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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.

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 μm) and high specific surface area (29.2 m^2/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 Akladious, 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 ± 2°C (16 hr)/20 ± 2°C (8 hr) and a light intensity of 425 ± 50 photons μmol/(m^2·sec). The relative humidity was adjusted to 65 ± 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 $cmol^+$/kg). The Pb concentration of the unpolluted soil was 25.5 ± 1.6 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 μm, specific area 29.2 m^2/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

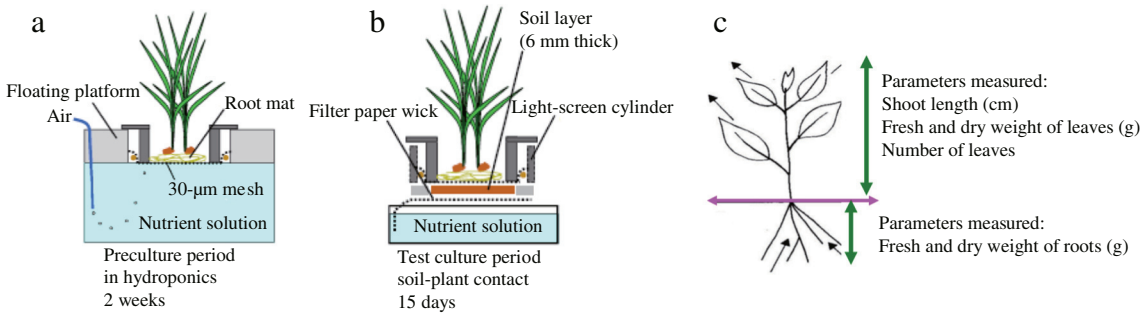


Fig. 1 – Steps for micro-culture by RHIZOtest® devices (a)and(b), and parameters used for biomass measurement (c).

concentrations reported in atmospheric fallouts near a secondary Pb smelter, which was 450 ± 7 mg/(m^2·month) (Xiong et al., 2014b), and concentrations occurring in a previous field experiment conducted by Schreck et al. (2013) in an industrial area (PM fallouts of 100 μg/(cm^2·week)). We define lettuces and cabbages exposed to 0 mg, 10 mg and 250 mg PbO as L_0, L_{10} and L_{250} and C_0, C_{10} and C_{250} respectively.

Furthermore, in order to precisely determine the quantity of pollutants deposited on the leaf surface, the applicator brush was rinsed with 20 mL of distilled water after PM deposition (Xiong et al., 2014a). The metal concentration of the rinse water was measured, and the final PM amounts actually deposited on the leaf surfaces were 0, 5.61 and 218.78 mg respectively for 0, 10 and 250 mg of PbO deposited with the brush.

1.2. Metal concentrations in plant leaves and roots

After harvest, root and shoot biomasses were separated and properly washed with deionized water based on a human ingestion scenario so as to remove particles deposited on the leaf surface (Birbaum et al., 2010; Uzu et al., 2010; Xiong et al., 2014a). The samples were first rinsed with deionized water, and then immersed for 10 min in deionized water and rinsed another time with deionized water (Hong et al., 2014). The root and leaf samples were oven-dried at 40°C to a constant weight (about 72 hr), ground and sieved to a <250 μm particle size. Mineralization of plant samples (0.125 g per sample) was performed in aqua-regia (1/4 HNO_3 and 3/4 HCl) at 80°C for 4 hr by a Digiprep® instrument (Xiong et al., 2014a). The digested samples were then filtered through 0.45 μm filters and stored at 4°C after volume adjustment. Total Pb concentrations in vegetable samples were measured by inductively coupled plasma-optical emission spectrometry ICP-OES (IRIS Intrepid II XXDL). The control method was performed with concurrent analysis of blank and standard reference materials (tobacco CTA-VTL2, peach leaves (1547)). Metal concentrations (mg/kg) are expressed on a dry weight (DW) basis.

Pb storage (μg) was calculated as:

$$\text{Pb storage} = C_{pb} \times M$$

where, C_{pb} (mg/kg) is Pb concentration, M (g) is plant dry weight.

Pb transfer kinetic (μg/day) is the dynamic change of metal storage capacity every day (Pb storage/exposure duration). Metal translocation factors (TF) were calculated as:

$$TF = [Pb]\ \text{roots}/[Pb]\ \text{leaves}$$

by dividing the metal concentrations in the root tissues ($[Pb]_{roots}$, mg/kg) by the metal concentrations accumulated in the aboveground tissues ($[Pb]_{leaves}$, mg/kg) (Marchiol et al., 2004; Soda et al., 2012): $TF = [Pb]_{roots} / [Pb]_{leaves}$.

1.3. Phytotoxicity

After exposure, plants were harvested every 5 days, defined as T_5, T_{10} and T_{15}, whereas the first day of exposure was defined as T_0. The exposure duration from one to 15 days was usually chosen for the study of metal accumulation as this period allows a significant metal uptake (Capelo et al., 2012; Xiong et al., 2014a).

1.3.1. Plant biomass

Plant biomass was then measured at T_0, T_5, T_{10} and T_{15}; it included the shoot length, the number of leaves, the fresh and dry weight of leaves and roots, the water content and the tolerance index (TI) of shoot growth.

Shoot length was measured from the bottom of the shoot to the endpoint of the highest leaf (Fig. 1c).

The plants were fresh weighted (FW) and oven-dried to determine DW; the percentage of water content was calculated according the fresh and dry weight of plants as Eq. (1):

$$\text{Water content} = \frac{FW-DW}{FW} \times 100\%. \quad (1)$$

TI evaluates plant tolerance against or susceptibility to the composite effects of metals. According to Mahmood et al. (2007), individual growth parameter data (G_x) were normalized (G_i) relative to the maximum value (G_{max}) of the parameter in the data set, $G_i = (G_x / G_{max})$. Summing all the G_i's and then dividing by the total number of G_i's calculated $TI = \Sigma(G_x / G_{max}) / n$. The TI of shoot growth ranged from 0 to 1, meaning highly tolerant when approaching 1 and extremely susceptible to the composite effects of heavy metals when approaching 0.

1.3.2. Fatty acid composition analysis

One centimeter square of fresh young leaf (secondary developed leaf) was collected for every sample and then immediately placed in screw-capped tubes containing 500 μL methanol acidified with 2.5% an H_2SO_4 solution. Samples were stored at 4°C before analysis. Fatty acid analysis and identification were performed according to Le Guédard et al. (2012a) and Schreck et al. (2013); leaf samples stored in acidified methanol were heated to 80°C for 1 hr, and then 1.5 mL of H_2O and 0.75 mL of hexane were added after cooling. Fatty acid methyl esters (FAMEs) were extracted into hexane by vigorous shaking, and a two-phase system was established by centrifugation (1500 g, 5 min). Separation of FAMEs in the hexane phase was performed by gas chromatography (Hewlett Packard 5890 series II) on a 15 m × 0.53 mm Carbowax column (Alltech) with flame ionization detection. The temperature ramp was performed (Le Guédard et al., 2009); the initial temperature of 160°C was held for 1 min, followed by a 20°C/min ramp to 190°C and a second ramp of 5°C/min to 210°C, and maintained for 6 min. FAMEs were identified by comparing their retention times with standards (Sigma Chemical, St. Louis, MO, USA).

In general, the lipid composition of leaves consisted of palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and alpha linolenic acid (C18:3). Lipid composition was dominated by polyunsaturated fatty acids, mainly represented by linolenic acid (C18:3), which accounted for 39.6–49.0% and 37.9–51.8% of the total fatty acid content in lettuce and cabbage respectively in our study.

The work done by Le Guédard et al. (2009, 2012a) showed that the fatty acid ratio (Eq. (2)) in plant leaves has indication characteristics of metabolic effect after plant exposure to metals and has been used to diagnose the impact of foliar metal uptake (Schreck et al., 2013). Moreover, the Z index, defined as the product of three fatty acid concentration ratios (C16:1/C16:0, C18:3/C18:0 and C18:1/C18:2, Eq. (3)) with different rates (1, 0.57, and 0.23 respectively), can be used when exploring the relationships between uptake and phytotoxicity (Schreck et al., 2013). Thus, a change in the ratio of lipids in leaves can be used as a biomarker of stress when plants are exposed to PbO PM by the foliar way.

$$\text{Fatty acid ratio} = \frac{C18:3}{C18:0 + C18:1 + C18:2} \times 100\% \quad (2)$$

$$Z = \left(\frac{C16:1}{C16:0}\right) \times \left(\frac{C18:3}{C18:0}\right)^{0.57} \times \left(\frac{C18:1}{C18:2}\right)^{0.23}. \quad (3)$$

1.3.3. Gas exchange parameters

Net photosynthesis (Pn) and stomatal conductance (gs), were recorded simultaneously using a portable infrared gas analyzer (LI-COR 6400 XT) for estimating the influence of metal stresses on photosynthesis. The infrared gas analyzer system (IRGAs) was equipped with a clamp-on leaf chamber that possessed 6 cm^2 of leaf area. According to Majer and Hideg (2012), IRGAs measure the reduction in transmission of infrared wavebands caused by the presence of CO_2 between the radiation source and a detector. The reduction in transmission is a function of the concentration of CO_2. Thus, the measurements of gas exchange are based on the differences in CO_2 and H_2O in an air stream that is flowing into a leaf cuvette (reference cell) compared to the air stream flowing out of it (sample cell). The rate of CO_2 uptake (μmol/(m^2·sec)) is used to assess the rate of photosynthetic carbon assimilation while the rate of water loss (mol H_2O/(m^2·sec)) is used to assess the rate of transpiration and stomatal conductance.

Gas exchange measurements were performed under irradiance at 425 μmol/(m^2·sec) and at a temperature of 23 ± 2°C. During measurement, humidity was fixed at 65%, and CO_2 concentration was maintained at a constant level of 380 μmol/mol using a LI-6400-01 CO_2 injector with a high-pressure liquid CO_2 cartridge source.

1.4. Statistical analysis

Total Pb concentrations in plants and the measured physiological parameters were subjected to analysis of variance (ANOVA) using Duncan's test at $p < 0.05$. The significances of the treatment effect were investigated by the use of 2-way ANOVA (duration × concentration). Data were tested for normal distribution and homogeneity of variance before the ANOVA analysis. The relationships between exposure conditions and different phytotoxicity parameters and the relationships among the parameters were analyzed by principal component analysis (PCA); «ρ» stands for the correlation coefficient between different parameters. Statistical analyses were carried out on the mean of four replicates for each exposure condition. Results are represented as mean ± SD (standard deviation).

2. Results

2.1. Foliar metal uptake and translocation towards roots in different plants and treatments

A significant Pb absorption, up to 3932 and 2039 mg/kg DW respectively for lettuce and cabbage (Fig. 2a) was observed. The uptake of metal by the two vegetables was very high compared to the control even during the first 5 days of exposure. From T_5 to T_{15}, the metal concentration increased rapidly in lettuce for the L_{10} and L_{250} treatments. Pb concentration was almost constant for the C_{10} and C_{250} treatments from T_5 to T_{10}. Lettuce showed a stronger metal absorption capacity than did cabbage.

The total Pb storage was variable and differed between the two plants during the exposure period (Fig. 2b). However, the final storage capacity at T_{15} was almost the same in lettuce and cabbage (1000 μg for L_{10} and C_{10}, 3000 μg for L_{250} and C_{250} respectively). The most significant increase of metal storage was observed from T_{10} to T_{15} in both plants (L_{250} and C_{250}).

The kinetics of Pb transfer increased in the first 5 days, from 0 to 23 μg Pb/day, 48 μg Pb/day, 67 μg Pb/day and 85 μg Pb/day for L_{250}, L_{10}, C_{10} and C_{250} respectively. In the second period of 5 days (from T_5 to T_{10}), the uptake rate was constant in L_{10}, C_{10} and C_{250}, but it significantly increased in L_{250} from 23 to 112 μg Pb/day. In the last 5 days (from T_{10} to T_{15}), transfer kinetics was significantly high in the highest PbO exposure group, 465 μg/day for L_{250} and 420 μg/day for C_{250} (Fig. 2c). Transfer kinetics is positively related to Pb storage ($\rho = 0.985$ in lettuce and $\rho = 0.934$ in cabbage) and Pb concentration ($\rho = 0.955$ in lettuce and $\rho = 0.973$ in cabbage) (Tables S1 and S2).

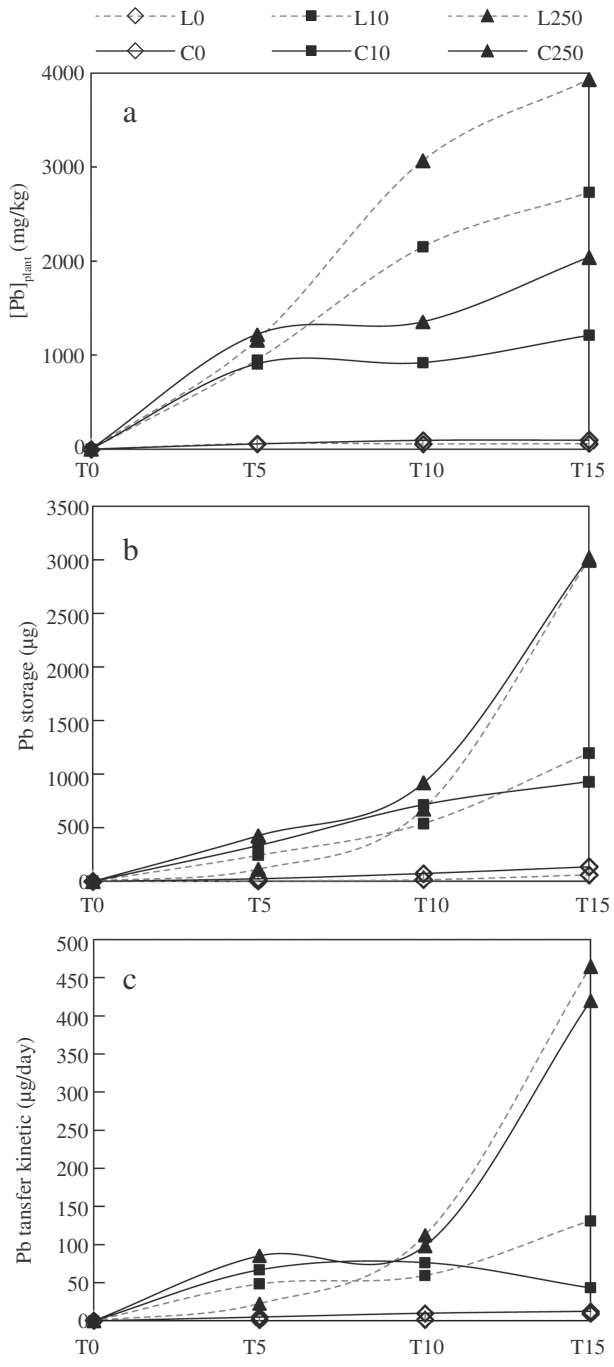


Fig. 2 – (a) Pb concentration ([Pb] $_{plant}$), (b) Pb storage and (c) Pb transfer kinetic in lettuces and cabbages exposed to PbO by foliar way. L0, L10 and L250 (and C0, C10 and C250) correspond to respectively 0, 10 and 250 mg of PbO deposited on lettuce (cabbage)) to various exposure durations (T0, T5, T10 and T15). Values are expressed as the mean of 4 replicates for each treatment.

Finally, the foliar transfer kinetics was quite similar in the two vegetables, and the absence of a saturation phenomenon was noticed (L_{250} and C_{250}).

Moreover, metal translocation factors (TF) from leaves to roots ranging from 0.06 to 0.21 were observed (Table 1). TF values were high during the first 5 days and then decreased with exposure duration.

2.2. Growth parameters for exposed plants

The shoot growth of lettuce and cabbage exposed to PbO is presented in Fig. 3. Lead phytotoxicity caused a significant reduction in shoot growth (in comparison to the control), about 49.9% and 23.6% respectively for lettuce (the more sensitive) and cabbage. In lettuce, the shoot growth in L_0 was about 0.55 cm (T_5), 0.875 cm (T_{10}) and 1.125 cm (T_{15}) while in L_{250}, shoot growth was reduced to 0.175 cm (T_5), 0.3 cm (T_{10}) and 0.4 cm (T_{15}), and the TI of lettuce dropped from 100% (control) to 31.8% (T_5), 34.3% (T_{10}) and 35.6% (T_{15}) respectively. The Pb phytotoxicity on shoot growth was dose and exposure time dependent (Fig. 3a).

In cabbage, the decrease of shoot growth was initially quick; during the first 5 days, a significant reduction was observed (from 100% (C_0) to 64.3% (C_{10}) and 60.7% (C_{250})). But, after 10 days, the decrease was quite moderate (Fig. 3b). The PCA analysis showed that the shoot growth of lettuce and cabbage is negatively related to PbO exposure quantity ($\rho = -0.781$ and -0.539 respectively for lettuce and cabbage) (Tables S1 and S2). This demonstrates high Pb toxicity to shoot growth.

The aerial biomass is presented in Fig. 3c. A significant difference of aerial biomass compared to control was only notable for T_{15} in lettuce and for C_{250}. The aerial biomass is significantly and negatively correlated to exposure quantity in cabbage ($\rho = -0.353$).

The water content ranges from 81% to 89% for lettuce and 67%–85% for cabbage; no significant differences were found among all treatments.

Obviously, cabbage acquiring a better shoot growth and aerial biomass than lettuce might indicate the better tolerance of cabbage. At the end of the 2 weeks of experiments, all the plants were alive. The shoot growth and aerial biomass of the two plants are positively correlated to exposure duration (shoot growth: $\rho = 0.341$ and 0.771 respectively for lettuce and cabbage; aerial biomass: $\rho = 0.909$ and 0.88 respectively for lettuce and cabbage), thus permitting plant growth even under high PbO stress (Fig. 6, Tables S1 and S2).

2.3. Gas exchanges at the leaf level after PbO exposure

Fig. 4 presents the results of Pn and gs. Photosynthesis activity showed a significant reduction after PbO exposure with different durations. In lettuce, at T_5, Pb caused a decrease of 25% and 58% in Pn respectively for L_{10} mg and L_{250}. For T_{10}, a 30% decrease in Pn was observed for L_{250}; no significant difference was found between L_{10} and the control (L_0). Finally, for T_{15}, 18% and 26% reductions were found respectively for L_{10} and L_{250}. The effect of the different levels of PbO exposure on Pn was more pronounced (linear) for T_5 compared to T_{10} and T_{15} (Fig. 4a).

The cabbage showed a different trend. Pn rates decreased significantly (about 50%) between control (C_0) and PbO treatments (C_{10} and C_{250}). No significant change was observed between C_{10} and C_{250} (Fig. 4a).

The gs value presented a notable decrease in the cabbage leaves at T_{10} and T_{15} (Fig. 4b). However, in lettuce leaves,

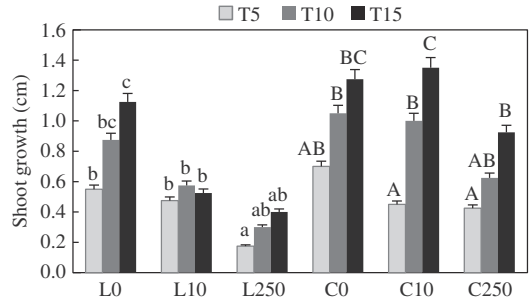
Table 1 – Mean Pb concentrations in roots and leaves of lettuces and cabbages and translocation factors (TF) from leaves to roots.

	Mean Pb concentration (mg/kg DW) and TF								
	T_5			T_{10}			T_{15}		
	Roots	Leaves	TF	Roots	Leaves	TF	Roots	Leaves	TF
L_0	60.4 ± 1.8^{a}	59.9 ± 0.8^{a}	1.01	57.9 ± 0.6^{a}	54 ± 1.7^{a}	1.07	62.7 ± 0.5^{a}	54 ± 0.4^{a}	1.16
L_{10}	333.1 ± 11.3^{b}	1568 ± 44.6^{c}	0.21	475.6 ± 15.7^{b}	3829 ± 27^{e}	0.12	310.2 ± 12.7^{b}	5149.4 ± 328.5^{f}	0.06
L_{250}	359.4 ± 14.4^{b}	1962.2 ± 34.3^{cd}	0.18	443.8 ± 15.1^{b}	5695.8 ± 108.1^{f}	0.08	472 ± 11^{b}	7392.3 ± 264.7^{g}	0.06
C_0	54.9 ± 1.8^{A}	56.5 ± 1.6^{A}	0.97	93.2 ± 5.3^{A}	96.2 ± 5.5^{A}	0.97	99.3 ± 3^{A}	94.1 ± 2.6^{A}	1.06
C_{10}	201 ± 5.1^{B}	1613.4 ± 38^{C}	0.12	282.8 ± 7.1^{B}	1558 ± 59.3^{C}	0.18	169.5 ± 6.0^{A}	2290 ± 92.4^{D}	0.07
C_{250}	237.2 ± 10.7^{B}	2202.6 ± 60.4^{D}	0.11	164.8 ± 7.9^{A}	2541.9 ± 34.2^{DE}	0.06	411.2 ± 16.4^{B}	3667.1 ± 139.5^{F}	0.11

T_5, T_{10} and T_{15} correspond respectively to 5, 10 and 15 days of exposure.
L_0, L_{10} and L_{250} (and C_0, C_{10} and C_{250}) correspond respectively to 0, 10 and 250 mg of PbO deposited on lettuce (cabbage) leaves.
The different letters indicate significant differences among the treatments at $p < 0.05$.
The different type of letters used correspond to the two different studied plants it means lettuce and cabbage.

gs appeared to be stable over time. But a slight drop was observed for L_{250} (Fig. 4b).

In all treatments, Pn was strongly correlated with exposure quantity ($\rho = -0.644$ and -0.666 respectively for lettuce and cabbage), shoot growth ($\rho = 0.807$ and 0.458 respectively for lettuce and cabbage) and gs ($\rho = 0.653$ and 0.418 respectively for lettuce and cabbage) (Tables S1 and S2).



Tolerance index (TI) %						
	T5		T10		T15	
	Lettuce	Cabbage	Lettuce	Cabbage	Lettuce	Cabbage
0(mg)	100	100	100	100	100	100
10(mg)	86.4	64.3	65.7	95.2	46.7	105.9
250(mg)	31.8	60.7	34.3	59.5	35.6	72.6

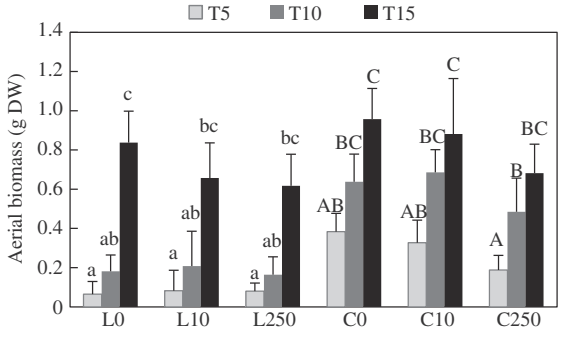


Fig. 3 – (a) Shoot growth, (b) tolerance index (TI) and (c) aerial biomass of lettuce and cabbage exposed to PbO by foliar way (L0, L10 and L250 (and C0, C10 and C250) correspond to respectively 0, 10 and 250 mg of PbO deposited on lettuce (cabbage)) to various exposure durations (T5, T10 and T15). The values are expressed as the mean of 4 replicates for each treatment (±SD), the different lowercase (capital) letters indicate significant differences at $p < 0.05$.

2.4. Effects on fatty acid composition

Fig. 5 presents the effect of Pb concentrations and exposure duration on the fatty acids composition in plant leaves. The values of the foliar fatty acid ratio showed no significant difference compared to the control in both species after PbO exposure, except a slight variation for C_{10}. However, the value of the foliar lipid biomarker decreased with the exposure duration ($\rho = -0.776$ and -0.651 respectively for lettuce and cabbage) (Tables S1 and S2).

The percentage of C18:3 also showed no significant correlation with PbO treatments, but it was negatively correlated with exposure duration ($\rho = -0.813$ and -0.768 respectively in lettuce and cabbage). Statistical analyses highlighted a positive correlation between the fatty acid ratio and the percentage of C18:3 in both vegetables (correlation coefficient $\rho = 0.939$ and 0.867 for respectively lettuce and cabbage (Tables S1 and S2). However, these results obtained for fatty acid measures can only be used as preliminary results as the procedure used here was not exactly the same currently described and used in normative way by Le Guédard et al. (2012a).

2.5. Relationship between exposure conditions, metal accumulation and phytotoxicity

The PCA results of lettuce and cabbage are shown in Fig. 6. For lettuce, the first two principal components accounted for 82.1% of the total variance. The first axis of the PCA (PC1 = 50.3% of total variance) was mainly associated with exposure duration, plant DW, Pb storage, aerial biomass, transfer kinetics and Pb concentration, in decreasing order of importance, as revealed by an examination of the correlation coefficient matrix. PC1 mainly represents the biomass and the metal accumulation with time. The second axis of the PCA (PC2 = 31.8% of total variance) was mainly associated with shoot growth, gs and Pn. Detailed examination of the PCA correlation coefficient matrix provided additional information on the parameter associations in the plants (Table S1).

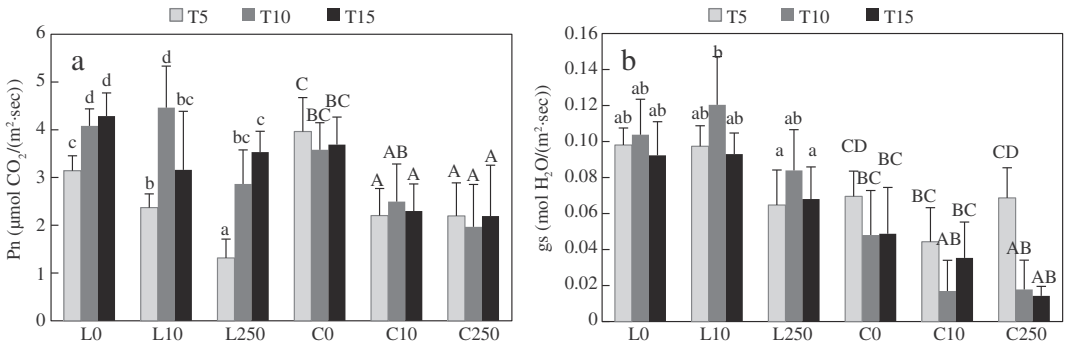


Fig. 4 – Measurement of gas exchange of (a) net photosynthesis (Pn), (b) stomatal conductance) (gs) at the leaf level of lettuce and cabbage after 5,10 and 15 days (T5, T10 and T15) of PbO exposure by foliar way (L0, L10 and L250 (and C0, C10 and C250) correspond to respectively 0, 10 and 250 mg of PbO deposited on lettuce (cabbage)). The values are expressed as the mean of 4 replicates (±SD), the different lowercase (capital) letters indicate significant differences at $p < 0.05$.

For cabbage, the first two principal components accounted for 75.6% of the total variance (eigenvalue >1). The first axis of the PCA (representing 39.8% of the total variance) was mainly associated with plant DW, aerial biomass, exposure duration, shoot growth, in decreasing order of importance, as revealed by the examination of the correlation coefficient matrix. The first principal component mainly represents the growth parameters with time. The second axis of the PCA (PC2 = 35.8% of total variance) was mainly associated with transfer kinetics, Pb concentration, Pb storage, exposure quantity, Pn and gs. Detailed examination of PCA correlation coefficient matrix provided additional information on the parameter associations in the plants (Table S2).

3. Discussion

3.1. *Metal uptake in different plants and treatments*

3.1.1. *Pb concentrations and storage capacity in plants*

According to Hu et al. (2011) and Schreck et al. (2012a), significant Pb accumulation was observed in plant shoots even after washing. It was positively correlated with exposure quantities and duration and negatively correlated with the morphological parameters of plant growth.

Pb concentration was higher in lettuce than in cabbage, but Pb storage in the first 10 days was higher for cabbage than for lettuce. There are two possibilities to explain these results. One, the thin and broad leaves of lettuce allowed quick metal absorption; at the same time, the lettuce grew slowly due to metal phytotoxicity, leading to a high Pb concentration. Two, the cabbage grew faster and acquired a bigger biomass than did the lettuce with a dilution effect. But the higher shoot growth and aerial biomass permitted the cabbage to store more metal per day. Rico et al. (2011) demonstrated that the uptake, translocation and accumulation of PM depend on the plant species and the life cycle stage of the plant. The total storage capacity (in T_{15}) is almost the same in lettuce and cabbage. This may be related to the high metal concentration in lettuce and the high biomass of cabbage (as storage capacity depends on both metal concentration and the plant biomass).

Lead transfer kinetics did not show a linear trend regardless of the PbO amount deposited on the leaf surface and the plant species. These results strongly suggest that, to some extent, the vegetables have defense mechanisms to limit Pb uptake during growth processes. The defense mechanisms may influence metal foliar uptake between T_5 and T_{10} since, during this period, Pb transfer kinetics was moderate. However, from T_{10} to T_{15}, Pb transfer kinetics strongly increased in L_{250} and C_{250}, suggesting some detoxification mechanisms. The existence of efficient storage and regulation/detoxification mechanisms allows the survival of vegetables under conditions of high accumulated metal concentrations (Phillips and Rainbow, 1988). Therefore, the change of transfer kinetics might be a process of accumulation (T_0 to T_5), regulation (T_5 to T_{10}) and adaptation (T_{10} to T_{15}) in vegetables.

Moreover, plants exposed to 500 mg of PbO were also tested in this study with lettuce (L_{500}) and cabbage (C_{500}); no significant difference was found in comparison with 250 mg of PbO exposure for cabbage. We suggest that there are adaption and saturation in the case of high PbO treatment.

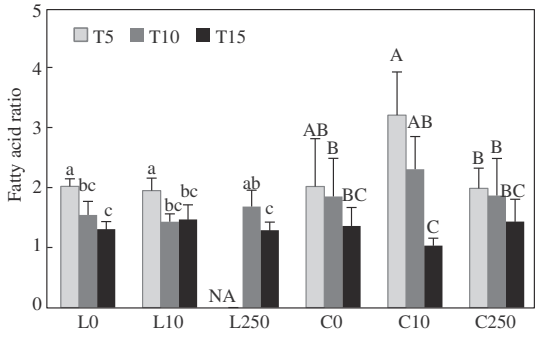


Fig. 5 – Mean available values of fatty acid ratio (C18:3/(C18:0 + C18:1 + C18:2)) in lettuce and cabbage exposed to PbO by foliar way (L0, L10 and L250 (and C0, C10 and C250) correspond to respectively 0, 10 and 250 mg of PbO deposited on lettuce (cabbage)) to various exposure durations (T5, T10 and T15). Values are expressed as the mean of 4 replicates (±SD), the different lowercase (capital) letters indicate significant differences at $p < 0.05$. NA: not available.

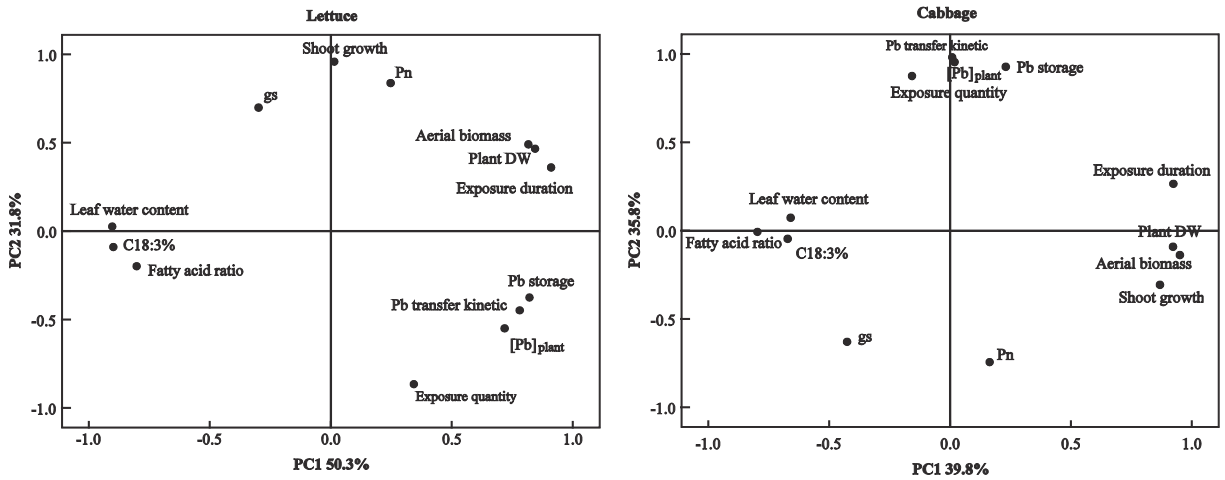


Fig. 6 – The first two principal component of the PCA (Principal Component Analysis) on 13 variables (Exposure quantity, Exposure duration, Pb concentration ($[Pb]_{plant}$), Pb storage, Pb transfer kinetic, Shoot growth, Aerial biomass, Plant DW, Leaf water content, Pn, gs, C18:3% and Fatty acid ratio in lettuce and cabbage after exposed with PbO PM.

For example, (i) the biomass (shoot growth: 0.93 cm for C_{250} and 0.73 cm for C_{500}; aerial biomass: 0.68 g for C_{250} and 0.62 g for C_{500}) and gaseous exchange (Pn: 2.2 μmol $CO_2/(m^2 \cdot sec)$ for C_{250} and 1.9 μmol $CO_2/(m^2 \cdot sec)$ for C_{500}; gs: 0.01 mol $H_2O/(m^2 \cdot sec)$ for C_{250} and C_{500}) showed no significant difference between 250 mg and 500 mg of PbO treatment ($p > 0.05$), and (ii) the Pb concentration is not always linear to exposure quantity. In cabbage, the Pb concentration even decreased for C_{500} (5173 mg Pb/kg) in comparison to C_{250} (5606 mg Pb/kg) at T_{15}. However, this dose is not often found in the environment and concerns only extreme situations or highly polluted contexts. Concerning the mechanisms involved in metal transfer *via* foliar application, Xiong et al. (2014a) concluded that biogeochemical transformations occurred on leaf surfaces. Internalization through the cuticle or penetration throughout the stomata apertures (Barber, 2004) are, therefore, proposed as the two major mechanisms involved in foliar uptake of PM. Thus, particle sizes as well as solubility are parameters that appear to strongly control physical transfer through the leaf surface (Eichert et al., 2008; Larue et al., 2012). If most of the PbO particles are in the assumed <10 μm diameter size range (original PbO size exposure on leaf surface), direct cuticular uptake would be minimal (cuticular pores are around 2 nm in diameter according to Uzu et al., 2011), and penetration through stomata openings could be the main uptake pathway.

In our previous study, CdO (<1 μm) and Sb_2O_3 (<1 μm) PM were observed near or inside the stomata apertures of cabbage after foliar exposure, which indicated the foliar transfer of metal(loid)s by the stomata (Xiong et al., 2014a). The changes to the particle and/or vegetable leaves induced by atmospheric gaseous pollutants (*i.e.* O_3, NO_2, SO_2) which are commonly found in urban or industrial tropospheres (Cicek and Koparal, 2004; Gandois et al., 2010; Chaparro-Suarez et al., 2011; Terzaghi et al., 2013), could favor the foliar transfer of metal(loid)s containing particles. However, Goix et al. (2014) observed by scanning electron microscopy that PbO particles could form aggregates; thus, particles may be trapped on the leaf surface of plants due to leaf hairs and the cuticular wax cover (Sæbø et al., 2012). Moreover, phyllosphere organisms release inorganic and organic compounds possessing acidifying, chelating and/or reductive abilities and may play an essential role in element mobilization and uptake at the leaf surface (Michaud et al., 2007). Metal concentrations and speciation in leaves could be modified by interactions on the phyllosphere between PM and microbes. Changes of temperature and humidity may transform PM at the leaf surface after contact with the leaf (Uzu et al., 2010; Schreck et al., 2012a). Pb solubilization (even if it may be low) could also be a potential mechanism for plant uptake.

3.1.2. *Translocation factor (TF)*

In agreement with previous studies from Xiong et al. (2014b), lead translocation from the leaves to roots was observed. Erenoglu et al. (2002) and Zhang et al. (2013) also studied inorganic element (zinc, copper, iron and manganese) translocation, and phloem Zn transport from leaves to roots was demonstrated by Haslett et al. (2001).

However, as it is not essential element, the TF of Pb ranges from 0.06 to 0.21, which is a value much lower than for the essential Zn element (Yoon et al., 2006; Amadi and Tanee, 2014).

3.2. *Phytotoxicity of deposited PM enriched in PbO*

3.2.1. *Impact of Pb-enriched PM on plant growth*

Shoot growth and aerial biomass decreased in both plant species after PbO exposure in a dose dependent manner. Lead-induced severe growth reduction in plants can be due to nutritional disturbances and probably to a direct Pb toxic effect. Lead phytotoxicity was proven in several previous studies, notably for morphological and growth parameters (Foucault et al., 2013; Shahid et al., 2014d). Capelo et al. (2012) showed a decrease of root and shoot growth in *Lactuca sativa* L. after Pb exposure. Abbas and Akladious (2012) reported a Pb-induced significant decrease in all growth parameters:

plant height (86.2%), root length (89.2%), number of leaves (96.9%), fresh weight of shoots (93.5%), fresh weight of roots (85.9%), dry weight of shoots (84.7%) and dry weight of roots (81.4%). Lead-induced decrease effects on plant growth parameters can be due to the following reasons: (1) inhibition of the Calvin cycle, (2) disruption in plastoquinone and carotenoid synthesis, (3) reduced activities of delta aminolevulinic acid dehydratase and ferredoxin NADP+ reductase, (4) interference in the electron transport chain, (5) inadequate CO_2 concentration due to stomatal closure, (6) replacement of vital bivalent cations by Pb and (7) a distorted chloroplast ultrastructure owing to Pb affinity for the S- and N-ligands of protein (Pourrut et al., 2011, 2013; Shahid et al., 2012, 2014c).

Generally, cabbage presents better growth compared to lettuce, suggesting a higher tolerance to Pb transfer and toxicity. Indeed, cabbage leaves possess a waxy coating; the epicuticular waxes are dense and are an important barrier to ion and water movement across the cuticle (Adams et al., 1989). In contrast, lettuce has thin and broad leaf surfaces (less cuticle thickness) and so may be more sensitive to metal contamination. In general, leaves with a lot of epicuticular wax are more hydrophobic, resulting in less contact between the surface of the cuticle and metals. The tolerance of cabbage may be an adjustment of the plant to metal stress (Shahid et al., 2013b). Finally, all plants were alive and the biomass increased with exposure duration ($T_{15} > T_{10} > T_5$), which is in agreement with the study of Lamhamdi et al. (2011). Thus, the plants can maintain basic growth, even under high Pb pollution.

The PCA analysis showed that the lettuce shoot growth and aerial biomass are positively correlated to Pn ($\rho = 0.807$, 0.508 respectively); the shoot growth of cabbage is significantly and positively correlated to exposure duration ($\rho = 0.771$) and Pn ($\rho = 0.458$), suggesting potential interactions between net photosynthesis and shoot growth.

3.2.2. *Impact of Pb-enriched PM on photosynthetic activities*

Both lettuce and cabbage showed a decline in Pn and gs value due to PbO foliar exposure. Photosynthesis is quite sensitive to Pb toxicity, and both *in vivo* and *in vitro* photosynthetic CO_2 fixations are affected by Pb (Sheoran and Singh, 1993; Pourrut et al., 2013). Lead-induced toxicity to photosynthesis can be due to multiple effects of PM. The immediate effect is on stomata closure, followed by chloroplastic changes (Pourrut et al., 2011). Lead may also affect photosynthesis indirectly *via* ROS production (Shahid et al., 2012). An enhanced accumulation of endogenous H_2O_2 was detected after treatment of *Lemna trisulca* L. with lead (Samardakiewicz et al., 2015). The results of long term Pb exposure are a reduction of leaf growth, a decrease of photosynthetic pigments, an alteration of chloroplast structure and a decrease of enzyme activities for CO_2 assimilation (Austruy et al., 2013). Larger metal agglomerates found nearby closed stomata, integrated into the surface wax or on the leaf surface (Birbaum et al., 2010), can decelerate plant net photosynthesis mainly *via* limiting stomata opening (the decrease of photon acquisition and CO_2 uptake).

3.2.3. *Impact of Pb-enriched PM on the leaf fatty acid composition*

The results obtained for leaf fatty acid composition can only be used as preliminary measures, as the procedure used was not exactly the same described by Le Guédard et al. (2012a). As the phytotoxicity was characterized with other indicators, the results of fatty acid composition were presented from complementary point of view. In our *ex situ* experiment, there is a slight decrease in the fatty acid ratio under high PbO treatment, but no significant effect was observed among the three different groups (0, 10 and 250 mg of PbO deposited). A similar phenomenon was observed by Nouairi et al. (2006) who reported that no significant change in the total fatty acid composition was observed in *Sesuvium portulacastrum* leaves under Cd treatment. Schreck et al. (2013) also found that, after 1 and 4 weeks of field exposure in the presence of soil and air Pb contamination (among various metal(loid)s in lower quantities), the fatty acid ratio was not significantly decreased. The results of Le Guédard et al. (2012a) showed that the fatty acid ratio is significantly and negatively correlated with Ni and Cr, but not with other metal levels (Cd, Cu, Pb and Zn). Generally, the level of trienoic fatty acids not changing with Pb pollution may indicate plant tolerance to oxidative stress (Kodama et al., 1994; Khodakovskaya et al., 2006; Dominguez et al., 2010).

By contrast, in their study conducted on a metallurgical landfill, Le Guédard et al. (2012b) showed that Pb and Cr leaf contents were anticorrelated to the C18:3/(C18:0 + C18:1 + C18:2) ratio in *Populus nigra* leaves. Our results for the *ex situ* experiment could be explained by differences in experimental and exposure conditions. Actually, in our study, Pb was used as a mono-metal, contrary to *in situ* studies in which multiple metal(loid)s were introduced, and it was applied to only favor the foliar pathway. Furthermore, we chose young leaves for fatty acid analysis. They may have less contaminated compared to old leaves exposed for a longer time. Finally, metal speciation, exposure duration and above all contamination sources (soil or atmosphere) and transfer pathways (by root or leaf) can also affect the lipid biomarker (Schreck et al., 2013).

Nevertheless, the variability of our Z values (Fig. S1), the index involved in the case of foliar exposure, suggested that Pb can still induce a disturbance to the fatty acid composition. This may be a plant response to oxidative stress and ROS formation (Shahid et al., 2012). PCA results showed that the fatty acid ratio decreased with the exposure duration; the change in fatty acid ratio is mainly dependent on the percentage of C18:3. α-linolenic acid (C18:3) is primarily associated with plastid lipids in leaves, notably in the chloroplasts, suggesting an inhibition by the metal of plastidial lysophosphatidylcholine acyltransferase catalyze acylation when lipids are imported from endomembranes to plastids in eucaryon (Akermoun et al., 2002). The decrease of the C18:3 content with exposure duration may also be related to a direct reaction of ROS with unsaturated lipids and be responsible for an alteration of chloroplast membrane structures, like photosystems, leading to an inhibition of photosynthetic activities (Austruy et al., 2013). Lead-induced toxicity to the lipid membrane *via* ROS production is well established in the literature (Shahid et al., 2014a).

3.3. *Relationship between exposure conditions, metal accumulation and phytotoxicity*

PCA results showed that PbO treatment is significantly and negatively correlated with shoot growth and gaseous exchanges (Pn and gs) and is not significantly or negatively correlated with

the lipid biomarker (Tables S2 and S3). These negative correlations, although insignificant, suggested an impact of Pb particles on the composition of membrane lipids in the leaves and metabolic stress after exposure to Pb particulates.

4. Conclusions and perspectives

This study highlights the influence of plant species on lead foliar transfer and phytotoxicity, some possible adaptation mechanisms and the related processes.

Our results could be applied to biomonitoring of atmospheric pollution and assessment of vegetable contamination, especially when vegetables are cultivated (in farms or kitchen gardens) near industrial or urban areas. Actually, populations living in such areas are exposed to high atmospheric quantities of PM enriched with metal(loid)s and can, therefore, incur health risks by consuming polluted plants. Educational projects such as "Réseau-Agriville" (Educational resources platform on urban agriculture to educate citizens about the parameters influencing vegetable quality and give advice to reduce exposure to pollutants) are, therefore, essential in order to favor an ecological transition at a global scale.

Furthermore, bioaccessibility experiments performed under different levels of soil and air pollution could help to build a database of metal(loid) bioavailability values for humans in a context of health risk assessments.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2015.08.029.

REFERENCES

Abbas, S.M., Akladious, S.A., 2012. Physiological and biochemical attributes of lettuce (*Lactuca sativa* L.) treated with lead in the presence or absence of ginger powder. Fresenius Environ. Bull. 21, 563–577.

Adams, B.Y.C.M., Simon, J.M., Hutchinson, D.T.C., 1989. Crystal occurrence and wax disruption on leaf surfaces of cabbage treated with simulated acid rain. New. Phytol. 114, 147–158.

Akermoun, M., Testet, E., Cassagne, C., Bessoule, J.J., 2002. Inhibition of the plastidial phosphatidylcholine synthesis by silver, copper, lead and mercury induced by formation of mercaptides with the lyso-PC acyltransferase. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1581, 21–28.

Amadi, N., Tanee, F.B.G., 2014. Efficacy of *Moringa oleifera* as a phytoextraction plant in the remediation of heavy metals polluted soil. African J. Plant Sci. 8, 546–553.

Austruy, A., Shahid, M., Xiong, T., Castrec, M., Payre, V., Niazi, N.K., et al., 2014. Mechanisms of metal-phosphates formation in the rhizosphere soils of pea and tomato: environmental and sanitary consequences. J. Soils Sediments 14, 666–678.

Austruy, A., Wanat, N., Moussard, C., 2013. Physiological impacts of soil pollution and arsenic uptake in three plant species: *Agrostis capillaris*, *Solanum nigrum* and *Vicia faba*. Ecotoxicol. Environ. Saf. 90, 28–34.

Barber, J., 2004. Current issues and uncertainties in the measurement and modelling of air–vegetation exchange and within-plant processing of POPs. Environ. Pollut. 128, 99–138.

Barrie, L.A., 1992. Scavenging Ratios: Black Magic or Useful Scientific Tool? In: Schwartzand, S.E., Slinn, W.G.N. (Eds.), Precipitation Scavenging and Atmosphere–Surface Exchange vol. 1. Hemisphere Publishing Corporation, Washington

Birbaum, K., Brogiolo, R., Schellenberg, M., Martinoia, E., Stark, W.J., Günther, D., 2010. No evidence for cerium dioxide nanoparticle translocation in maize plants. Environ. Sci. Technol. 44, 8718–8723.

Bravin, M.N., Michaud, A.M., Larabi, B., Hinsinger, P., 2010. RHIZOtest: a plant-based biotest to account for rhizosphere processes when assessing copper bioavailability. Environ. Pollut. 158, 3330–3337.

Cao, H., Chen, J., Zhang, J., Zhang, H., Qiao, L., Men, Y., 2010. Heavy metals in rice and garden vegetables and their potential health risks to inhabitants in the vicinity of an industrial zone in Jiangsu, China. J. Environ. Sci. (China) 22, 1792–1799.

Capelo, A., Santos, C., Loureiro, S., Pedrosa, M.A., 2012. Phytotoxicity of lead on *Lactuca sativa* : effects on growth, mineral nutrition, photosynthetic activity and oxidant metabolism. Fresenius Environ. Bull. 21, 450–459.

Chaparro-Suarez, I.G., Meixner, F.X., Kesselmeier, J., 2011. Nitrogen dioxide (NO_2) uptake by vegetation controlled by atmospheric concentrations and plant stomatal aperture. Atmos. Environ. 45 (32), 5742–5750.

Cicek, A., Koparal, A.S., 2004. Accumulation of sulfur and heavy metals in soil and tree leaves sampled from the surroundings of Tunc bilek thermal power plant. Chemosphere 57 (8), 1031–1036.

De Leon, C.P., Sommer, I., Cram, S., Murguia, F., Hernandez, M., Vanegas, C., 2010. Metal uptake in a peri-urban *Lactuca sativa* cultivated area. J. Environ. Sci. Health A 45, 111–120.

Diop, C., Dewaelé, D., Diop, M., Touré, A., Cabral, M., Cazier, F., et al., 2014. Assessment of contamination, distribution and chemical speciation of trace metals in water column in the Dakar coast and the Saint Louis estuary from Senegal, West Africa. Mar. Pollut. Bull. 86, 539–546.

Dominguez, M., Hernandez, L., Pennycooke, J.C., Jimenez, P., Martinez-Rivas, J.M., Sanz, C., et al., 2010. Increasing omega-3 desaturase expression in tomato results in altered aroma profile and enhanced resistance to cold stress. Plant Physiol. 153, 655–665.

Eichert, T., Kurtz, A., Steiner, U., Goldbach, H.E., 2008. Size exclusion limits and lateral heterogeneity of the stomatal foliar uptake pathway for aqueous solutes and water-suspended nanoparticles. Physiol. Plant. 134 (1), 151–160.

Erenoglu, B., Nikolic, M., Römheld, V., Cakmak, I., 2002. Uptake and transport of foliar applied zinc (^{65}Zn) in bread and durum wheat cultivars differing in zinc efficiency. Plant Soil 241, 251–257.

Foucault, Y., Lévêque, T., Xiong, T., Schreck, E., Austruy, A., Shahid, M., et al., 2013. Green manure plants for remediation of soils polluted by metals and metalloids: ecotoxicity and human bioavailability assessment. Chemosphere 93, 1430–1435.

Freer-Smith, P.H., Sophy, H., Goodman, A., 1997. The uptake of particulates by an urban woodland: site description and particulate composition. Environ. Pollut. 95, 27–35.

Gandois, L., Tipping, E., Dumat, C., Probst, A., 2010. Canopy influence on trace metal atmospheric inputs on forest ecosystems: speciation in throughfall. Atmos. Environ. 44 (6), 824–833.

Gillespie, P., Tajuba, J., Lippmann, M., Chen, L.C., Veronesi, B., 2013. Particulate matter neurotoxicity in culture is size-dependent. Neurotoxicology 36, 112–117.

Goix, S., Lévêque, T., Xiong, T.T., Schreck, E., Baeza-Squiban, A., Geret, F., et al., 2014. Environmental and health impacts of fine and ultrafine metallic particles: assessment of threat scores. Environ. Res. 133, 185–194.

Gupta, S.K., Chabukdhara, M., Kumar, P., Singh, J., Bux, F., 2014. Evaluation of ecological risk of metal contamination in river Gomti, India: A biomonitoring approach. Ecotoxicol. Environ. Saf. 110, 49–55.

Haslett, B.S., Reid, R.J., Rengel, Z., 2001. Zinc mobility in wheat: uptake and distribution of zinc applied to leaves or roots. Ann. Bot-London 87, 379–386.

Hong, J., Peralta-videa, J.R., Rico, C., Sahi, S., Viveros, M.N., Bartonjo, J., et al., 2014. Evidence of translocation and physiological impacts of foliar applied CeO_2 nanoparticles on cucumber (*Cucumis sativus*) plants. Environ. Sci. Technol. 48, 4376–4385.

Honour, S.L., Bell, J.N.B., Ashenden, T.W.A., Cape, J.N., Power, S.A., 2009. Responses of herbaceous plants to urban air pollution: effects on growth, phenology and leaf surface characteristics. Environ. Pollut. 157, 1279–1286.

Hu, X., Zhang, Y., Luo, J., Xie, M., Wang, T., Lian, H., 2011. Accumulation and quantitative estimates of airborne lead for a wild plant (*Aster subulatus*). Chemosphere 82, 1351–1357.

Hurtevent, P., Thiry, Y., Levchuk, S., Yoschenko, V., Henner, P., Madoz-Escande, C., et al., 2013. Translocation of ^{125}I, ^{75}Se and ^{36}Cl to wheat edible parts following wet foliar contamination under field conditions. J. Environ. Radioact. 12, 43–54.

Khan, S., Aijun, L., Zhang, S., Hu, Q., Zhu, Y.G., 2008. Accumulation of polycyclic aromatic hydrocarbons and heavy metals in lettuce grown in the soils contaminated with long-term wastewater irrigation. J. Hazard. Mater. 152, 506–515.

Khodakovskaya, M., McAvoy, R., Peters, J., Wu, H., Li, Y., 2006. Enhanced cold tolerance in transgenic tobacco expressing a chloroplast omega-3 fatty acid desaturase gene under the control of a cold-inducible promoter. Planta 223, 1090–1100.

Kodama, H., Hamada, T., Horiguchi, C., Nishimura, M., Iba, K., 1994. Genetic enhancement of cold tolerance by expression of a gene for chloroplast omega-3 fatty acid desaturase in transgenic tobacco. Plant Physiol. 105, 601–605.

Lamhamdi, M., Bakrim, A., Aarab, A., Lafont, R., Sayah, F., 2011. Lead phytotoxicity on wheat (*Triticum aestivum* L.) seed germination and seedlings growth. Cr. Biol. 334, 118–126.

Larue, C., Laurette, J., Herlin-Boime, N., Khodja, H., Fayard, B., Flank, A.M., et al., 2012. Accumulation, translocation and impact of TiO_2 nanoparticles in wheat (*Triticum aestivum* spp.): influence of diameter and crystal phase. Sci. Total Environ. 431, 197–208.

Le Guédard, M., Bessoule, J.J., Boyer, V., Ayciriex, S., Velours, G., Kulik, W., et al., 2009. PSI1 is responsible for the stearic acid enrichment that is characteristic of phosphatidylinositol in yeast. FEBS J. 276, 6412–6424.

Le Guédard, M., Faure, O., Bessoule, J.J., 2012a. Soundness of in situ lipid biomarker analysis: early effect of heavy metals on leaf fatty acid composition of *Lactuca serriola*. Environ. Exp. Bot. 76, 54–59.

Le Guédard, M., Faure, O., Bessoule, J.J., 2012b. Early changes in the fatty acid composition of photosynthetic membrane lipids from *Populus nigra* grown on a metallurgical landfill. Chemosphere 88, 693–698.

Little, P.E., 1978. Deposition of Exhaust Lead and its Impact on Plants. Symposium "The Impact of Road Traffic on Plants" — 11–13 September 1978, TRRL, Supplementary Report. 513, pp. 49–54.

Lombi, E., Scheckel, K.G., Kempsond, I.M., 2011. In situ analysis of metal(loid)s in plants: state of the art and artefacts. Environ. Exp. Bot. 72, 3–17.

Luo, C., Liu, C., Wang, Y., Liu, X., Li, F., Zhang, G., et al., 2011. Heavy metal contamination in soils and vegetables near an e-waste processing site, South China. J. Hazard. Mater. 186, 481–490.

Ma, X., Geiser-Lee, J., Deng, Y., Kolmakov, A., 2010. Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. Sci. Total Environ. 16, 3053–3061.

Mahmood, T., Islam, K.R., Muhammad, S., 2007. Toxic effects of heavy metals on early growth and tolerance of cereal crops. Pak. J. Bot. 39, 451–462.

Majer, P., Hideg, É., 2012. Existing antioxidant levels are more important in acclimation to supplemental UV-B irradiation than inducible ones: studies with high light pretreated tobacco leaves. Emir. J. Food Agric. 24, 598–606.

Marchiol, L., Assolari, S., Sacco, P., Zerbi, G., 2004. Phytoextraction of heavy metals by canola (*Brassica napus*) and radish (*Raphanus sativus*) grown on multicontaminated soil. Environ. Pollut. 132, 21–27.

Mateos-Naranjo, E., Andrades-Moreno, L., Redondo-Gómez, S., 2012. Tolerance to and accumulation of arsenic in the cordgrass *Spartina densiflora* Brongn. Bioresour. Technol. 104, 187–194.

Michaud, A.M., Bravin, M.N., Galleguillos, M., Hinsinger, P., 2007. Copper uptake and phytotoxicity as assessed in situ for durum wheat (*Triticum turgidum durum* L.) cultivated in copper-contaminated, former vineyard soils. Plant Soil 298, 99–111.

Mombo, S., Foucault, Y., Deola, F., Gaillard, I., Goix, S., Shahid, M., et al., 2015. Management of human health risk in the context of kitchen gardens polluted by lead and cadmium near a lead recycling company. J. Soils Sediments http://dx.doi.org/10.1007/s11368-015-1069-7.

Monni, S., Uhlig, C., Junttila, O., Hansen, E., Hynynen, J., 2001. Chemical composition and ecophysiological responses of *Empetrum nigrum* to aboveground element application. Environ. Pollut. 112, 417–426.

Monteiro, M.S., Santos, C., Soares, A.M.V.M., Mann, R.M., 2009. Assessment of biomarkers of cadmium stress in lettuce. Ecotoxicol. Environ. Saf. 72, 811–818.

Nouairi, I., Ghnaya, T., Ben Youssef, N., Zarrouk, M., Habib Ghorbel, M., 2006. Changes in content and fatty acid profiles of total lipids of two halophytes: *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum* under cadmium stress. J. Plant Physiol. 163, 1198–1202.

Perrone, M.G., Gualtieri, M., Ferrero, L., Lo Porto, C., Udisti, R., Bolzacchini, E., 2010. Seasonal variations in chemical composition and in vitro biological effects of fine PM from Milan. Chemosphere 78, 1368–1377.

Phillips, D., Rainbow, P., 1988. Barnacles and mussels as biomonitors of trace elements: a comparative study. Mar. Ecol. Prog. Ser. Oldend. 49, 83–93.

Pierart, A., Shahid, M., Séjalon-Delmas, N., Dumat, C., 2015. Antimony bioavailability: knowledge and research perspectives for sustainable agricultures. J. Hazard. Mater. 289, 219–234.

Polichetti, G., Cocco, S., Spinali, A., Trimarco, V., Nunziata, A., 2009. Effects of particulate matter (PM_{10}, $PM_{2.5}$ and PM_1) on the cardiovascular system. Toxicology 261, 1–8.

Pourrut, B., Shahid, M., Douay, F., Dumat, C., Pinelli, E., 2013. Molecular Mechanisms Involved in Lead Uptake, Toxicity and Detoxification in Higher Plants. In: Gupta, D.K., Corpas, F.J., Palma, J.M. (Eds.), Heavy Metal Stress in Plants. Springer, Berlin Heidelberg, pp. 121–147.

Pourrut, B., Shahid, M., Dumat, C., Winterton, P., Pinelli, E., 2011. Lead uptake, toxicity, and detoxification in plants. Rev. Environ. Contam. Toxicol. 213, 113–136.

Rico, C.M., Majumdar, S., Duarte-Gardea, M., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2011. Interaction of nanoparticles with edible plants and their possible implications in the food chain. J. Agric. Food Chem. 59, 3485–3498.

Sæbø, A., Popek, R., Nawrot, B., Hanslin, H.M., Gawronska, H., Gawronski, S.W., 2012. Plant species differences in particulate matter accumulation on leaf surfaces. Sci. Total Environ. 427–428, 347–354.

Samardakiewicz, S., Krzeszowiec-Jeleñ, W., Bednarski, W., Jankowski, A., Suski, S., Gabryœ, H., et al., 2015. Pb-induced avoidance-like chloroplast movements in fronds of *Lemna trisulca* L. PLoS One 10 (2), e0116757. http://dx.doi.org/10.1371/journal.pone.0116757.

Schreck, E., Bonnard, R., Laplanche, C., Leveque, T., Foucault, Y., Dumat, C., 2012b. DECA: a new model for assessing the foliar uptake of atmospheric lead by vegetation, using *Lactuca sativa* as an example. J. Environ. Manag. 112, 233–239.

Schreck, E., Dappe, V., Sarret, G., Sobanska, S., Nowak, D., Nowak, J., et al., 2014. Foliar or root exposures to smelter particles: consequences for lead compartmentalization and speciation in plant leaves. Sci. Total Environ. 476-477, 667–676.

Schreck, E., Foucault, Y., Geret, F., Pradere, P., Dumat, C., 2011. Influence of soil ageing on bioavailability and ecotoxicity of lead carried by process waste metallic ultrafine particles. Chemosphere 85, 1555–1562.

Schreck, E., Foucault, Y., Sarret, G., Sobanska, S., Cécillon, L., Castrec-Rouelle, M., et al., 2012a. Metal and metalloid foliar uptake by various plant species exposed to atmospheric industrial fallout: mechanisms involved for lead. Sci. Total Environ. 427-428, 253–262.

Schreck, E., Laplanche, C., Le Guédard, M., Bessoule, J.J., Austruy, A., Xiong, T., et al., 2013. Influence of fine process particles enriched with metals and metalloids on *Lactuca sativa* L. leaf fatty acid composition following air and/or soil–plant field exposure. Environ. Pollut. 179, 242–249.

Seinfeld, J.H., 1986. Atmospheric Chemistry and Physics of Air Pollution. Wiley-Interscience, New York.

Shahid, M., Dumat, C., Pourrut, B., Sabir, M., Pinelli, E., 2014c. Assessing the effect of metal speciation on lead toxicity to *Vicia faba* pigment contents. J. Geochem. Explor. 144, 290–297.

Shahid, M., Dumat, C., Pourrut, B., Silvestre, J., Laplanche, C., Pinelli, E., 2014b. Influence of EDTA and citric acid on lead-induced oxidative stress to *Vicia faba* roots. J. Soils Sediments 14, 835–843.

Shahid, M., Dumat, C., Silvestre, J., Pinelli, E., 2012. Effect of fulvic acids on lead-induced oxidative stress to metal sensitive *Vicia faba* L. plant. Biol. Fertil. Soils 48, 689–697.

Shahid, M., Ferrand, E., Schreck, E., Dumat, C., 2013b. Behavior and impact of zirconium in the soil–plant system: plant uptake and phytotoxicity. Rev. Environ. Contam. Toxicol. 221, 107–127.

Shahid, M., Pinelli, E., Pourrut, B., Dumat, C., 2014d. Effect of organic ligands on lead-induced oxidative damage and enhanced antioxidant defense in the leaves of *Vicia faba* plants. J. Geochem. Explor. 144, 282–289.

Shahid, M., Pinelli, E., Pourrut, B., Silvestre, J., Dumat, C., 2011. Lead-induced genotoxicity to *Vicia faba* L. roots in relation with metal cell uptake and initial speciation. Ecotoxicol. Environ. Saf. 74, 78–84.

Shahid, M., Pourrut, B., Dumat, C., Nadeem, M., Aslam, M., Pinelli, E., 2014a. Heavy-metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. Ecotoxicol. Environ. Saf. 232, 1–44.

Shahid, M., Xiong, T., Castrec-Rouelle, M., Leveque, T., Dumat, C., 2013a. Water extraction kinetics of metals, arsenic and dissolved organic carbon from industrial contaminated poplar leaves. J. Environ. Sci. (China) 25, 2451–2459.

Sheoran, I.S., Singh, R., 1993. Effect of Heavy Metals on Photosynthesis in Higher Plants. Photosynthesis: Photoreactions to Plant Productivity. Springer, Netherlands, pp. 451–468.

Soda, S., Hamada, T., Yamaoka, Y., Ike, M., Nakazato, H., Saeki, Y., et al., 2012. Constructed wetlands for advanced treatment of wastewater with a complex matrix from a metal-processing plant: bioconcentration and translocation factors of various metals in *Acorus gramineus* and *Cyperus alternifolius*. Ecol. Eng. 39, 63–70.

Stampoulis, D., Sinha, S.K., White, J.C., 2009. Assay-dependent phytotoxicity of nanoparticles to plants. Environ. Sci. Technol. 43, 9473–9479.

Terzaghi, E., Wild, E., Zacchello, G., Cerabolini, B.E.L., 2013. Forest filter effect: role of leaves in capturing/releasing air particulate matter and its associated PAHs. Atmos. Environ. 74, 378–384.

Uzu, G., Schreck, E., Xiong, T., Macouin, M., Lévêque, T., Fayomi, B., et al., 2014. Urban market gardening in Africa: foliar uptake of metal(loid)s and their bioaccessibility in vegetables; implications in terms of health risks. Water Air Soil Pollut. 225, 2185.

Uzu, G., Sobanska, S., Aliouane, Y., Pradere, P., Dumat, C., 2009. Study of lead phytoavailability for atmospheric industrial micronic and sub-micronic particles in relation with lead speciation. Environ. Pollut. 157, 1178–1185.

Uzu, G., Sobanska, S., Sarret, G., Munoz, M., Dumat, C., 2010. Foliar lead uptake by lettuce exposed to atmospheric fallouts. Environ. Sci. Technol. 44, 1036–1042.

Uzu, G., Sobanska, S., Sarret, G., Sauvain, J.J., Pradère, P., Dumat, C., 2011. Characterization of lead recycling facility emissions at various workplaces: major insights for sanitary risk assessment. J. Hazard. Mater. 186, 1018–1027.

Waisberg, M., Black, W.D., Waisberg, C.M., Hale, B., 2004. The effect of pH, time and dietary source of cadmium on the bioaccessibility and adsorption of cadmium to/from lettuce (*Lactuca sativa* L. cv. ostinata). Food Chem. Toxicol. 42, 835–842.

Ward, N.I., Savage, J.M., 1994. Metal dispersion and transportational activities using food crops as biomonitors. Sci. Total Environ. 146–147, 309–319.

Xiong, T., Leveque, T., Austruy, A., Goix, S., Schreck, E., Dappe, V., et al., 2014a. Foliar uptake and metal(loid) bioaccessibility in vegetables exposed to particulate matter. Environ. Geochem. Health 36, 897–909.

Xiong, T., Leveque, T., Shahid, M., Foucault, Y., Mombo, S., Dumat, C., 2014b. Lead and cadmium phytoavailability and human bioaccessibility for vegetables exposed to soil or atmospheric pollution by process ultrafine particles. J. Environ. Qual. 43, 1593–1600.

Yin, L., Cheng, Y., Espinasse, B., Colman, B.P., 2011. Auffan M,Wiesner MMore than the ions: the effects of silver nanoparticles on *Lolium multiflorum*. Environ. Sci. Technol. 45, 2360–2367.

Yoon, J., Cao, X., Zhou, Q., Ma, L.Q., 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci. Total Environ. 368, 456–464.

Zhang, H., Yang, H., Wang, Y., Gao, Y., Zhang, L., 2013. The response of ginseng grown on farmland to foliar-applied iron, zinc, manganese and copper. Ind. Crop. Prod. 45, 388–394.

Zhao, H., Xia, B., Fan, C., Zhao, P., Shen, S., 2012. Human health risk from soil heavy metal contamination under different land uses near Dabaoshan Mine, Southern China. Sci. Total Environ. 417-418, 45–54.

Zia, M.H., Codling, E.E., Scheckel, K.G., Chaney, R.L., 2011. In vitro and in vivo approaches for the measurement of oral bioavailability of lead (Pb) in contaminated soils: a review. Environ. Pollut. 159, 2320–2327.