



Mercury uptake into poplar leaves



Mohamad Assad^a, Julien Parelle^b, David Cazaux^c, Frédéric Gimbert^b, Michel Chalot^{a,d}, Fabienne Tatin-Froux^{b,*}

^a Laboratoire Chrono-Environnement, UMR CNRS 6249, Université de Bourgogne Franche-Comté, Pôle Universitaire du Pays de Montbéliard, 4 Place Tharradin, BP 71427, 25211 Montbéliard, France

^b Laboratoire Chrono-Environnement, UMR CNRS 6249, Université de Bourgogne Franche-Comté, 16 Route de Gray, Besançon Cedex, 25030 France

^c Solvay Electrolyse France, Tavaux Cedex, 39500 France

^d Université de Lorraine, Faculté des Sciences et Technologies, BP 70239, Vandoeuvre-les-Nancy, 54506 France

HIGHLIGHTS

- Poplar cuttings were grown on Hg-contaminated or control substrates.
- The experimental design allowed discrimination between root and leaf uptake.
- Mercury accumulation is dependent on leaf age.
- Uptake of Hg by poplar occurs exclusively through the atmospheric pathway.
- Experimental factors affecting Hg accumulation pathway in plant leaves are reviewed.

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ABSTRACT

Tailings dumps require mercury stabilization to prevent air pollution by evaporated mercury, which can be achieved through plant covers. Plants are considered a net sink for atmospheric Hg via incorporation into leaf tissues. However, most studies related to Hg uptake by plants have considered plants exposed to only atmospheric Hg, whereas in the case of tailings dumps, plants are potentially exposed to both soil and atmospheric Hg. The goal of this work is to evaluate the relative contributions of root and atmospheric pathways by growing poplar (*Populus trichocarpa* X *Populus maximowiczii*/var *Skado*) cuttings on either control or polluted substrates and under either natural or controlled exposure conditions. We showed that foliar Hg concentrations significantly increased with age, reaching 120 ng g⁻¹ dry mass when poplars were exposed to Hg-contaminated substrate under natural exposure. Remarkably, we did not observe significantly different Hg concentrations in poplar leaves grown on either the control or polluted substrates when cultivated together in growth chambers. Our set of data prompted us to conclude that Hg entry into poplar leaves is exclusively through an atmospheric pathway. Our results are discussed in line with existing literature.

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1. Introduction

It is estimated that more than 300 million tons of mining and quarrying waste is generated annually in the European Union according to a reference document elaborated by the European Commission (European Commission, 2009) as a follow-up to the tailings dam bursts that occurred at Baia Mare in Romania and

Aznalcóllar in Spain. After the dams were abandoned, these large dumps that store industrial tailings can generate significant unvegetated surfaces, which suggests biologically unfertile areas. Chlor-alkali industries have generated mercury-contaminated wastes (Xu et al., 2015) and sludge during the past half-century, where mercury-contaminated wastes have been excavated from sewage sludge sediments and then stocked in licensed tailings dumps. Such tailings dumps are equipped with an impermeable membrane to prevent transfer of mercury by lixiviation to the environment (see Mukherjee et al., 2004 for a review). The global biogeochemical cycle of Hg is dynamic and complex because Hg can be readily

* Corresponding author.

E-mail address: fabienne.tatin-froux@univ-fcomte.fr (F. Tatin-Froux).

transported through the atmosphere and cycle through terrestrial and aquatic ecosystems. Elemental gaseous mercury (Hg^0) is the principal form of mercury in the atmosphere, where Hg^0 is extremely volatile and can be oxidized photochemically to mercuric ion (Hg^{2+}) and methylated by microorganisms (Bacci et al., 1994; Guimarães et al., 1998). Hg evaporates easily as Hg^0 species due to its low boiling point (357°C at atmospheric pressure, Mukherjee et al., 2004). Soil gaseous mercury depends on the organic matter content, redox potential, and temperature (Moore and Castro, 2012). Moreover, recent studies showed a daytime Hg^0 emission and a night-time deposition linked to the photochemical cycle and evasion from litter fall (Obrist et al., 2014). A recent paper summarized the natural and anthropogenic sources that have contributed to the increase of Hg concentration in soil and reviews major remediation techniques and their applications to control soil Hg contamination (Xu et al., 2015).

Tailings dumps require mercury stabilization to prevent air pollution by evaporated mercury (Lee and Lee, 2012). A spontaneous recolonization by endogenous plant species is often observed after sediment deposition has ceased, which may impact the local cycling of mercury. Stamenkovic and Gustin (2009) showed that plants are considered as a net sink and that atmospheric mercury is incorporated into the leaf tissue. On the basis of their work, we hypothesized that the plant cover offered by a poplar plantation would intercept part of the volatile mercury, which has been observed for other metals/tree combination (Fantozzi et al., 2013). The use of an aided revegetation strategy would therefore be an appropriate and cost effective method to stabilize the mercury and prevent putative outputs to the local environment and threats to local populations. Molina et al. (2006) hypothesized that variations in Hg uptake by a vegetation cover depend on a large number of factors, including the Hg contents in the soil and atmosphere, plant species, organs, and season. However, results on uptake pathways into plant biomass are also unclear because the data reported in the literature have been obtained from various and mostly artificial exposure conditions using different Hg levels and various plant species.

In the present study, we combined field and laboratory approaches to determine Hg uptake pathways in leaves of *Populus trichocarpa* X *Populus maximowiczii*/var Skado. More precisely, the goal of this work is to evaluate the relative contributions of root and atmospheric pathways by growing poplar cuttings either on control or polluted substrates, which thus emphasizes natural rather than artificial exposure. The age-related accumulation patterns and possible Hg uptake pathways are discussed in detail and compared with the scientific literature, which takes into account the source of variations of the conclusions.

2. Materials and methods

2.1. Site description

The polluted site was a tailings dump located at Saint Symphorien-sur-Saône in the Bourgogne region in France (lat. $47^\circ 5' 5.985''$ N – long. $5^\circ 19' 44.0322''$ E), which has a total surface of 12 ha. The region is characterized by an annual average temperature of 11°C and 75% moisture. From the 1950's to 2003, this site was exploited as a storage area for sediments from the adjacent sedimentation basin. These sediments originated from effluents produced during electrolytic processes based on an Hg cell chlor-alkali process used to produce chlorine until 2012. The tailings dump was confined by 5-m high dikes to preserve the surrounding environment and is composed of a multi-contaminated calcareous and alkaline anthropogenic soil (Zappellini et al., 2015). The control site was located at Montbéliard-France (lat. $47^\circ 29' 42.9''$ N – long. $6^\circ 48' 9.684''$ E), distant from the polluted site of 140 km.

2.2. Field experiment

Cuttings of *Populus trichocarpa* x *Populus maximowiczii*/var Skado were planted in May 2013 in 4-L pots either in control or in polluted substrates (Fig. 1a). The polluted substrate was collected from the polluted site after discarding the roots and surface vegetation cover and after homogenization of the first 20-cm layer. The control substrate was made of a loam (Brill substrate GmbH, Georgsdorf, Germany) and sand mixture (50:50, v:v). Substrate sub-samples were collected and freeze-dried to determine the Hg initial concentrations (T_0). Poplar pots were left for various lengths of time, i.e., 66 days at the polluted site and 142 days at the control site. Pots were left for a longer period at the control site to reach detectable levels of Hg in the leaves. A leaf age of one day was defined when a leaf has a foliar index equal to three (corresponding to expanded leaf), as defined by Larson and Isebrands (1971). Leaf ages are provided in the result section.

2.3. Growth chamber experiments

For the second set of sequential experiments, poplar Skado cuttings were planted in 3-L pots either in control or in polluted substrates (Fig. 1b) and incubated in a growth chamber. The first experiment began in March 2014, which consisted of growing poplar cuttings in control substrates, as detailed above except that 9 g of osmocote fertilizer (N/P/K 11/11/18, osmocote exact, Heerlen, Netherlands) was added to each pot at the beginning of the experiment. Poplar cuttings were grown for 45 days, and the second experiment began immediately after in April 2014, which consisted of growing poplar Skado cuttings in either control or polluted substrates, as described above. Polluted and control pots were mixed in the growth chamber as detailed in Fig. 1b. Poplar cuttings were grown for 46 days. Climatic conditions for the growth chamber experiments were as follows: day illumination duration of 16 h; night time of 8 h; day temperature of 22°C ; night temperature of 18°C ; day and night humidity of 60%; and day illumination intensity of 100% with a photon flux density of $250\text{--}300 \mu\text{mol m}^{-2} \text{s}^{-1}$. Two to three leaves representative of different ages were sampled on each plant as described above. The Hg content of the growth chamber experiments were estimated using coal trap at the end of the experiments and found to be $2.190 \pm 0.356 \text{ ng m}^{-3}$ and $8.775 \pm 0.764 \text{ ng m}^{-3}$ for control and polluted chambers, respectively.

2.4. Hg analysis in substrate and biomass

The extraction of Hg from Hg-contaminated substrates was performed in a 0.5-g aliquot of each substrate sample via mineralization with 2 mL HNO_3 and 5 mL HCl in closed tubes placed in a block digestion system (DigiPREP, SCP Sciences, Courtaboeuf, France). A gradual heating mode was used to achieve a final temperature of 100°C (total run of 265 min). Then, ultrapure water (Millipore Milli-Q Integral 3 system, Molsheim, France) was added to a final volume of 50 mL. Finally, a filtration to $1 \mu\text{m}$ was performed. Hg concentrations were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, Radial ICAP 6500 Model, Thermo Fischer Scientific, Courtaboeuf, France), and all samples were analysed in triplicate and performed together with a certified reference material (loamy clay soil, CRM052, LGC Promochem, Molsheim, France, $2.40 \pm 0.125 \mu\text{g g}^{-1}$ dry mass, average recovery: 99%). The detection limit was 132 ng g^{-1} dry mass. The control substrates and freeze-dried leaves were analysed after milling using an advanced mercury analyser (AMA 254, Courtage Analyses Services, Mont Saint Aignan, France), and all samples were performed together with a certified reference

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