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Nature of fly ash amendments differently influences oxidative stress alleviation in four forest tree species and metal trace element phytostabilization in aged contaminated soil: A long-term field experiment

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ABSTRACT

Aided phytostabilization using coal fly ashes (CFAs) is an interesting technique to clean-up polluted soils and valorizing industrial wastes. In this context, our work aims to study the effect of two CFAs: silico-aluminous (CFA1) and sulfo-calcic (CFA2) ones, 10 years after their addition, on the phytostabilization of a highly Cd (cadmium), Pb (lead) and Zn (zinc) contaminated agricultural soil, with four forest tree species: *Robinia pseudoacacia*, *Alnus glutinosa*, *Acer pseudoplatanus* and *Salix alba*. To assess the effect of CFAs on trees, leaf fatty acid composition, malondialdehyde (MDA), oxidized and reduced glutathione contents ratio (GSSG: GSH), 8-hydroxy-2′-deoxyguanosine (8-OHdG), Peroxidase (PO) and Superoxide dismutase (SOD) activities were examined. Our results showed that CFA amendments decreased the $CaCl_2$-extractable fraction of Cd and Zn from the soil. However, no significant effect was observed on metal trace element (MTE) concentrations in leaves. Fatty acid percentages were only affected by the addition of sulfo-calcic CFA. The most affected species were *A. glutinosa* and *R. pseudoacacia* in which C16:0, C18:0 and C18:2 percentages increased significantly whereas the C18:3 decreased. The addition of sulfo-calcic CFA induced the antioxidant systems response in tree leaves. An increase of SOD and POD activities in leaves of trees planted on the CFA2-amended plot was recorded. Conversely, silico-aluminous CFA generated a reduction of lipid and DNA oxidation associated with the absence or low induction of anti-oxidative processes. Our study evidenced oxidative stress alleviation in tree leaves due to CFA amendments. MTE mobility in contaminated soil and their accumulation in leaves differed with the nature of CFA amendments and the selected tree species.

1. Introduction

Soil metal contamination presents a serious threat for environment and human health. This kind of contamination is mainly due to anthropogenic activities such as pesticide manufacturing and their application, mining and siderurgical activities, combustion of fossil fuel and industrial waste (Kushwaha, 2015). Aided phytostabilization is a promising technique proposed to phytoremediate large area contaminated with metal trace elements (MTE). This technique aims to immobilize MTE in the rhizosphere through the use of metal-tolerant plants and mineral or organic soil amendments to decrease the mobility of pollutants and by increasing soil fertility (Kidd et al., 2009). Amongst the amendments used for MTE stabilization in soils, coal fly ashes (CFA) have been described in previous studies (Haynes, 2009; Lopareva-Pohu et al., 2011b; Pandey et al., 2009; Ram and Masto, 2014). CFA are oxidized, non-combustible residues generated from combustion of coil in thermal power plant. They are amorphous silicates with a very similar matrix to the soil (Pandey and Singh, 2010). Coal fly ash accounts for 5–20% of burned coal weight equivalent to an annual worldwide production of 750 million tons of solid waste materials (Yao

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et al., 2015). The huge quantity of CFA produced annually not only poses serious environmental concerns but also requires large areas of land for its storage and disposal (Shaheen et al., 2014). Significant research efforts have been made to develop fly ashes recycling methods and to limit the discharge of CFA into ash ponds, lagoons or landfills surrounding energy plants. They include the use of CFA in construction industry, ceramic industry, zeolite synthesis, catalysis or metal extraction (Ahmaruzzaman, 2010). However, these applications have small recycling capacities that do not cover the utilization of all CFA produced (Yao et al., 2015). Pandey et al. (2010) mentioned that the use of CFA in agriculture, forestry, horticulture, and floriculture sectors could be a viable option. Coal fly ashes are characterized by a low bulk density, a higher water-holding capacity, a favorable pH and an important amounts of essential plant nutrients making it a potential amendment for soils (Pandey and Singh, 2010). Indeed, numerous organic and mineral soil amendments can reduce metal mobility and bioavailability through adsorption, complexation and/or precipitation (Mench et al., 2010). In soils amended with alkaline materials, such as CFA, precipitation and increased sorption were identified as the main mechanisms involved in MTE retention (Lee et al., 2009; Lombi et al., 2002). Therefore, a better growth of *Lolium perenne* and *Trifolium repens* on a highly lead (Pb), zinc (Zn) and cadmium (Cd) contaminated soil amended with CFA was reported by Lopareva-Pohu et al. (2011b). Likewise, Krzaklewski et al. (2012) showed that the mixture of fly ash and lignite culm had a positive effect on the survival and growth of *Alnus glutinosa* (L.) Gaertn as well as *Alnus incana* (L.) Moench. High levels of MTE could have drastic effects on plant physiology because of the production and accumulation of reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radicals and hydrogen peroxide. Inside leaves, accumulation of MTE promotes ROS production by interfering with different plant physiological and metabolic processes such as inactivation of the antioxidant defense and photosynthetic systems (Hossain et al., 2012). ROS excess exposes the cell to uncontrolled oxidative stress leading to lipid peroxidation, loss of membrane integrity, ion permeability leakage as well as protein alteration and DNA-strand cleavage which result in necrosis and chlorosis of the tissue (Shahid et al., 2014). To protect cells against the toxic effect of ROS, plants developed many anti-oxidant systems such as superoxide dismutases, ascorbate–glutathione cycle and NADP-dependent dehydrogenases that play an important role in the detoxification reactions of living cells (Halliwell and Gutteridge, 2000; Del Río et al., 2002). It was demonstrated that plant leaf fatty acid composition is a trustful parameter to study the effects of metal (loid) contamination on plants (Schreck et al., 2013). In a previous work carried out by Le Guédard et al. (2008) on lettuce seedlings grown on metal contaminated soil, a decrease in tri-unsaturated fatty acid percentage and an increase in C18:2, C18:1 and C18:0 percentages were observed. Similar results were obtained by Ben Youssef et al. (2005) in leaves of *Brassica napus* cultivated on nutrient solution supplied by $CdCl_2$. Few studies reported the effect of high level of MTE on tree species (Gaudet et al., 2011; Upchurch, 2008; Le Guédard et al., 2012). In addition, stabilization of MTE in soil might have beneficial consequences on health of forest species growing on highly contaminated sites. *In situ* MTE immobilization by means of CFA soil amendments is now being recognized as a relevant and robust remediation technique (Shaheen et al., 2014). Whilst characteristics of CFA are determined by its elemental and structural composition which varies with the types and sources of coal burned (Comberato et al., 1997) and evolve over time in amended soil, noticeable and persistent effects on MTE mobility that could be observed a decade after their application in contaminated site (Bidar et al., 2016).

The present work aims to study the effects of two types of coal fly ashes (silico-aluminous and sulfo-calcic CFA), 10 years after their addition to a highly Cd, Pb and Zn contaminated agricultural soil, on the phytostabilization with four forest tree species: *Robinia pseudoacacia*, *Alnus glutinosa*, *Acer pseudoplatanus* and *Salix alba*. To investigate the response of the different tree species to the amended soils, leaf fatty acid composition and the most important oxidant stress indicators: malondialdehyde (MDA), 8-hydroxy-2′-deoxyguanosine (8-OHdG), oxidized and reduced glutathione contents ratio (GSSG: GSH), Peroxidase (PO) and Superoxide dismutase (SOD) activities were examined.

2. Material and methods

2.1. Experimental site description

The experimental site (50°26′N, 3°01′E) is located in an agricultural field in the North of France (Noyelles-Godault) in the surrounding area of an old formerPb and Zn smelter. MTE emissions during the 100 years of the smelter activity caused metal contamination of the agricultural lands. Pb, Cd and Zn concentrations in topsoils of the experimental field exceed up to 20–50 fold of those usually reported for the regional arable lands (Sterckeman et al., 2002). In 2000, the experimental field was divided in 3 plots. The first one was not amended and was considered as the polluted reference plot (R). The two other plots were amended with two different CFA provided by Surschiste Ltd. (Mazingarbe, France). Plot F1 was amended with CFA1, a silico-aluminous CFA Sodeline1 (Carling thermal power plant, France) and the second plot (F2) was amended with CFA2, a sulfo-calcic CFA Soproline1 (Gardanne thermal power plant, France). CFA were added at a rate of $23.3\ kg\ m^{-2}$ then ploughed up to a 25- to 30-cm soil depth. The main physico-chemical parameters of non-amended (R) and amended plots (F1 and F2) are presented in Table 1. Experimental plots were planted with a tree mix: black locust (*Robinia pseudoacacia* L.), black alder (*Alnus glutinosa* L.), sycamore maple (*Acer pseudoplatanus* L.), white willow (*Salix alba* L.) and pedunculate oak (*Quercus robur* L.). The latter tree species will not be considered, due to the low growth of plants observed during the experiment. About 1800 trees were planted altogether according to an experimental design described by Lopareva-Pohu et al. (2011a).

2.2. Plant and soil sampling

11 years after tree plantation, in September 2011, five leaves from each tree species were sampled in each experimental plot: R, F1 and F2. Fresh leaves were immediately frozen in liquid nitrogen on the field and then stored at −80 °C until further analyses. For each tree species, nine soil samples were realized with a gouge at 0–25 cm soil depth near tree trunk, in each experimental plot.

2.3. Metal Trace Element determination in the soil

After root removal, all top soil samples were dried at 40 °C and then

Table 1
Main physico-chemical parameters of the three plots: non-amended plot (R), plot amended with the silico-aluminous coal fly ash (F1) and plot amended with the sulfo-calcic coal fly ash (F2) (Lopareva-Pohu et al., 2011a).

		R	F1	F2
pH		$7.31^{b} \pm 0.20$	$7.95^{a} \pm 0.03$	$7.74^{ab} \pm 0.02$
CEC	$Cmol^{+}kg^{-1}$	$14.5^{a} \pm 0.5$	$13.6^{a} \pm 0.2$	$14.6^{a} \pm 0.4$
Corg	$g\ kg^{-1}$	$23.8^{c} \pm 0.7$	$25.9^{b} \pm 0.6$	$29.8^{a} \pm 1.1$
Ntot	$g\ kg^{-1}$	$1.76^{a} \pm 0.06$	$1.68^{a} \pm 0.04$	$1.70^{a} \pm 0.06$
C/N		$13.5^{c} \pm 0.1$	$15.5^{b} \pm 0.1$	$17.5^{a} \pm 0.3$
P_2O_5	$g\ kg^{-1}$	$0.20^{ab} \pm 0.02$	$0.18^{b} \pm 0.01$	$0.24^{a} \pm 0.03$
$CaCO_3$ total	$g\ kg^{-1}$	$1.5^{c} \pm 0.8$	$5.8^{b} \pm 0.9$	$71.6^{a} \pm 6.5$
K_2O	$g\ kg^{-1}$	$0.30^{b} \pm 0.01$	$0.41^{a} \pm 0.01$	$0.40^{a} \pm 0.04$
MgO	$g\ kg^{-1}$	$0.19^{b} \pm 0.01$	$0.34^{a} \pm 0.01$	$0.16^{b} \pm 0.01$
Fe	$g\ kg^{-1}$	$7.3^{c} \pm 0.2$	$8.8^{b} \pm 0.4$	$10.0^{a} \pm 0.4$
Mn	$mg\ kg^{-1}$	$25.6^{a} \pm 5.7$	$10.1^{b} \pm 0.5$	$8.2^{c} \pm 0.3$

Data are means ± SD; a, b and c denote significant differences between plots, analysis was carried out separately for each year (Kruskal–Wallis test, $p < 0.05$, $n = 3$).