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Foliar or root exposures to smelter particles: Consequences for lead compartmentalization and speciation in plant leaves

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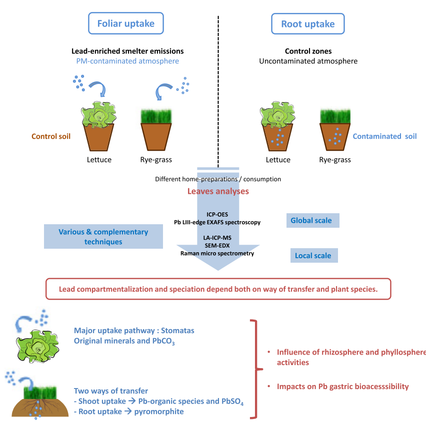
HIGHLIGHTS

- Plants were exposed to factory process particles via atmosphere or soil transfer.
- Pathways of Pb uptake were investigated using microscopy and spectroscopy.
- Contrary to lettuces, Pb speciation has changed in the leaf surfaces of rye-grass.
- In the case of root exposure, pyromorphite was formed in the leaves of rye-grass.
- Pb compartmentalization and speciation depend on transfer way and plant species.

GRAPHICAL ABSTRACT



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ABSTRACT

In urban areas with high fallout of airborne particles, metal uptake by plants mainly occurs by foliar pathways and can strongly impact crop quality. However, there is a lack of knowledge on metal localization and speciation in plants after pollution exposure, especially in the case of foliar uptake. In this study, two contrasting crops, lettuce (*Lactuca sativa* L.) and rye-grass (*Lolium perenne* L.), were exposed to Pb-rich particles emitted by a Pb-recycling factory via either atmospheric or soil application. Pb accumulation in plant leaves was observed for both ways of exposure. The mechanisms involved in Pb uptake were investigated using a combination of microscopic and spectroscopic techniques (electron microscopy, laser ablation, Raman microspectroscopy, and X-ray absorption spectroscopy).

The results show that Pb localization and speciation are strongly influenced by the type of exposure (root or shoot pathway) and the plant species. Foliar exposure is the main pathway of uptake, involving the highest concentrations in plant tissues. Under atmospheric fallouts, Pb-rich particles were strongly adsorbed on the leaf surface of both plant species. In lettuce, stomata contained Pb-rich particles in their apertures, with some deformations of guard cells. In addition to PbO and $PbSO_4$, chemical forms that were also observed in pristine particles, new

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species were identified: organic compounds (minimum 20%) and hexagonal platy crystals of $PbCO_3$. In rye-grass, the changes in Pb speciation were even more egregious: Pb–cell wall and Pb–organic acid complexes were the major species observed.
For root exposure, identified here as a minor pathway of Pb transfer compared to foliar uptake, another secondary species, pyromorphite, was identified in rye-grass leaves. Finally, combining bulk and spatially resolved spectroscopic techniques permitted both the overall speciation and the minor but possibly highly reactive lead species to be determined in order to better assess the health risks involved.

1. Introduction

Atmospheric contamination by airborne particles (PM) enriched with metals has increased in regions with intense industrial activity (Douay et al., 2008; Perrone et al., 2010; Polichetti et al., 2009; Pruvot et al., 2006) and different megalopoleis in the world, particularly in Asia, Africa, and Latin America (Moffet et al., 2008; Park and Dam, 2010; Sammut et al., 2010; Shi et al., 2012; Waheed et al., 2011). Health risks associated with these atmospheric contaminations may arise mainly from inhalation of particles and consumption of polluted vegetables (Morman and Plumlee, 2013). For example, in industrial areas, Pb concentrations in crop plant tissues were reported to be two orders of magnitude higher than the European threshold of 0.3 mg Pb kg^{-1} fresh weight (Caggiano et al., 2005; Gonzalez-Miqueo et al., 2010; Honour et al., 2009; Tomasevic et al., 2005).

Metals can be accumulated in plant leaves through both root transfer and translocation (Estrella-Gómez et al., 2009; Piechalak et al., 2002; Uzu et al., 2009; Yanqun et al., 2004; Zhang et al., 2008) and/or foliar transfer after deposition of atmospheric particles on the leaf surfaces (Schreck et al., 2012a, and references therein). According to the pollution context, the foliar transfer of metals can be neglected, or in contrast appears as the main pathway of pollution, particularly when ultra-fine particles interact with plant leaves (De Temmerman et al., 2012; Feng et al., 2011; Hu et al., 2011; Schreck et al., 2012a) and especially as lead has relatively low mobility in soil and is generally weakly phytoavailable by root uptake. Recently, working on lead uptake, Schreck et al. (2013) showed that root and shoot metal uptake pathways are independent, with an additive effect in terms of phytotoxicity. However, even if the foliar pathway is better known nowadays, mechanisms involved in metal uptake, transfer, and trafficking in leaf tissues are still less investigated (Zangi and Filella, 2012). The type of exposure (root or shoot) may have different impacts on both metal compartmentalization (distribution at the tissue and cellular scale) and speciation in plants (Birbaum et al., 2010; Lei et al., 2008; Raskin et al., 1994; Sarret et al., 2013) and consequently metal bioavailability and toxicity. Thus, supplementary studies are needed to compare the two pathways of exposure in terms of metal transfer, their localization and speciation in plant tissues, and finally their environmental and health impacts.

Thus, the aim of this present study was to compare Pb compartmentalization and speciation in crops cultivated in the vicinity of a secondary Pb smelter and contaminated by Pb-enriched particles via either atmosphere–plant or soil–plant pathways.

In order to highlight the involved changes potentially occurring in the phyllosphere or rhizosphere and then in the plant (with possible bio-transformations), two morphologically contrasting plant species were chosen: *Lactuca sativa* L. (lettuce, cultivar Batavia), with large, soft, frilly, and smooth leaves forming a loose leaf head, and *Lolium perenne* L. (rye-grass), with smooth and glossy leaves on the lower surface and parallel sides and prominent parallel veins on the upper surface.

These two plants are relevant models in terms of sanitary risk assessment, and are handled differently depending on their use and consumption. *L. sativa* L. is one of the most frequently self-grown vegetables, whereas *L. perenne* L. is consumed as fodder by cattle, with possible human impacts through dairy products and meat (Schreck et al., 2012a). Pb compartmentalization and speciation were studied at various scales, from macro- to micro- and molecular-level scales. After carrying out bulk inductively coupled plasma mass spectrometry (ICP-MS) analyses, Pb concentrations in leaf spots were determined by laser ablation (LA)–ICP-MS. Major Pb chemical forms were determined by Pb LIII-edge X-ray absorption spectroscopy (XAS), and Pb speciation at the microscopic scale was studied by scanning electronic microscopy coupled with energy dispersive X-ray microanalysis (SEM–EDX) and Raman microspectrometry (RMS). Pristine and aged particles from the secondary Pb smelter were also studied. To our knowledge, this is the first comparison of metal compartmentalization and speciation performed with an unprecedented combination of techniques as a function of the exposure pathway: soil versus atmospheric pollution, and in addition this study was done on plants from a field experiment.

2. Materials and methods

2.1. Description of the field exposure area and characterization of atmospheric fallouts

Plant exposure experiments were performed in the courtyard of a secondary Pb smelter that recycles car batteries, located in the southwest of France in the peri-urban area of Toulouse (43°38′12″ N, 01°25′34″ E). According to the French authorities (DREAL), the yearly emission of the smelter industry is 328 kg of total suspended particles. These emitted PMs have been previously characterized (Uzu et al., 2011). The size distribution of PM is mainly in the 1–100 μm range (89% by volume fraction) with 9, 50, 20, and 21% in the $PM_{>10}$, PM_{10}, $PM_{2.5}$, and PM_1 fractions, respectively. Pb is the major element in these atmospheric fallouts, comprising 33% of the total metal content, and its speciation was found to be mainly PbS, $PbSO_4$, $PbO \cdot PbSO_4$, and PbO (Uzu et al., 2011). During the vegetable exposure experiments, Pb concentration in industrial atmospheric fallouts was controlled in the smelter courtyard by collecting wet depositions in Owen gauges (Taylor and Witherspoon, 1972), following the procedure described by Gandois et al. (2010), Munksgaard and Parry (1998), and Schreck et al. (2012b).

2.2. Plant characteristics and exposure experiments

Two separate experiments, that is, foliar and root metal transfers, were performed on both lettuce and rye-grass.

For the *root transfer experiment*, plants were cultivated individually in 5-l pots each containing 4 kg of soil collected in the vicinity of the smelter facility. The physico-chemical properties of the soil were previously characterized: $pH_{water} = 8.5$, CEC = 6.9 $cmol^{(+)}$ kg^{-1}, amounts of soil organic matter and carbonates ($CaCO_3$) were respectively 6 and 4 g kg^{-1}. This soil was contaminated by metals and metalloids due to atmospheric deposition with a concentration of Pb of 2000 mg kg^{-1} of dried soil. These plants were cultivated under an unpolluted atmosphere. Twenty-five plants of each species were cultivated for six weeks.

For the *foliar transfer experiment*, 45 pots, containing one plant each, were placed for six weeks in the smelter courtyard and exposed to Pb atmospheric fallouts. The Pb concentration in soil was checked and found to be less than 25.5 ± 1.6 mg kg^{-1} of dried soil. A geotextile membrane was placed on the top of the soil to protect it from atmospheric fallouts and thus to avoid soil contamination and metal transfer