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Kinetic study of phytotoxicity induced by foliar lead uptake for vegetables exposed to fine particles and implications for sustainable urban agriculture

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ABSTRACT

At the global scale, foliar metal transfer occurs for consumed vegetables cultivated in numerous urban or industrial areas with a polluted atmosphere. However, the kinetics of metal uptake, translocation and involved phytotoxicity was never jointly studied with vegetables exposed to micronic and sub-micronic particles (PM). Different leafy vegetables (lettuces and cabbages) cultivated in RHIZOtest® devices were, therefore, exposed in a greenhouse for 5, 10 and 15 days to various PbO PM doses. The kinetics of transfer and phytotoxicity was assessed in relation to lead concentration and exposure duration. A significant Pb accumulation in leaves (up to 7392 mg/kg dry weight (DW) in lettuce) with translocation to roots was observed. Lead foliar exposure resulted in significant phytotoxicity, lipid composition change, a decrease of plant shoot growth (up to 68.2% in lettuce) and net photosynthesis (up to 58% in lettuce). The phytotoxicity results indicated plant adaptation to Pb and a higher sensitivity of lettuce in comparison with cabbage. Air quality needs, therefore, to be considered for the health and quality of vegetables grown in polluted areas, such as certain megacities (in China, Pakistan, Europe, etc.) and furthermore, to assess the health risks associated with their consumption.

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Introduction

Urban agriculture has been progressively developed in order to respond to the sustainable aims of cities. However,

environmental pollution needs to be assessed and reduced (Mombo et al., 2015; Pierart et al., 2015). Particularly, the proportion of metal(loid) micronic and sub-micronic particles (PM), including nanoparticles, has increased in the atmosphere

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with the expansion of urban areas and the development of industries (Austruy et al., 2014; Luo et al., 2011; Uzu et al., 2014; Zhao et al., 2012). According to Seinfeld (1986), natural sources of PM enriched with metals are generally coarse particles while the metals in fine and ultrafine PM are related to anthropogenic sources. Fine and ultrafine PM are often highly reactive due to their large surface-area-to-volume ratio (Barrie, 1992; Gillespie et al., 2013). These PM can interact with terrestrial ecosystems (Schreck et al., 2012a, 2014), waters (Diop et al., 2014; Gupta et al., 2014), soils (Stampoulis et al., 2009; Schreck et al., 2011; Shahid et al., 2011, 2013a) and plants (Uzu et al., 2010; Hu et al., 2011; Xiong et al., 2014a). Thus, metal(loid)s carried by PM can induce a sanitary risk linked to polluted plants (Polichetti et al., 2009; Perrone et al., 2010; Xiong et al., 2014b).

Owing to an increased use in numerous anthropogenic activities, lead is widely observed in all ecosystems at the global scale (Pourrut et al., 2011). Due to its strong (eco)toxicity and persistence, Pb is, therefore, considered by various European regulations, such as the REACH law. Fine PbO particles currently observed in the environment are highly reactive due to their low size ($<10\text{ }\mu\text{m}$) and high specific surface area ($29.2\text{ m}^2/\text{g}$) (Goix et al., 2014). According to Zia et al. (2011), food is the major source of human exposure to Pb due to possible Pb bioaccumulation in the edible parts of vegetables. Lead pollution of leafy vegetables can be caused both by root transfer from polluted soils (Ma et al., 2010; Yin et al., 2011; Lombi et al., 2011) and by direct foliar uptake (Honour et al., 2009; Uzu et al., 2010; Schreck et al., 2012a and 2012b). As atmospheric pollution has increased over the decades, foliar uptake is currently taken into account (Xiong et al., 2014a). But most studies focus on metal accumulation or shoot/leaf elongation (Little, 1978; Ward and Savage, 1994; Abbas and Akladios, 2012).

The PM-retention abilities of vegetables depend on several factors, such as leaf surface area, leaf longevity and cuticular structure (Freer-Smith et al., 1997; Barber, 2004; Rico et al., 2011; Schreck et al., 2012a).

Metals cause damage on plant leaves, stomata and leaf proteins (Pourrut et al., 2013; Xiong et al., 2014a). At a cellular level, Pb toxicity results in the overproduction of reactive oxygen species (ROS) (Shahid et al., 2014a, 2014b). Morphological and growth parameters showed a decrease in root and shoot growth (Schreck et al., 2011) and alterations in root branching pattern in *Lactuca sativa* L. after Pb treatment (Capelo et al., 2012). Physiological processes, such as photosynthesis and water status, are particularly sensitive to metals (Monni et al., 2001; Austruy et al., 2013; Mateos-Naranjo et al., 2012; Shahid et al., 2014c). Moreover, Le Guédard et al. (2012a) reported that leaf fatty acid composition is a considerable biomarker of the early effect of metals.

In this context, the kinetics of foliar transfer and Pb phytotoxicity was studied in controlled conditions with lettuce (*L. sativa* L.) and cabbage (*Brassica oleracea* L. var. *capitata* cv. *Snowball*), leafy vegetables, which are currently cultivated and consumed at the global scale. The vegetables were exposed to PbO fine particles; thus, metal accumulation, biomass (leaf elongation, aerial mass), gaseous exchanges (Pn, gs) and leaf fatty acid composition were measured through the function of exposure time.

1. Materials and methods

1.1. Experimental conditions and set up

Micro-culture was performed with a RHIZOtest® device (ISO 16198) in this study (Bravin et al., 2010). The device used hydroponic solutions for pre-culture (Fig. 1a). During this period, root and foliar growth were fast without contamination. In the test culture period (Fig. 1b), the device allowed soil–plant contact indirectly; roots separated from the soil by a membrane can absorb nutrients from soil. Thereafter, the different compartments of a plant can be analyzed separately. The nutrient solutions according to the phases of RHIZOtest® are present in Table S3. The experiments were carried out in a controlled chamber with a day/night temperature regime of $25 \pm 2^\circ\text{C}$ (16 hr)/ $20 \pm 2^\circ\text{C}$ (8 hr) and a light intensity of $425 \pm 50\text{ photons }\mu\text{mol}/(\text{m}^2\cdot\text{sec})$. The relative humidity was adjusted to $65 \pm 5\%$.

Leafy vegetables (lettuces and cabbages) are widely cultivated for human consumption and regularly grown in farms in China, Europe and other countries (Waisberg et al., 2004; Khan et al., 2008; Uzu et al., 2009; Schreck et al., 2012a). They have a short life cycle and large surface interception, features which are useful to investigate the atmospheric transfer of metals and, therefore, have been the subject of several studies of metal transfer (Monteiro et al., 2009; Cao et al., 2010; De Leon et al., 2010; Schreck et al., 2013). The experimental design consisted of 36 plants for each plant species, including three durations, three exposure quantities and four replicates.

After pre-culture, plants were grown for 2 weeks in a control unpolluted soil, which exhibited the following physico-chemical characteristics as described by Schreck et al. (2011): high contents of organic matter (44.7 g/kg), an optimum pH (6.5) and an average cationic exchange capacity ($12.3\text{ cmol}^+/\text{kg}$). The Pb concentration of the unpolluted soil was $25.5 \pm 1.6\text{ mg/kg DW}$ as described by Uzu et al. (2010) and Schreck et al. (2012a). Because this study focused on the foliar transfer of metals, a geotextile membrane was placed between root and shoot parts to protect the roots from PM fallouts and then to avoid metal transfer via root uptake (Hurtevent et al., 2013; Xiong et al., 2014a).

Vegetable leaves were then exposed to PbO particles using an applicator brush applied to the entire leaf surface (Xiong et al., 2014a). Note that the leaf surfaces were moistened with a hand spray first, and the brush was only used to deposit the PM onto the leaf surfaces without spreading. The homogeneity of the solution distribution on the leaves and the reproducibility of the technique were confirmed in previous tests (Xiong et al., 2014a). We used this method because the deposition of dry PM with an applicator brush without pre-wetting can mechanically bring the particles into the leaf and favor PM uptake (Xiong et al., 2014a). The PbO characteristics were as follows: CAS number 1317-36-8, PM size $<10\text{ }\mu\text{m}$, specific area $29.2\text{ m}^2/\text{g}$, purity $>99.99\%$ and a molecular weight of 223.19 g/mol . The metal foliar exposure was performed at 5, 10 and 15 days to allow PbO uptake by the plant leaves. Three treatments were defined based on the function of metal quantities deposited: a control condition without any metal input (0 mg) and 10 mg and 250 mg PbO inputs. These concentrations correspond to the Pb

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