Effects of soil contamination by trace elements on white poplar progeny: seed germination and seedling vigour

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Abstract

Seed germination is considered a critical phase in plant development and relatively sensitive to heavy metals. White poplar (*Populus alba*) trees tend to accumulate Cd and Zn in their tissues. We tested if soil contamination can affect *P. alba* progeny, reducing seed germination, and explored the distribution of mineral elements in the seed. For this purpose fruits and seeds from female *P. alba* trees were selected from two contaminated and one non-contaminated areas. Seeds from all the sites were germinated using only water or a nutritive solution (*in vitro*). Concentrations of nutrients and trace elements in the fruits and seeds were analysed. Seedling growth *in vitro* was also analysed. Finally, a mapping of different elements within the poplar seed was obtained by particle Induced X ray emission (PIXE). Germination was similar between different progenies, refuting our hypothesis that seeds from a contaminated origin would have reduced germination capacity compared to those from a non-contaminated site. Seedling growth was not affected by the contaminated origin. Cadmium and Zn concentrations in fruits produced by *P. alba* trees in the contaminated sites were higher than by those from the non-contaminated site. However, the nutritional status of the trees was adequate in both cases. Cd in seedlings was higher in those from contaminated soils although lower than in fruits, indicating a certain exclusion from seeds. Preliminary results of the PIXE

technique showed that Al and Zn were distributed uniformly in the seeds (Cd was not detected with this technique) while the nutrients P and S were concentrated in the cotyledons.

Keywords: element distribution, metal toxicity, micro-PIXE, plant analysis, Populus alba

1. Introduction

Atmospheric and soil pollution are regarded as important factors contributing to forest decline (Hüttermann et al. 1999; Brydges et al. 2000; FAO 2005; Domínguez et al. 2010a,b). In these contaminated environments, as well as in natural mineral deposits rich in trace metals, plants and other organisms have to cope with chemical and physiological constraints by different strategies, either excluding or accumulating metals in their tissues (Baker 1981). Adult plants, like trees, must be adapted not only to survive adverse conditions, but also must be able to produce viable seeds and recruit a new generation of seedlings and saplings to complete the life cycle.

High concentrations of non-essential metals in the seeds can have toxic effects and there are several regulatory mechanisms to avoid or mitigate them. For example, 60% of Cd in seeds of the hyperaccumulator *Thlaspi praecox* is complexed with thiol-containing compounds. A similar mechanism has been proposed for As accumulation in rice grains (Mendoza-Cózatl et al. 2011). After uptake from the soil, metals are mostly sequestered into the vacuoles of root and shoot tissues. Essential metals, like Fe and Zn, are transported to seeds via the phloem. Meanwhile non-essential metals can also reach the developing seeds through transporters of essential metals due to their ionic similarities or as a consequence of broad substrate specificity (Kranner and Colville 2011). As a result, considerable amounts of metals can be accumulated in the seeds of different species (e. g., Gross et al. 1986). An extreme case is *Viola* species (*V. arsenica, V. macedonica,* and *V. allchariensis*) from a Macedonian mine, reaching more than 1000 mg kg^{-1} of As and Tl in their seeds (Bačeva et al. 2014). Trees in the Salicaceae family, such as *Populus* and *Salix* species, tend to accumulate Cd and Zn in leaves and even in fruits in contaminated areas (Madejón et al. 2004, 2013). In contrast, other plant species such as *Helianthus annuus* show effective barriers preventing metals from reaching their seeds in contaminated soils (Madejón et al. 2003).

New techniques like Particle Induced X ray Emission (PIXE) allow mapping the distribution of non-essential elements in the seeds, which has biological relevance. For example, the accumulation of Ni in seeds of *Thlaspi indicum*, mostly in the micropylar region of the testa, a sensitive part of the seed, has been interpreted as a possible chemical defence against seed predators (Psaras and Manetas 2001).

Seed germination is one of the most important and critical early phases in plant demography (Harper 1977); however, comparatively little information is available on how it is affected by high concentrations of heavy metals (Kranner and Colville 2011). In general, seed germination is considered a process sensitive to heavy metals compared to other stages in plant development (Ernst 1998).

The technique of *in vitro* culture may help to assess the potential negative effects of metal toxicity on seedling growth and the vigour of metal-enriched seeds. There have been some previous studies with poplars. Iori et al. (2012) culturing callus of two parental clones of *Populus nigra* in different Cd concentrations (0, 150 and 250 µM), found that Poli clone was more tolerant. Bittsánszky et al. (2005) showed that transgenic *Populus canescens* (*Populus tremula* x *Populus alba*) plants were more suitable for the phytoextraction of soils contaminated with Zn ($^{2+}$) than wild-type plants, using *in vitro* leaf discs cultures. Di Lonardo et al. (2011) assessed the response of clones of *Populus alba* to high levels of cadmium, zinc and copper with *in vitro* micro-shoots. Castiglione et al. (2007) investigated *in vitro* the tolerance to high concentrations of Zn of a commercial clone of *Populus alba* on the basis of leaf chlorosis and the rate of adventitious root formation from shoot cuttings, indicating that the threshold of tolerance is 1 mM Zn, and that the metal is taken up and translocated to the shoots. Kališová-Špirochová et al. (2003) studied *in vitro* that aspen (*Populus tremula*×*tremuloides*) had an accumulation capacity of about 70% of Pb^{2+} originally present in the solution, without a negative influence on the plants at a level of 0.1 mM. However, there is no information about the differential response of poplar seeds developed in mother trees grown naturally in metal-polluted soils.

Our plant-model, *Populus alba* (white poplar), is a deciduous tree common in riparian forests under Mediterranean climate. A single mature female poplar can produce thousands or even millions of wind- and water-dispersed, non-dormant, short-lived tiny seeds (about 1 mm in length) annually (Karrenberg and Suter 2003). Seed viability is high (90%) and seed germination is fast, usually within a period of 24 h (Siegel and Brock 1990; van Splunder et al. 1995; Karrenberg and Suter 2003). However, facilitating the natural regeneration of *Populus* from seed is a largely unexplored means for reintroducing this tree into disturbed areas where its vegetative regeneration is difficult (Schott et al., 2014).

In this paper we tested the hypothesis that soil contamination would negatively affect the white poplar progeny. In particular we asked several questions: 1. Do seeds from contaminated trees have a retarded and reduced germination rate? 2. Do the seedlings from a contaminated origin have reduced vigour and growth? 3. How are the elements distributed within the seeds? 4. Are non-essential elements accumulated in fruits and seeds of poplars grown in contaminated soils?

2. Materials and methods

2.1. Study area

To assess the effects of soil contamination on white poplar progeny we selected two sites, locally named Doblas (DO, 37º 23’ 40” N, 6º 13’ 35” W) and Soberbina (SO, 37º 27’ 10” N, 6º 13’ 5” W) in the Guadiamar Green Corridor (Sevilla, Spain), which were contaminated by

trace elements (As, Cd, Cu, Pb, Zn) after a mine spill occurred in 1998 (the Aznalcóllar mine accident, Grimalt et al. 1999). For comparison, a non-contaminated site was selected in Ribera de Huelva (RHU, 37º 29' 5" N, 6º 01' 34" W). The spill-affected zone was cleaned-up, remediated and afforested, being transformed from agricultural use to a protected area (Domínguez et al., 2008). More information about the study area and the three sampling sites can be found in Madejón et al. (2004, 2013).

2.2. Sampling strategy

At each sampling site five female *Populus alba* trees were selected and marked with a metal tag. Mean values for tree features in each site are shown in Table S1.

Soil samples were taken from the root zone of each selected tree, about 2 m from the trunk and at a depth of 0–25 cm. Three subsamples were taken to make a composite soil sample per tree.

Fruits of the selected trees were collected in April 2013. Those fruits were analysed for nutrients and trace elements. In April 2015, completely mature reproductive structures from three trees on each site were sampled to obtain mature seeds.

2.3. Seed germination and seedling performance

Seed germination experiments were carried out under two conditions:

a) In water. Thirty seeds of each tree (three replicates per site) were placed in a Petri dish with 3 ml of water. They were incubated in a culture chamber at 23±1°C, 30 $\mu Em^{-2}s^{-1}$ light intensity and a16 h photoperiod. The number of germinated seeds was recorded at 24 h, 48 h and 72 h. The vigour (size) of the seedlings was measured at 72 h.

b) *In vitro*. A subsample of seeds was stored in the cold (two weeks) before being germinated *in vitro*. Sixteen seeds (Fig S1) from each tree (three trees per site, as described above) were disinfected by immersion in absolute ethanol for 5 seconds and then in sodium hypochloride (0.7% active chlorine) for 30 seconds, followed by three rinses of 4 min each with sterilized water. Afterwards the seeds were placed individually in test tubes (150 x 25 mm) with 10 ml of 1/4 strength MS medium (Murashige and Skoog 1962) plus 2.5% sucrose and 100 mg L^{-1} of inositol. The medium was adjusted to pH 5.7. All the tubes were covered with a plastic cap, sealed with parafilm, and placed in a culture chamber at 23±1°C, 30 $\mu Em^{-2}s^{-1}$ light intensity and a 16 h photoperiod. The percentage of germination was measured every 3 days for 30 days.

Seedling performance was evaluated for the *in vitro* grown plants. Stem length was measured every three days for 30 days. At the end of the experiment, fresh and dry weights of each plant were quantified.

2.4. Plant and soil chemical analyses

Fruit samples (from five tree-replicates per site, year 2013) and seed samples (from three trees per site, year 2015, after germinating a minimum of 1000 seeds per tree to generate sufficient initial seedling biomass) were prepared for analysis. They were dried at 70ºC for at least 48 h, and ground using an agate pestle (due to the small amount of plant material).

The plant material was analysed for N by Kjeldahl digestion (only for fruits). Trace elements (Al, As, Cd, Cu, Fe, Mn, Pb, and Zn) and macronutrients (Ca, K, Mg, N, P and S) were determined by wet oxidation with concentrated HNO_3 under pressure in a microwave digester (Jones and Case 1990). The analysis of macronutrients and trace metals in the digests was performed by ICP-OES (inductively coupled plasma spectrophotometry). The accuracy of the analytical method was determined using a plant reference material: NCS DS 73348 (poplar leaves). Recovery rates for reference plant samples were between 90 and 110%.

Soil samples were oven-dried at 40ºC for at least 48 h, crushed to pass a 2 mm sieve, and then ground to <60 µm for trace element analysis. Soil pH was determined using a pH meter (CRISON micro pH 2002). Values of pH were measured in a 1/2.5 sample/1M KCl extract after shaking for 1 h. The total content of trace elements was determined by ICP-OES, after digesting the samples with a mixture of concentrated HNO_3 and HCl (*aqua regia*). The concentration of trace elements thus obtained provided an estimate of their total (also called pseudo-total) content in the soil (Vidal et al. 1999).

To evaluate the soil pollution severity, we used the pollution load index (*PLI*) as defined by Tomlinson et al. (1980). This index is based on the concentration factor (*CF*) of each metal in the soil. The *CF* is the ratio obtained by dividing the concentration of each metal in the soil by the base line or background values (the concentrations of As, Cu, Pb and Zn for 'normal' soil according to Bowen (1979) were used). For each sampling site, *PLI* is calculated as the *n*th root of the product of the obtained *nCF*. Values of *PLI* close to one indicate heavy metal loads near the background level, while values above one indicate soil pollution (Cabrera et al. 1999).

2.5. Mapping elements in seeds by PIXE

Compositional analysis and mapping of the main elements in white poplar seeds from the most contaminated site (DO) were obtained by Particle Induced X ray Emission (PIXE). The elemental analysis was carried out using a 3 MV tandem accelerator, described in detail elsewhere (García-López et al. 2000). The acquisition setup is based on an Oxford microbeams end-station OM2000 and on the Oxford Microbeams DAQ system (Grime et al. 1991), respectively. The samples were irradiated with a 2.8 MeV proton beam of size 4*4 μm^2 and a beam current of ~800 pA. A retractable Gresham Si (Li) detector (active area 80 mm^2, resolution 145 eV) with a 50 µm thick Mylar filter placed at 135º was used, together with a Titan

amplifier, for PIXE measurements. Elemental maps were done in scanning mode using a field 300*300 μm^2.

2.6. Statistical analysis

The differences between sites for trace element concentrations in soils and fruits, morphological characteristics of the trees and growth parameters *in vitro* were analysed by ANOVA considering the site as factor. The normality of the data was tested prior to analysis. Significant differences in all variables between the different sites were established by post-hoc Tukey's test. All statistical analyses were carried out with the program SPSS 15.0 for Windows.

3. Results and discussion

Soil analyses confirmed the high concentrations of non-essential elements in the contaminated DO and SO sites compared with the non-contaminated RHU site. In particular, the "pollution load index" (PLI) was 36 fold greater in the DO site than in the RHU site. The high mean concentrations of trace elements in the DO site were remarkable: 344 mg kg^{-1} As, 2.6 mg kg^{-1} Cd and 759 mg kg^{-1}Pb (see comparison with normal values in Table 1).

Despite the work of cleaning-up and remediation after the mine-spill, most environmental reports on the Guadiamar Green Corridor have documented residual contamination by trace elements in soils, being very irregular in distribution and intensity (Domínguez et al. 2008, 2016).

The high total concentrations of As, Cd, Cu, Pb and Zn in soils of the contaminated DO and SO sites are potentially toxic for most plants (see Ross 1994 and Table 1). However, the effective toxicity will be caused only by the bioavailable fraction of the trace elements taken up by the plants (Burgos et al. 2013; Ciadamidaro et al. 2013). Given the low soil pH ($\leq$5) in the DO and SO sites we expect a high bioavailability of cationic metals (Domínguez et al. 2010b). Previous surveys in this area have proved that under acidic conditions the solubilisation of the more mobile metals, Cd and Zn, may reach as high as 10–15 % of their total concentrations, which in the case of the more contaminated DO site would suppose soluble concentrations in the ranges of 0.2–0.4 mg kg^{-1} (Cd) and 70–100 mg kg^{-1} (Zn), excessive for most plants (Domínguez et al., 2016).

3.1. Seed germination

Germination is perhaps the most sensitive stage in the life cycle of a plant, and there is no consistent test or measurable parameter valid for all possible conditions at the time of sowing, with *in vitro* germination tests being in general of little predictive value for field growth of plants (Naylor and Hutcheson 1986). However, it is conceivable that if certain toxic elements

are accumulated in fruits and seeds to a threshold level they could adversely affect plant germination under any conditions.

Nearly all the seeds (more than 90%) germinated in water after 24 hours, irrespective of the origin and contamination environment of the mother tree (Table 2). Seed germination *in vitro* was also completed by seeds from the three sites before 72 h.

These results refuted our hypothesis that seeds from trees grown in contaminated soils would have a reduced and retarded germination rate compared with those from non-contaminated soils. Under a condition of Cd hyperaccumulation in seeds of *Thlaspi praecox* collected from highly polluted soils, a consistent reduction in seed biomass and germination, and increased dormancy, was observed (Kachenko et al. 2009), which seems not to be the case in the poplar seeds studied here.

3.2. Seedling growth and vigour

In the *in vitro* situation the greatest plantlet growth was from the DO and RHU sites at both 72 hours and at the end of experiment (30 days, Table 3), with stem lengths that were statistically similar and significantly greater than those of plantlets from the SO site.

Seedlings from poplar trees in the RHU (non-contaminated) site grew larger (6.4 mg) during 30 days in nutrient solution compared with contaminated site SO seedlings (2.2 mg) (Table 3). However, there were no significant differences between RHU and the other contaminated site DO (Table 3). This latter result again refutes our hypothesis that seedlings from a contaminated origin would have less growth than those from the non-contaminated site.

3.3. Chemical composition of seeds

The chemical composition of the seeds (of very small size) was estimated by analysing seedlings just germinated in water (Table 4). Cadmium concentrations were higher in seeds from contaminated sites (around 3 mg kg^{-1}) than from the non-contaminated site (0.3 mg kg^{-1}). These results indicate that a notable proportion of Cd can reach seeds; despite this, germination was not affected, according to the results in Table 2. It is possible that a portion of the Cd is complexed with thiol-containing compounds as reported by (Mendoza-Cózatl et al. 2011) for different plants, a matter of study that must be confirmed in the case of *Populus*.

With regards to the elements Cu and Zn, which had higher concentrations in contaminated soils (Table 1), it is remarkable that there were no significant differences in their concentrations in poplar seeds (Table 4). In this case, being micronutrients, they could be accumulated in seeds, even when the soil concentration is lower, like in the non-contaminated site. The case of Pb is different, because it is non-essential and toxic, and could be excluded from the seeds of poplars growing in contaminated sites to levels significantly different from those in the non-contaminated site (Table 4). In general, most Pb taken up by plants is

accumulated in the roots and not translocated into above-ground parts; that is, entry of Pb into the food web tends to be impeded by the 'soil-plant barrier' (Chaney, 1989). This was proven by Madejón et al. (2003) in sunflower; there was no significant accumulation of Pb in seeds, flower heads and leaf petioles in plants from polluted soils compared with non-polluted plants.

Macronutrient elements showed similar concentrations in seeds from the three sites, apparently not being affected by the soil contamination of the mother tree. Phosphorus concentration in seeds (1.6–1.9 g $100g^{-1}$) was richer than in fruits (average of 0.6 g $100g^{-1}$; 0.6–0.7 in 2011, Madejón et al., 2013), indicating preferential allocation to seeds.

3.4. Distribution of elements in seeds

The distribution of different elements in poplar seeds from a contaminated site was studied by Particle Induced X ray Emission (PIXE) and results are presented in Figure 1. These types of X-ray spectrometry techniques are increasingly used because classical analysis require expensive equipment, highly trained analysts, contamination free reagents and extensive sample preparation (Vogel-Mikuš et al. 2007). For example, micro-PIXE technique has been employed for elemental localization within fruits, seeds and vegetative tissues of metal hyperaccumulating plants (Mesjasz- Przyby1owicz et al. 1997, 1999; Bhatia et al. 2003, 2004; Przyby1owicz et al. 2004).

It is well known that nutrients for seed formation are mainly transported from the leaves via phloem, which may also deliver certain metals. However, sequestration of metals in the vacuoles of root and shoot tissues of metal tolerant plants can restrict this movement to the seeds, where metal concentrations may thus be comparatively low (Vogel-Mikuš et al. 2007). This could be the case in poplar seeds, where the trace elements Al and Zn seemed to show low concentrations and uniformly distributed. Restriction of Zn uptake into the seeds has also been detected by micro-PIXE in other plants, e.g., the hyperaccumulator *Thlaspi praecox* (Vogel-Mikuš et al. 2007).

Cadmium in seeds was not detected by this technique. It has been proven as effective and robust for very high Cd concentrations in seeds, e.g., up to 4.5×10^3 mg kg^{-1} (dry weight) in cotyledons of the hyperaccumulator *Thlaspi caerulescens* (Kachenko et al. 2009). However, in this case Cd concentrations were below the detection limit.

Nutrient and metal distribution in seeds can vary depending on the plant species, the element involved and seed anatomy (Kranner and Colville 2011). Thus, there is no single pattern for all plants. In the seed of contaminated poplar nutrients like P and S were mostly concentrated in the cotyledons whereas Ca and K were found in the whole seed, including the coats (Fig 1). In *Amaranthus hypochondriacus* L. seeds P, K, and Mg were exclusively localized in embryonic tissues whereas Ca was mostly present in seed coats and the boundary between the perisperm and embryo, suggesting that Ca is associated with the pectins that

constitute the network structure of the cell wall (Konishi et al. 1998). This seems to corroborate the importance of Ca as a constituent of cell walls.

3.5. Chemical composition of fruits

A consistent accumulation of metals such as Cd and to a lesser extent Zn in the seeds (Table 4) could derive from a consistent transfer of these elements from the soil to the leaves and the reproductive structures. Trace elements in the fruits certainly responded to the soil contamination in the case of Cd and Zn, with the highest significant concentrations at the DO site compared to the RHU site (Fig. 2). The high concentration of trace elements in soils of the DO site and the low pH must induce a high availability of metals that are transferred to plants, including their reproductive structures. Compared with the previous monitoring in 2011 (two years before; Madejón et al. 2013) slightly higher values were found, due to the natural variability.

Concentrations of Cd and Zn in *P. alba* fruits were relatively high in comparison with fruits of other plant species growing in a soil with the same level of pollution. In the case of sunflower (*Helianthus annuus*) fruits, Cd concentrations were 0.24 mg kg^{-1} and 112 mg kg^{-1} for Zn (Madejón et al. 2003) and for *Hirschfeldia incana* fruits they were 0.18 mg kg^{-1} and 74.8 mg kg^{-1} for Zn (Madejón et al. 2007). These concentrations were much lower in the case of the tree holm oak (*Quercus ilex*): their fruits accumulated concentrations of 0.02 mg kg^{-1} Cd and 20 mg kg^{-1} of Zn (Madejón et al. 2006). These results reinforce the fact that *P. alba* fruits can accumulate higher amounts of both elements in comparison with other species. This could be due to a comparatively more efficient translocation from root to shoot and further phloem transport of metals to poplar seeds, and also to their small size and smaller amounts of sugars and oils, which may increase metal concentrations in comparison to other species, an aspect that requires being studied in detail.

Comparing the "relative accumulation" of different trace elements in poplar fruits, calculated as the metal concentration in contaminated fruits divided by that in the 'control' fruits, the highest value was for Cd, up to 15, and the second highest for Zn, up to 3. These results confirm the capacity of *Populus alba* to uptake and transfer especially Cd and, to a lesser extent, Zn from leaves to fruits (Madejón et al. 2004, 2013). The further transfer to seeds seemed to be somewhat lower, according the data of Table 4.

There were no effects of soil contamination on the concentrations of Cu and Pb in poplar fruits (Fig 2, although Cu in the SO site was higher) or in the further transfer to seeds (Table 4). Both elements, Cu and Pb, were abundant in the mine sludge contaminating the soils (Cabrera et al. 1999); however, plant monitoring throughout the affected zone has shown only low transfer of Cu and Pb from soil to plants of different species (Madejón et al. 2002; Domínguez et al. 2008).

The concentrations of Al and Mn in poplar fruits differed between the two contaminated sites. Fruits from the DO soils showed significant higher Al and Mn concentrations than in the contaminated SO soil (Fig 2). Neither element was enriched in the mine spill; concentrations of Mn were even higher in RHU compared with SO soil, although the differences in soil were not significant (Table 1). Moreover, both elements are known for their high availability in acid soils. In general, Mn concentrations in plants show a negative relationship with increasing soil pH; on the other hand, high levels of Al are related to increasing levels of Mn, among other trace elements (Kabata-Pendias and Pendias 2001).

Nutritional levels of poplar fruits (Fig 2) showed that soil contamination did not impair the nutritional status of the trees. Concentrations of N, Ca, K and Mg in fruits were similar at the three sites. Moreover, fruits from contaminated sites were richer in P and S than in those from the non-contaminated site. These values are in accord with those reported in a previous monitoring of white poplar (Madejón et al. 2013). A certain 'fertilizing' effect of the slurry, especially noticeable in the case of P, was reported earlier by Murillo et al. (1999) when studying the crops (sunflower and sorghum) that were initially affected by the accidental spill.

On the other hand, the greater S concentration in polluted fruits may be a reflection of the high S concentration in the spill-affected soils (Table 1) close to the riverbank, where S solubilisation may reach values up to 15% of the total content (more than 1000 mg kg^{-1} in the soil solution) (Domínguez et al., 2016). Plants are rather insensitive to high sulphate concentrations in the soil medium (except in the case of saline soils) and, when the sulphate taken up exceeds the demand for S for the synthesis of organic compounds, it can be stored in plant tissues without much damage (Marschner, 1995).

In the presence of heavy metals, S accumulation may facilitate the synthesis of S-compounds like thiols, which in turn facilitate their safe storage. Sulphur uptake and assimilation are crucial for the synthesis of cysteine (Cys), a precursor of glutathione (GSH) biosynthesis. GSH, a non-protein thiol, acts as an important antioxidant in mitigating Cd and other heavy metal-induced oxidative stress. It also plays an important role in phytochelatin and metallothionein synthesis, which has a proven role in the detoxification of different heavy metals (Grill et al., 1987; Prasad, 1999; Mendoza-Cózatl et al., 2011; Gill and Tuteja, 2011; Gilabel et al., 2014). In fact, somewhat greater transfer of S to seeds was detected in the contaminated sites, compare to the control site, although differences were not significant; as point out before, this element was then mostly confined in the cotyledons, basic structures for the photosynthesis of the plantlets.

Although in the case of *Populus* the metabolic response to high concentrations of heavy metals such as Zn and Cd is still not clearly understood (Romeo et al. 2014), results reported by Di Baccio et al. (2005) and Jakovljević et al. (2014), among others, suggest that besides various

physiological processes, glutathione metabolism is involved in the adaptive response of this plant.

4. Conclusions

The first results indicated that *P. alba* can accumulate Cd in their seeds, showing that this tree has no complete "barriers" impeding toxic elements, such as Cd, from reaching the seeds. It was not possible to determine the precise location of the Cd within the seed due to its low concentration, below the detection limit of PIXE techniques. Concentrations of nutrients in seeds from contaminated sites did not reflect any nutritional unbalance.

Despite the higher Cd concentration in seeds from contaminated sites germination and seedling vigour were not affected. These results seem to indicate a noticeable tolerance of *Populus* to the presence of Cd and Zn in their reproductive structures, including seeds, which did not affect the seed germination and, possibly, further seedling growth. It is necessary to stress on the preliminary nature of some of these results, which should be confirmed in further studies. In particular, it would be very interesting to know how this species copes with a consistent concentration of Cd in its seeds (for example by a specific allocation) and regenerates when grown in a contaminated area.

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Table 1.Total concentrations of trace elements of the soils (0-25 cm depth, mean values ± standard error, N=5). PLI is pollution load index; DO, SO and RHU are abbreviation of the site names (see Materials and Methods).

Site	pH	As (mg kg^{-1})	Cd (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	S (g Kg^{-1})	PLI
DO	4.2 ± 0.9 b	344 ± 116 a	2.57 ± 1.70 a	261 ± 54 a	497 ±69.5 a	759 ± 437 a	725 ± 463 a	10.5 ± 5.2 a	9.46
SO	5.0 ± 0.5 ab	168 ± 26 a	1.06 ± 0.38 a	116 ± 20 b	375 ± 49.8 a	284 ± 32 a	375 ± 110 a	6.23 ± 1.31 a	4.21
RHU	6.8 ± 0.1 a	8.37 ± 0.31 b	0.01 ± 0.002 b	21.1 ± 0.95 c	527 ± 22.5 a	16.7 ± 1.05 b	61.8 ± 3.9 b	0.11 ± 0.01 b	0.26
UNA GC*		18.9	0.33	30.9	678	38.2	109	-	
NS**		6	0.35	30	1000	35	90	-	
TP***		20	3-8	60-125	1500-3000	100-400	70-400	-	

*UNA GC: Values for unaffected soils along the Green Corridor (Cabrera et al., 1999)

** NS: Values (median) in normal soils (Bowen, 1979)

*** TP: values toxic for plants (Ross, 1994)

Table 2. Germination of *Populus* seeds in water (Petri dishes) and in nutritive solution (*in vitro* culture). Mean percentage values (%) for each site.

	In water		In nutritive solution
Site	24 h	72 h	72 h
DO	94 ± 6 a	95 ± 4 a	100 ± 0 a
SO	91 ± 6 a	95 ± 2 a	100 ± 0 a
RHU	90 ± 1 a	93 ± 0 a	100 ± 0 a

Table 3. Growth parameters of *Populus* seedlings cultivated in nutrient solution (*in vitro* culture) during 30 days. Mean values. Values followed by the same letter in the same column do not differ significantly ($p<0.05$).

Site	Stem length (cm)		Fresh weight (mg)	Dry weight (mg)
	(72 h)	(30 days)		
DO*	0.26 b	1.79 b	90 b	5.0 b
SO*	1.08 a	0.55 a	32 a	2.2a
RHU*	0.21 ab	1.77 b	89 b	6.4b

*Abbreviation of the sites names in materials and methods

Table 4 Concentrations of nutrients and trace elements in water-germinated seedlings (mean values ± standard error; n= 3).

Site	Trace elements (mg kg^{-1})				
	Cd	**Cu**	**Mn**	**Pb**	**Zn**
DO	2.78 ± 0.54 b	34.7 ± 1.46 a	62.9 ± 9.32 a	1.15 ± 0.07 a	287 ±28.1 a
SO	3.40 ± 0.94 b	42.0 ± 11.8 a	32.6 ± 3.04 a	1.89 ± 0.75 a	300 ± 57.1 a
RHU	0.30 ± 0.08 a	36.4 ± 5.68 a	40.1 ± 8.60 a	2.02 ± 0.17 a	225 ±14.3 a

Site	Nutrients (g 100 g^{-1})				
	Ca	**K**	**Mg**	**P**	**S**
DO	0.91 ±0.02 a	1.08 ±0.13 a	0.52 ± 0.03 a	1.59 ± 0.14 a	0.46 ± 0.03 a
SO	0.76 ± 0.09 a	1.21 ±0.25 a	0.64 ± 0.11 a	1.78 ± 0.55 a	0.50 ± 0.07 a
RHU	1.06 ± 0.14 a	1.23 ±0.01 a	0.55 ± 0.05 a	1.94 ± 0.19 a	0.44 ± 0.04 a

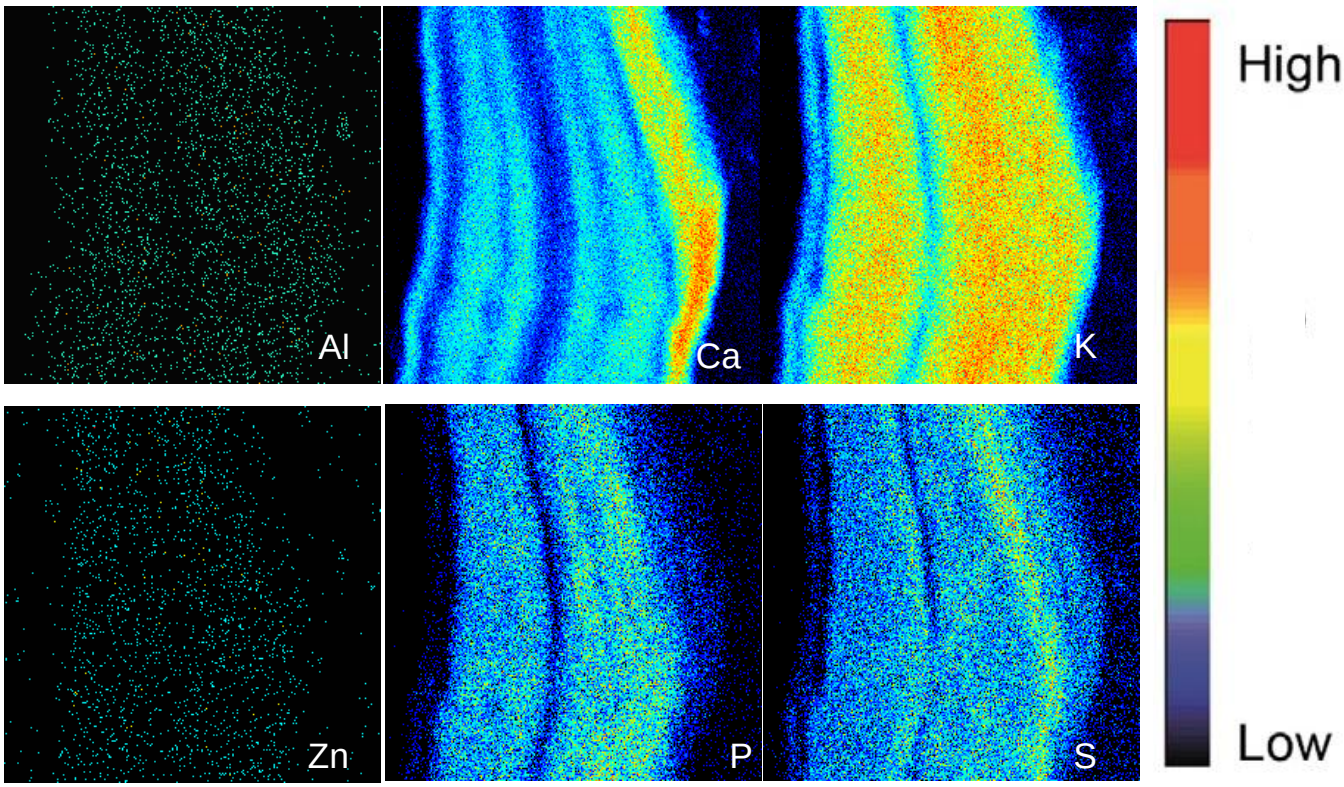

Figure 1. Bidimensional map of the distribution and relative concentration of different elements in the poplar seeds from the more contaminated DO site determined by Proton Induced X-ray Emission. Mapping signal is shown as a gradient of concentration.

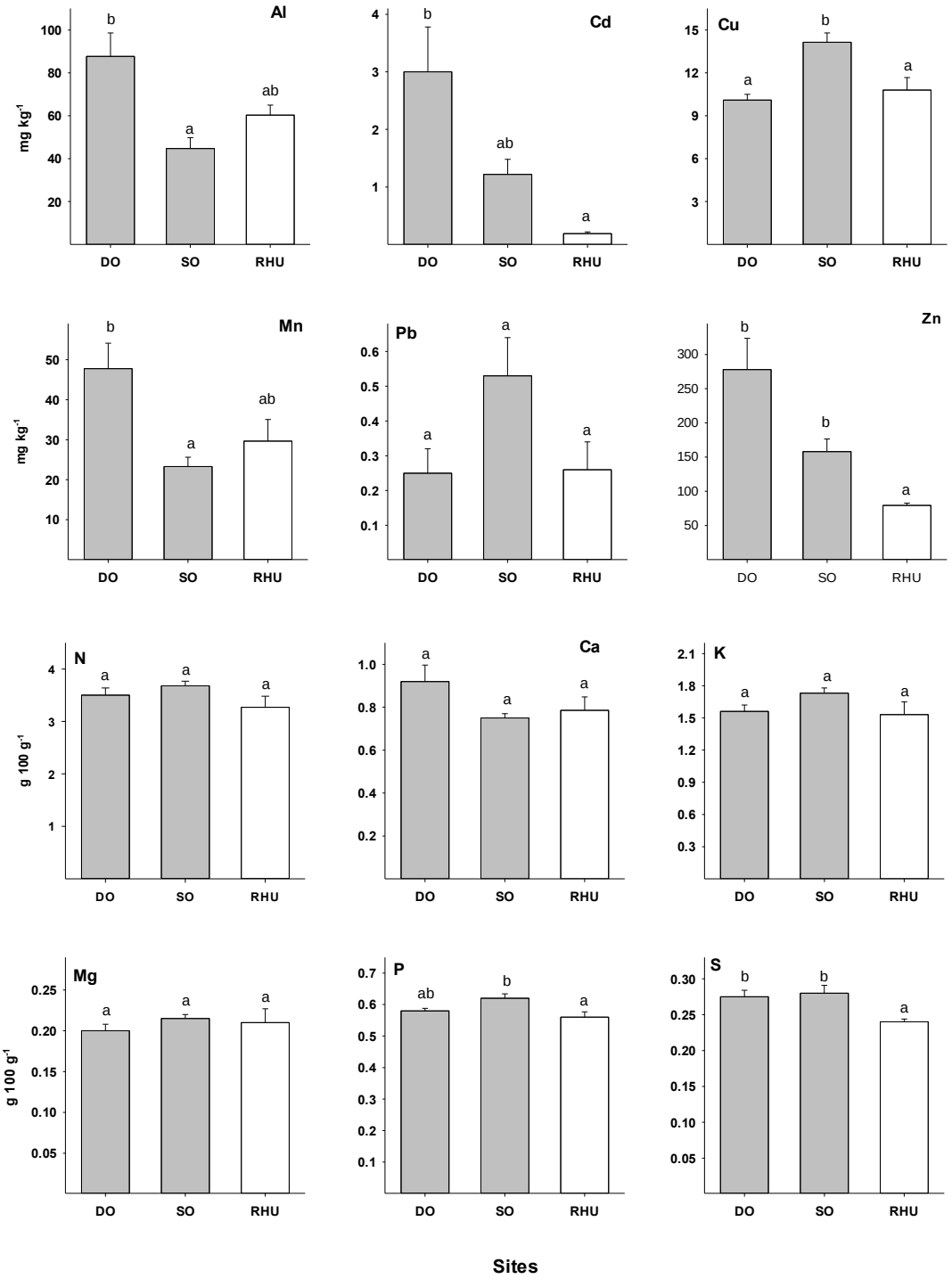

Figure 2.Trace element and nutrient concentrations of *Populus alba* fruits collected at the three sites (RHU was non-contaminated whereas SO and DO were contaminated by a mine spill). Mean values ± standard error. Columns with the same letter do not differ significantly ($p<0.05$).