arbuscular mycorrhizal fungi inoculation had no significant effect on Cd accumulation in poplars shoots used for phytoextraction. In most of the less contaminated areas of our study, PTE concentrations of poplar leaves did not vary under inoculation. Interestingly, our results showed a significant decrease in foliar concentrations of Zn, Cu, Pb and Cr for the poplar Skado clone grown on the most highly polluted areas, which confirms fungal mycorrhizal symbiosis dynamics at elevated soil PTE concentrations (Audet and Charest, 2007; Bojarczuk and Kieliszewska-Rokicka, 2010). Therefore, the inocula and poplar cultivars used in this study were strongly influenced by the sites conditions (levels of PTEs in soil, OM, CEC, and certainly native microbial communities, vegetation, etc.). With the aim of managing risk at contaminated sites, further studies are needed to isolate fungal strains able to reduce Cd and Zn transfers in the aboveground parts of poplars, to reach baseline concentrations in tissues. Tripartite relationship including bacteria and fungi should also be considered to optimize root colonization by mycorrhizal fungi and reduce PTE transfers in poplars (Labbé et al., 2014).

Despite the positive effect of the inoculation observed in Skado clones *in situ*, the high Cd and Zn concentrations measured in poplar leaves is of concern since PTEs may disseminate in the environment after leaf abscission. Litter decomposition rates may be strongly reduced by the metal pollution, affecting consequently soil physicochemical and biological conditions (Berg et al., 1991). Therefore, a risk assessment is required to characterize Cd and Zn accumulation in the litter layer, as well as their potential transfers to other organisms since changes in metal speciation can be expected (Nirola et al., 2016). Based

on the outcomes, PTEs could be removed from contaminated sites by collecting the leaves after abscission. Also, the use of poplars for energy production in phytomanagement strategies requires low concentrations of PTEs in wood, in agreement with current local regulations. As poplars accumulate lower amounts of PTEs in wood than in leaves, we assume that Cd concentrations in wood are 1.2 to 2-fold lower than in leaves, and Zn concentrations, 4 to 10-fold lower considering similar foliar concentrations and moderately contaminated soils (Laureysens et al., 2004; Madejón et al., 2004; Migeon et al., 2009). In comparison with reference values obtained from a non-contaminated area (Chalot et al., 2012), Cd and Zn estimated concentrations in the wood of the present poplars may be in excess by the end of the first rotation. Valorization options (torrefaction, pyrolysis) of non-inoculated 7-year-old Skado poplars were investigated at a Zn and Cd contaminated site in Northern France (Bert et al., 2017). The Leforest site showed close physico-chemical characteristics, especially the soil texture and low CaCl<sub>2</sub>-extractable fractions, compared to the Fresnes site (Foulon et al., 2016). Wood concentrations of Cd and Zn after the first harvest were  $5.66 \pm 0.51$  mg Cd kg<sup>-1</sup> DW and  $64.10 \pm 11.61$  mg Zn kg<sup>-1</sup> DW, suggesting that Cd could be of concern for Skado poplar conversion processes as the concentration of Cd exceeded its baseline value ( $0.53 \pm 0.1$  mg Cd kg<sup>-1</sup> DW; Bert et al. (2017)). In addition, in France, a threshold value for Cd was stated at 5 mg kg<sup>-1</sup> DW to regulate the use of wood as fuel in combustion plants (MEDDE, 2013). In some plants covered by this regulation, Cd concentration in Skado poplar could limit its use for combustion. Concerning the use of Cd-enriched poplar wood as feedstock for pyrolysis and torrefaction, the potential limitation due to Cd could be overcome if end-products can be treated or upgraded. Due to the short duration of the study (3 years), we were not able to harvest poplars and report the Cd concentration of the woody biomass, poplar SRC being usually harvested on a cycle of 6 to 8 years (Marron et al., 2012). Nevertheless, we expect that the upcoming results would be comparable to those obtained in Bert et al. (2017). One can expect that inoculated poplars with efficient mycorrhiza for Cd sequestration could contribute to decrease the Cd wood accumulation in poplars. These hypotheses need to be assessed in further long-term field studies.

#### 4. Conclusion

The implementation of large field trials is a key stage to provide theoretical and technical knowledge in phytotechnologies. Our large-scale study pointed out the complexity of environmental processes that are not taken into account in greenhouse experiments. With



the objective of limiting poplar metal accumulation in the aboveground parts of poplars and optimizing their growth *in situ*, mycorrhizal fungi inoculation revealed to be effective in reducing Zn, Cu, Pb and Cr accumulation in Skado leaves after two growing seasons, in highly polluted areas. The same trend was also observed for most plots planted with I-214 or Skado clones. Therefore, mycorrhizal fungi interacted differently with poplars depending on the cultivars, soil physico-chemical properties and certainly other conditions specific to each site (vegetation, native microbial communities, etc.). In terms of territorial and sustainable development, the use of mycorrhizal poplars tends to be efficient in heavily polluted areas but further research is needed to identify and isolate fungal strains that have the capacity to reduce Cd and Zn accumulation in poplar tissues, to reach baseline concentrations for risk management and valorization purposes. Otherwise, poplar wood and leaves have both to be safely managed to prevent food chain contamination and pollutant emissions. Despite these promising results, a better understanding of interactions between mycorrhizal fungi, poplars and soil conditions in the field are still needed to allow operational implementations of phytomanagement options.

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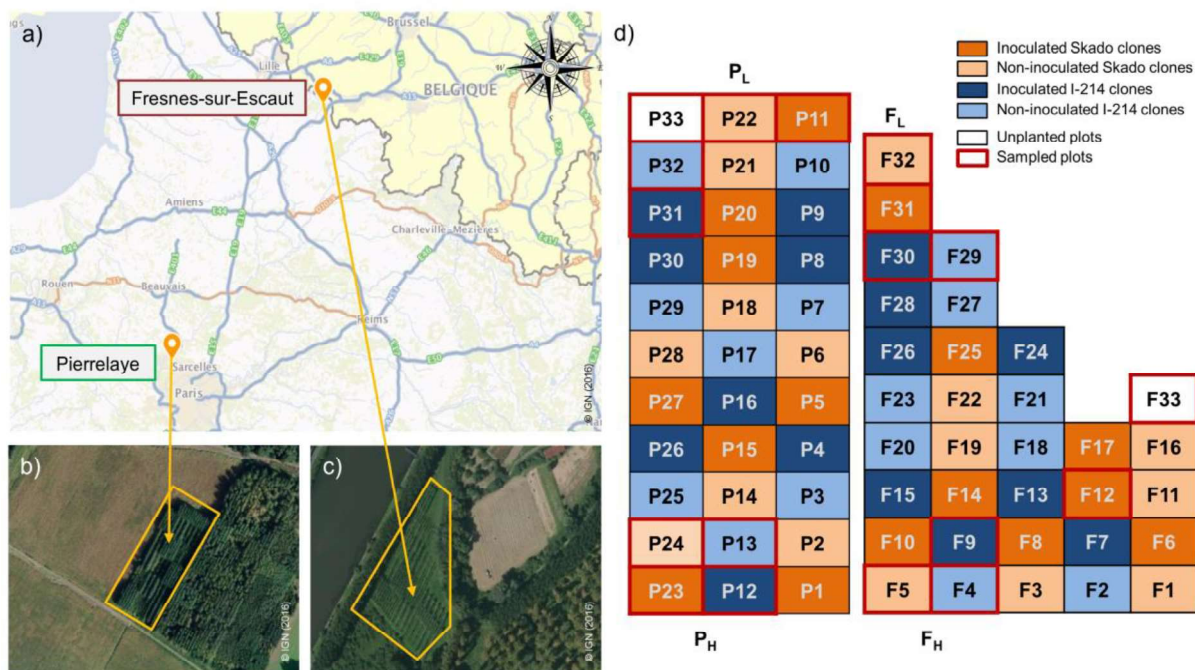
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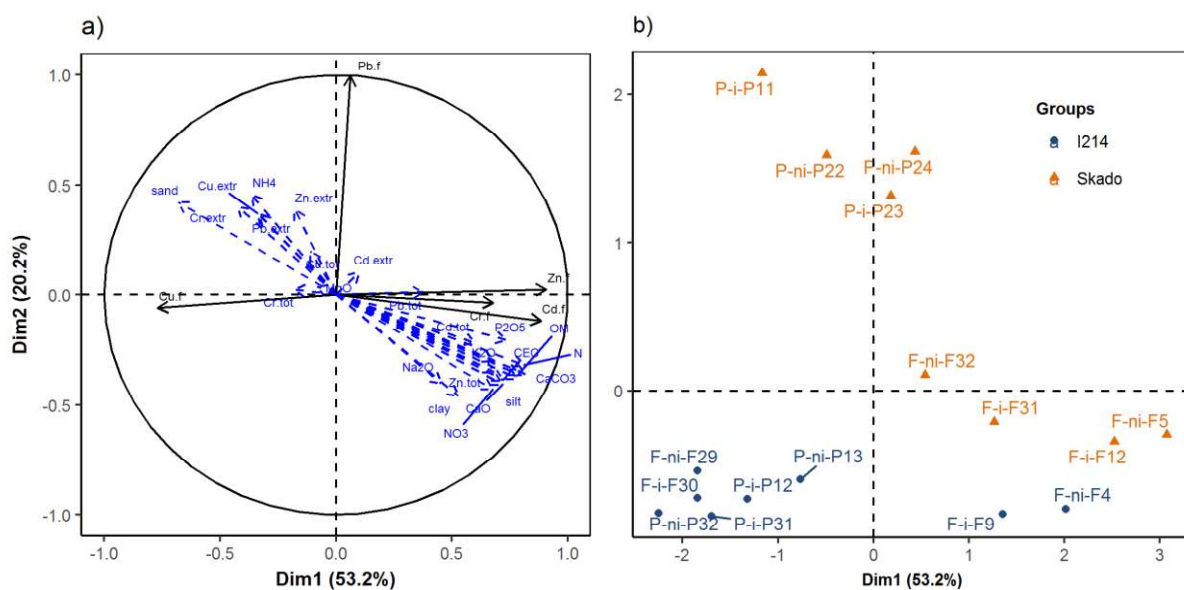
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**Fig. 1.** a) Location of the two sites. b) Aerial view of the Pierrelaye site. c) Aerial view of the Fresnes site. d) Experimental designs of the field trials which indicate the sampled plots in yellow boxes. On the left, the experimental design of the Pierrelaye site. On the right, the experimental design of the Fresnes site. P<sub>L</sub> and F<sub>L</sub> refer to the least contaminated areas at the Pierrelaye and Fresnes sites, respectively whereas P<sub>H</sub> and F<sub>H</sub> refer to the most contaminated areas of the two sites.

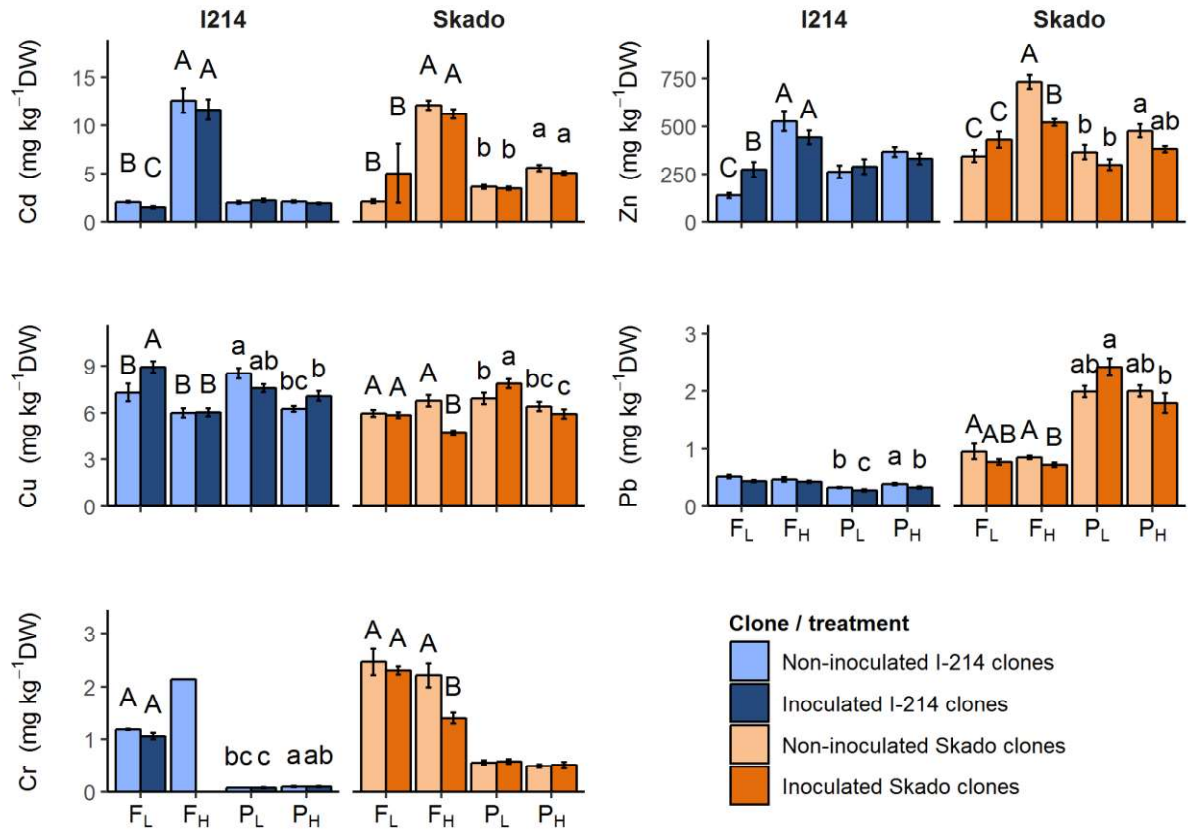
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**Fig. 2.** a) Graph of active (black solid arrows; leaf metal contents) and supplementary variables (blue dotted arrows; soil physico-chemical parameters). b) Graph of individuals corresponding to inoculated ("i") or non-inoculated ("ni") Skado and I-214, of Pierrelaye (P) and Fresnes (F) sites.

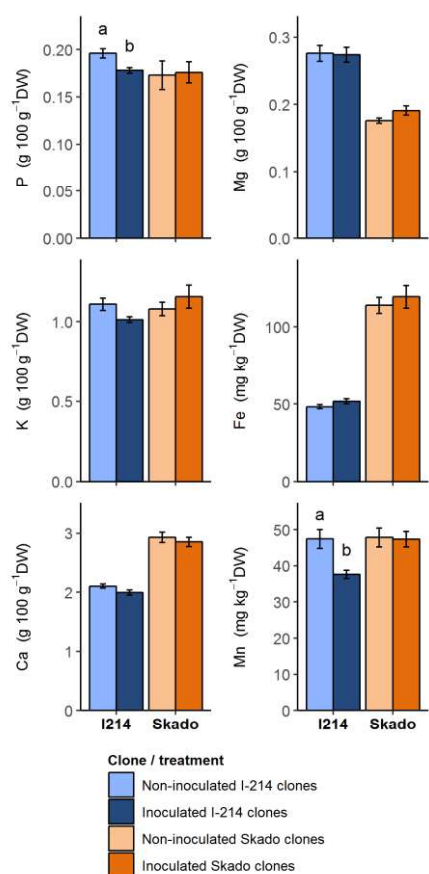
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**Fig. 3.** Foliar concentrations of Cd, Zn, Cu, Pb and Cr in Skado and I-214 clones. P<sub>L</sub> and F<sub>L</sub> refer to the least contaminated areas at the Pierrelaye and Fresnes sites, respectively whereas P<sub>H</sub> and F<sub>H</sub> refer to the most contaminated areas of the two sites. Data are means ± SE. Different letters indicate significant differences between the treatments for each site among each clone (Kruskal-Wallis test,  $p < 0.05$ ). No letter indicates no difference. Data below the LQ are not represented (LQ = 1-2 mg kg<sup>-1</sup> for Cr).

➤ 2-column fitting image



**Fig. 4.** Foliar concentrations of macronutrients P, K, Ca, Mg, Fe and Mn in Skado and I-214 clones. Data are means  $\pm$  SE. Different letters indicate significant differences between the treatments for each site, among each clone (Kruskal-Wallis test,  $p < 0.05$ ). No letter indicates no difference.

➤ 1.5-column fitting image

**Table 1.** Physico-chemical characteristics of composite topsoil samples collected in both sites at the time of the plantation (2011). P<sub>L</sub> and F<sub>L</sub> refer to the least contaminated areas at the Pierrelaye and Fresnes sites, respectively whereas P<sub>H</sub> and F<sub>H</sub> refer to the most contaminated areas of the two sites.

Site	Pierrelaye												Fresnes											
	P <sub>L</sub>						P <sub>H</sub>						F <sub>L</sub>						F <sub>H</sub>					
	P32	P31	P22	P11	P13	P12	P24	P23	F29	F30	F32	F31	F4	F9	F5	F12								
Sand (%)	91	91	91	91	85	81	83	80	16	15	22	19	24	21	24	22								
Silt (%)	4.8	5.3	5.2	5.6	8.5	11	11	11	67	68	62	65	61	61	59	61								
Clay (%)	3.9	4.2	3.8	3.7	6.1	8	6.1	8.3	18	17	17	16	15	17	17	17								
CaCO <sub>3</sub> (%)	0.7	0.7	0.6	0.7	1.5	1.9	1.3	1.8	2.3	2.0	2.9	2.1	5.3	4.8	7.9	4.6								
OM (%)	2.2	2.4	2.6	2.0	3.8	5.4	3.9	4.6	3.4	3.6	4.1	3.8	6.9	8.7	7.2	7.7								
pH-H <sub>2</sub> O	7.8	7.8	7.9	7.6	7.5	7.6	7.7	7.6	7.4	7.7	7.6	7.7	7.5	7.6	7.6	7.3								
N (g kg <sup>-1</sup> )	1.1	1.8	1.9	1.2	3.4	3.2	2.8	3.2	2.2	2.3	2.6	2.8	3.8	5.0	4.7	4.6								
NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> )	33	21	37	30	42	62	46	64	113	99	108	110	129	145	132	121								
NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> )	0.4	0.6	0.5	0.5	0.3	0.4	0.3	0.7	0.2	0.1	0.1	0.2	0.1	0.3	0.1	0.3								
C/N	11	7.5	7.8	10	6.5	10	7.9	8.3	8.9	8.8	8.9	7.9	11	10	8.8	10								
CEC (meq kg <sup>-1</sup> )	61	66	62	57	115	148	121	149	138	138	147	138	169	206	187	193								
B (mg kg <sup>-1</sup> )	0.5	0.6	0.5	0.5	0.8	1.0	0.9	0.9	0.4	0.5	0.5	0.5	0.4	0.4	0.6	0.4								
P <sub>2</sub> O <sub>5</sub> (mg kg <sup>-1</sup> )	106	107	115	107	164	228	154	216	120	125	188	157	195	227	220	285								
K <sub>2</sub> O (mg kg <sup>-1</sup> )	131	151	133	96	231	314	215	270	177	164	197	181	320	258	439	248								
MgO (mg kg <sup>-1</sup> )	181	181	140	135	131	175	151	166	95	97	126	115	126	148	160	125								
CaO (mg kg <sup>-1</sup> )	3430	3399	3215	3697	4888	5528	5352	5671	6987	6755	7227	6882	8189	8527	8183	8271								
Na <sub>2</sub> O (mg kg <sup>-1</sup> )	16	16	5.0	9.0	13	19	16	26	14	16	17	17	23	24	18	20								

**Table 2.** Total concentrations of trace and major elements of composite topsoil samples from the two sites ( $\text{mg kg}^{-1}$  DW), Pierrelaye and Fresnes at the time of the plantation (2011). Local usual agricultural concentrations (UAC; Lamy et al. (2006)) and threshold values used to assess the quality of dredged canal sediment (S1; MEDD (2006)) are indicated for comparison. P<sub>L</sub> and F<sub>L</sub> refer to the least contaminated areas at the Pierrelaye and Fresnes sites, respectively whereas P<sub>H</sub> and F<sub>H</sub> refer to the most contaminated areas of the two sites. P33 and F33 refer to unplanted plots.

Site	Area	Plot	Al	As	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Na	Ni	Pb	Zn
Pierrelaye	P <sub>L</sub>	P32	13600	4.52	6850	1.69	6.52	44.8	130	13500	3320	1170	759	21.4	243	352
		P31	9130	4.14	6110	1.61	5.38	42.8	123	8500	3300	980	745	17.4	214	365
		P22	7790	3.41	5160	1.47	5.5	36.4	94.4	6440	2870	769	615	16.4	207	289
		P11	8530	4.22	5730	1.40	5.22	38.2	107	8030	3190	803	760	17.18	236	218
		P13	13500	10.7	12500	3.85	7.49	59.0	229	11400	4190	1670	970	22.8	318	561
	P <sub>H</sub>	P12	16300	13.4	17000	5.93	7.83	76.9	281	14000	4603	2023	1148	27.6	361	525
		P24	11600	8.53	8290	3.26	5.87	53.7	179	9700	3950	1410	931	17.8	263	268
		P23	16600	15.9	13600	6.11	9.07	92.1	340	14300	4690	2083	1557	31.3	488	427
		P33	4.07	2.97	2.75	28.7	51.0	9.83	244	677						
		UAC		0.19-0.42	3.0-7.7	15-29	8-19	6-20	18-43	34-63						
Fresnes	F <sub>L</sub>	F29	337	9.16	13900	0.49	10.1	46.6	19.6	183	141	3012	5620	20.9	40.5	79.0
		F30	345	8.69	13200	0.46	9.40	53.0	25.5	184	143	2890	5860	18.8	44.5	92.0
		F32	424	10.7	19200	0.52	12.2	51.9	29.3	224	159	3230	6500	22.7	52.1	172
		F31	373	10.2	15200	0.49	10.6	50.4	27.8	196	145	3010	6060	21.3	47.0	137
		F4	505	23.5	30400	67.1	10.4	9.00	114	374	168	3750	3440	17.3	459	2560
	F <sub>H</sub>	F9	505	24.3	29600	10.1	18.2	86.1	114	395	128	3710	3570	40.6	497	2580
		F5	483	21.0	39300	60.1	8.60	7.5	101	348	131	3807	3527	15.7	390	2023
		F12	448	19.1	19000	0.93	14.5	67.8	62.6	277	141	3460	4990	29.3	224	1105
		F33				6.52			65.4							
		S1		30	2	150	100	50	100	300						

**Table 3.** Ca(NO<sub>3</sub>)<sub>2</sub>-extractable PTE concentrations of topsoil samples from the two sites (mg kg<sup>-1</sup> DW), Pierrelaye and Fresnes. P<sub>L</sub> and F<sub>L</sub> refer to the least contaminated areas at the Pierrelaye and Fresnes sites, respectively whereas P<sub>H</sub> and F<sub>H</sub> refer to the most contaminated areas of the two sites. Soil samples were first collected in 2011 at the time of plantation (composites made of 10 topsoil samples), then in 2012 (means ± sd; n=10) and 2013 (means ± sd; n=10) at the Fresnes and Pierrelaye sites, respectively. “-ni” refers to non-inoculated clones; “-i” refers to inoculated clones. Different letters indicate significant differences between the treatments (with or without inoculation) for each site and each clone, for 2012 and 2013 data (p < 0.05). No letter indicates no difference (for 2012 and 2013 data).

Site	Area	Plot	Clone-treatment	As			Cd			Cr			Cu			Ni			Pb			Zn		
				2011	2012 / 2013	2013	2011	2012 / 2013	2013	2011	2012 / 2013	2013	2011	2012 / 2013	2013	2011	2012 / 2013	2013	2011	2012 / 2013	2013	2011	2012 / 2013	2013
Pierrelaye	P <sub>L</sub>	P3	I214-ni	0.02	0.031	<0.00	0.002	0.007	0.014	<0.01	0.202	<0.01	0.032	<0.00	0.015	<0.06	0.411							
				3	(0.003) <sup>c</sup>	5	(0.000) <sup>c</sup>	0.007	(0.005) <sup>b</sup>	5	(0.027) <sup>b</sup>	5	(0.006) <sup>c</sup>	6	(0.005) <sup>c</sup>	<0.06	(0.156) <sup>b</sup>							
		P3	I214-i	0.02	0.039	0.011	0.003	0.007	0.024 (0.006) <sup>a</sup>	0.024	0.220	<0.01	0.048	0.115	0.045	1.556	0.779 (0.377) <sup>a</sup>							
				2	(0.005) <sup>b</sup>	0.011	(0.000) <sup>c</sup>	0.007	0.024 (0.006) <sup>a</sup>	0.024	(0.028) <sup>b</sup>	<0.01	0.013 <sup>b</sup>	0.115	(0.028) <sup>a</sup>	0.045	0.779 (0.377) <sup>a</sup>							
		P2	Skado-ni	0.01	0.040	<0.00	0.003	0.020	0.018	0.024	0.223	<0.01	0.040	<0.00	0.031	0.252	0.756							
				2	(0.005) <sup>c</sup>	5	(0.001) <sup>B</sup>	0.020	(0.003) <sup>B</sup>	0.024	(0.027) <sup>C</sup>	0.223	<0.01	0.040	<0.00	0.031	0.252	0.756						
	P1	Skado-i	0.02	0.033	<0.00	0.002	0.007	0.018	<0.01	0.206	0.032	<0.01	0.032	<0.00	0.039	0.484								
			1	(0.005) <sup>D</sup>	5	(0.001) <sup>C</sup>	0.007	(0.003) <sup>B</sup>	5	(0.020) <sup>C</sup>	0.206	<0.01	0.032	<0.00	0.039	<0.06	0.484							
	P <sub>H</sub>	P1	I214-ni	0.03	0.047	<0.00	0.004	0.014	0.020 (0.003) <sup>a</sup>	0.121	0.355	<0.01	0.059	<0.00	0.026	0.182	0.653 (0.165) <sup>a</sup>							
				4	(0.006) <sup>a</sup>	5	(0.001) <sup>b</sup>	0.014	0.020 (0.003) <sup>a</sup>	0.121	(0.036) <sup>a</sup>	5	(0.005) <sup>a</sup>	<0.00	0.026	0.182	0.653 (0.165) <sup>a</sup>							
		P1	I214-i	0.02	0.047	<0.00	0.004	0.008	0.024 (0.009) <sup>a</sup>	0.064	0.355	<0.01	0.067	<0.00	0.044	0.288	0.889 (0.381) <sup>a</sup>							
				6	(0.008) <sup>a</sup>	5	(0.001) <sup>a</sup>	0.008	0.024 (0.009) <sup>a</sup>	0.064	(0.047) <sup>a</sup>	5	(0.015) <sup>a</sup>	<0.00	0.044	0.288	0.889 (0.381) <sup>a</sup>							
P2		Skado-ni	0.02	0.052	<0.00	0.003	0.010	0.023	0.055	0.309	<0.01	0.058	<0.00	0.032	0.123	0.916								
			4	(0.008) <sup>A</sup>	5	(0.000) <sup>B</sup>	0.010	(0.004) <sup>A</sup>	0.055	(0.056) <sup>B</sup>	0.309	<0.01	0.058	<0.00	0.032	0.123	0.916							
P2	Skado-i	0.02	0.044	<0.00	0.004	0.013	0.022	0.091	0.384	<0.01	0.059	<0.00	0.022	0.232	0.797									
		4	(0.006) <sup>B</sup>	5	(0.001) <sup>A</sup>	0.013	(0.004) <sup>A</sup>	0.091	(0.053) <sup>A</sup>	0.384	<0.01	0.059	<0.00	0.022	0.232	0.797								
Fresnes	F <sub>L</sub>	P3	Unplanted	0.02	0.025 (0.002)	<0.00	0.002	0.006	0.015 (0.004)	<0.01	0.182	<0.01	0.027	<0.00	0.027 (0.014)	<0.06	0.425 (0.175)							
				3	(0.000)	5	(0.000)	0.006	0.015 (0.004)	<0.01	(0.016)	5	(0.002)	<0.00	0.027 (0.014)	<0.06	0.425 (0.175)							
		F2	I214-ni	0.01	0.015	<0.00	<0.002	<0.00	0.004 (0.000) <sup>c</sup>	<0.01	0.036	<0.01	0.016	<0.00	0.003	<0.06	0.060 (0.022) <sup>c</sup>							
				6	(0.005) <sup>a</sup>	5	<0.002	<0.00	0.004 (0.000) <sup>c</sup>	<0.01	(0.004) <sup>c</sup>	5	(0.002)	<0.00	0.003	<0.06	0.060 (0.022) <sup>c</sup>							
		F3	I214-i	0.01	0.017	<0.00	<0.002	<0.00	0.005	<0.01	0.057	<0.01	0.015	<0.00	0.003	<0.06	0.073 (0.056) <sup>c</sup>							
				4	(0.004) <sup>a</sup>	5	<0.002	<0.00	0.005	<0.01	(0.018) <sup>b</sup>	5	(0.002)	<0.00	0.003	<0.06	0.073 (0.056) <sup>c</sup>							
	F3	Skado-ni	0.02	0.014	<0.00	<0.002	<0.00	0.005	<0.01	0.051	<0.01	0.018	<0.00	0.003	<0.06	0.084								
			7	(0.004) <sup>B</sup>	5	<0.002	<0.00	0.005	<0.01	(0.010) <sup>B</sup>	5	(0.002)	<0.00	0.003	<0.06	0.084								
	F3	Skado-i	0.02	0.020	<0.00	<0.002	<0.00	0.006	<0.01	0.055	<0.01	0.019	<0.00	0.003	<0.06	0.071								
			3	(0.004) <sup>A</sup>	5	<0.002	<0.00	0.006	<0.01	(0.021) <sup>B</sup>	5	(0.005)	<0.00	0.003	<0.06	0.071								

F4	I214-ni	0.01	0.015	<0.00	0.003	<0.00	0.006	<0.01	0.085	<0.01	0.020	<0.00	0.010	0.151	0.402 (0.141) <sup>a</sup>
		0	(0.002) <sup>ab</sup>	5	(0.001)	6	(0.002) <sup>b</sup>	5	(0.014) <sup>a</sup>	5	(0.011)	6	(0.005) <sup>a</sup>		
F9	I214-i	0.01	0.013	0.014	0.003	<0.00	0.007 (0.003) <sup>a</sup>	<0.01	0.084	<0.01	0.017	<0.00	0.011	0.770	0.276
		2	(0.001) <sup>b</sup>		(0.001)	6		5	(0.016) <sup>a</sup>	5	(0.009)	6	(0.004) <sup>a</sup>		(0.108) <sup>b</sup>
F <sup>H</sup>	F5 Skado-ni	0.01	0.018	<0.00	0.003	<0.00	0.007	<0.01	0.084	<0.01	0.022	<0.00	0.014	0.278	0.353
		3	(0.004) <sup>A</sup>	5	(0.001) <sup>A</sup>	6	(0.003) <sup>A</sup>	5	(0.012) <sup>A</sup>	5	(0.009)	6	(0.006) <sup>A</sup>		(0.114) <sup>A</sup>
F1	Skado-i	0.01	0.014	<0.00	0.002	<0.00	0.006	<0.01	0.073	<0.01	0.015	<0.00	0.014	0.234	0.311
		2	(0.002) <sup>B</sup>	5	(0.001) <sup>B</sup>	6	(0.003) <sup>B</sup>	5	(0.008) <sup>A</sup>	5	(0.004)	6	(0.005) <sup>A</sup>		(0.060) <sup>A</sup>
F3	Unplante	-	0.011	0.005	<0.002	-	0.005	0.027	0.057	-	0.016	0.002	0.002	1.107	0.312
	<sup>d</sup>			(0.002				(0.008				(0.001	0.002	(0.843	)

**Table 4.** Bioconcentration factors of Cd and Zn based on their foliar concentrations in poplar leaves ( $\text{mg kg}^{-1}$  DW) and total concentrations in soil ( $\text{mg kg}^{-1}$  DW).

Site	Area	Clone	Treatment	Plot	Cd	Zn
Pierrelaye	P <sub>L</sub>	I214	Not inoculated	P32	1.17	0.74
			Inoculated	P31	1.36	0.79
	Skado	Not inoculated	P22	2.46	1.26	
		Inoculated	P11	2.46	1.37	
	P <sub>H</sub>	I214	Not inoculated	P13	0.54	0.65
			Inoculated	P12	0.32	0.63
Skado		Not inoculated	P24	1.71	1.78	
		Inoculated	P23	0.83	0.89	
Fresnes	F <sub>L</sub>	I214	Not inoculated	F29	4.13	1.73
			Inoculated	F30	3.24	2.97
	Skado	Not inoculated	F32	4.02	2.00	
		Inoculated	F31	10.3	3.14	
	F <sub>H</sub>	I214	Not inoculated	F4	0.19	0.21
			Inoculated	F9	1.16	0.17
Skado		Not inoculated	F5	0.20	0.36	
		Inoculated	F12	12.1	0.47	