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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

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Keywords:
- Uptake pathways
- Mixture of metal(loid)s
- Leaf fatty acid composition
- Statistical index

We investigate the effect of both foliar and root uptake of a mixture of metal(loid)s on the fatty acid composition of plant leaves. Our objectives are to determine whether both contamination pathways have a similar effect and whether they interact. *Lactuca sativa* L. were exposed to fine process particles enriched with metal(loid)s in an industrial area. Data from a first experiment were used to conduct an exploratory statistical analysis which findings were successfully cross-validated by using the data from a second one. Both foliar and root pathways impact plant leaf fatty acid composition and do not interact. A Z index (dimensionless quantity), weighted product of fatty acid concentration ratios was built up from the statistical analyses. It provides new insights on the mechanisms involved in metal uptake and phytoxicity. Plant leaf fatty acid composition is a robust and fruitful approach to detect and understand the effects of metal(loid) contamination on plants.

1. Introduction

Nowadays, as reported by Donisa et al. (2000) or Ma et al. (2010), atmospheric fallouts of fine particles enriched with metal(loid)s (denoted PM, in the present study) involve significant contaminations of ecosystem compartments: soils, organisms, ground and surface waters. The released metal(loid)s are highly persistent in the environment and can cause adverse effects for ecosystems and human health (Komárek et al., 2013). According to Bermudez et al. (2012), they can accumulate in vegetables and crops, with therefore health risks in relation with food chain contamination. Both soil-plant (Alexander et al., 2006; Polichetti et al., 2009) and air-plant (Perrone et al., 2010; Schreck et al., 2012a) transfers of inorganic pollutants are involved. Mechanisms of soil-plant (or root) transfers have been well studied for several years (Lin and Xing, 2007, 2008; Stampoulis et al., 2009; Ma et al., 2010; Yin et al., 2011; Lombi et al., 2011) whereas air-plant (or foliar) transfers have been scarcely investigated until recently (Uzu et al., 2010; Bermudez et al., 2011; Hu et al., 2011; Schreck et al., 2012a,b). Actually, the use of combined microscopy and spectroscopy techniques for tissue observations has brought some advances in the understanding of metal pathways (Schreck et al., 2012a). It has been demonstrated that metals can enter root cells: working on Cd particles, Isaure et al. (2006) reported that this metal is localized in vascular bundles of roots and coordinated to sulphur ligands, due to their high affinity with metallic element such as Cd or Hg. Straczek et al. (2008) showed that Zn could have different localizations in roots: intracellular (bound with oxalate) or in the cell walls (linked to COOH/OH groups) and finally bound to intracellular organic acids. Birbaum et al. (2010) reported that finest metallic particles may be incorporated into leaves, whereas large agglomerates are trapped on the surface wax. Depending on plant physiology or environmental factors, metals can cross the cuticle (Chamberlain, 1983; Ward and Savage, 1994; Nair et al., 2010; Uzu et al., 2010). After diffusion through the cuticle, ultrafine particles

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may interact with plant cells (Birbaum et al., 2010) and may be internalized during endocytosis process with phytotoxicity induced (Nair et al., 2010; Schreck et al., 2012a). Li et al. (2012) reported too that Cd exposure involve cellular changes such as modifications in photosynthetic pigments, electrolyte leakage, malondialdehyde (MDA) and antioxidants (ascorbic acid and glutathione) in Artemisia annua L. Phytotoxicity induced by inorganic pollutants was largely studied with the objective of health plants survey (Alt Ali et al., 2004; Ma et al., 2010; Violante et al., 2010). Moreover, in Europe, the development of biotests to highlight media quality is required by the Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as reported by Pereira et al. (2009), Bravin et al. (2010) and Schreck et al. (2011). Actually, the aims of REACH regulation are to ensure a high level of protection of human health and the environment from the risks caused by hazardous materials. Following plant exposure to metal(loid)s, the phytotoxicity of metal(loid)s are also linked to soil environment (Serbula et al., 2012). Actually, the phytotoxicity involved cell damage and varies from species to species. The recovery rate (100%) of plants was recorded (within 14 days) by Serbula et al. (2012). The toxicity induced by Cd exposure involves cellular changes such as modification in membrane antioxidant enzymes (ascorbic acid and glutathione) in fresh tissue (Upchurch, 2008). According to Djebali et al. (2005), foliar fatty acid ratio (C18:3/(C18:2 + C18:1 + C18:0)) is nowadays available to diagnose soil contamination by metals ex situ (AFNOR, 2012) and it was also successfully used in field (Le Guédard et al., 2012a,b). A decrease in the amount of tri-unsaturated fatty acids in higher plants was also observed for tomato seedlings grown in culture solution containing copper or cadmium (Ouariti et al., 1997; Djebali et al., 2005), as well as for pepper and rape seedlings grown in nutrient solution supplemented with Cd (Jemal et al., 2000; Ben Youssef, 2005). However, plants may be exposed to pollutants through both air and soil transfers (Sanità di Toppo and Gabbrigli, 1999; Serbula et al., 2012). To our knowledge, foliar fatty acid composition has not been used so far to diagnose foliar metal uptake, whereas a global approach of plants exposure to metal(loid)s is necessary in order to better assess environmental and health risks (Dmuchowski et al., 2011).

In that scientific context, we intend to answer the following three main issues: does foliar metal(loid) uptake impact the leaf fatty acid composition? Do foliar fatty acid composition in regards to fatty acid composition? Do the two transfer pathways interact? We finally aim to improve the knowledge on the mechanisms of metal(loid) uptake and phytotoxicity. For that purpose, plants were exposed in situ in the immediate vicinity of metal processing company in order to investigate the impact of a complex mixture of metal(loid)s. The industrial study site is firstly described; two complementary field experiments were conducted via both foliar and root pathways. These experiments as well as chemical analyses (fatty acid composition and metal contents) are later described. Finally, statistical analyses explore and confirm relationships between variables and then allow presenting and discussing new insights on mechanisms of metal(loid) transfer and phytotoxicity.

2. Materials and methods

2.1. Industrial site: smelter emissions and courtyard soil

The industrial site chosen for the experimentation was a secondary lead smelter that recycles car batteries: the Society of Chemical Treatments of Metals (STCM, Schreck et al., 2012a,b). It is located in Toulouse, Southwest France in the peri-urban area (43°38′12″N, 01°25′34″E). The rate of bulk atmospheric deposition was estimated to be about 325.2 ± 12.8 mg cm⁻² week⁻¹, with 139.4 ± 7.8 mg cm⁻² week⁻¹ of lead, which is the most concentrated metal. The size of the particles from the channelled emissions of rotary furnaces was already determined: expressed as volume fractions, the majority of PM (89%) was in the 1–100 μm range, 7% were inferior to 1 μm (sub-micronic and nanoparticles) and 4% were superior to 100 μm (Uzu et al., 2011; Schreck et al., 2011). The STCM smelter emissions are assimilated to a mixture of various metals and metalloids. The major elements in the PM were, by mass: Pb (27%), O (15%) and S (7.5%) with no significant differences according to particle size (Schreck et al., 2011). Several other secondary metals: Cd (2.5%), Zn (0.5%), Fe (0.1–0.4%) and Sb (0.1%) were also present (Uzu et al., 2011). The remaining elements to complete 100% were for the most part Cl, Na, Ca and other metals such as Al, As, Cu and Ni recorded as traces. According to Uzu et al. (2009, 2011), particles are mainly composed of metallic sulphides, sulphates, oxides and chorides; lead speciation was, in decreasing order of abundance: PbS, PbSO₄, PbO, Pb₂SO₄, ZnO, Pb₆O₁₃.

Industrial atmospheric fallouts in the smelter courtyard were measured using Owen gauges that enable wet and dry atmospheric depositions to be recorded (Taylor and Witherspoon, 1972; Candois et al., 2010). Two gauges were left exposed throughout the entire experimental period in order to determine the metal(loid)s contents in atmospheric deposits (Schreck et al., 2012b), according to NF EN 1492 (2005).

In the smelter courtyard, the soils are largely polluted: the concentrations of lead vary from 100 to 290 000 mg kg⁻¹ of soil according to sampling sites (parking area, near the batteries storage area, proximity to an infiltration point, etc.). Thus, the soil chosen for the experimentation was sampled in a homogenous polluted zone (Foucault et al., 2013). Pb, Cd, Cu, Zn, As and Sb concentrations were respectively 1650 ± 10.2, 0.8 ± 0.1, 11.9 ± 1.2, 12.2 ± 1.3, 11.9 ± 1.4 and 86.8 ± 3.2 mg kg⁻¹ of dry weight of soil. This historically polluted soil was sampled from the top 0–25 cm soil layer, air-dried at ambient temperature for a week, disaggregated and, finally, sieved to keep aggregates smaller than 2 mm before plant cultivation.

2.2. Experimental set-up

Two complementary field experiments were conducted by exposing lettuce (Lactuca sativa L.) to processes. The first experiment (denoted E₁) considered both foliar and root transfer whereas the second one (denoted E₂) focused only on foliar uptake. Lettuce (Lactuca sativa L.) was chosen for field exposure experiments (E₁ and E₂), metal transfer and phytotoxicity studies. This leafy vegetable, largely consumed by humans (Lebeda et al., 2007), has been widely used as biotest to study soil–plant or air-plant transfers of metals (Waisberg et al., 2004; Alexander et al., 2006; Uzu et al., 2010; Schreck et al., 2012a) with in particular the measure of fatty acid composition in the context of polluted soils (Le Guédard et al., 2008; Le Guédard et al., 2012a,b).

For the experiment E₁, seed germination was carried out with commercial lettuce seeds (cultivar “Battavia Blonde Dorée”), firstly immersed in a 10% sodium hypochlorite solution for 10 min to ensure surface sterility (according to Lin and Xing, 2007; Schreck et al., 2011). After seed germination, young plants were then grown 15 days in a greenhouse on an uncontaminated or a polluted soil, according to the chosen exposure way in the experimental design: air and/or soil-plant uptake (see Fig. 1). Then, two-week-old plants were transferred in different individual pots, containing 4 kg of polluted or not polluted soil, accordingly to exposure experiments. A geotextile membrane was placed on the soil in view of avoiding direct transfer between air and soil in the experiment, as previously described by Uzu et al. (2010) and Schreck et al. (2012a).

The design of the first experiment (E₁) was reported in Fig. 1. The plants were segregated into 4 different batches to study the different transfer pathways and their potential interactions: (condition-1) no pollution or control; (condition-2) soil pollution only; (condition-3) air pollution only and (condition-4) both air and soil pollutions. For conditions (1) and (2), plants were placed in a control area, without any atmospheric contamination. For conditions (3) and (4), plants were exposed to atmospheric fallouts enriched in metals, in the smelter courtyard.

Plant exposure was performed during 4 weeks and the influence of the way of transfer (foliar, root or the 2 both ways at once) was studied each week by determining fatty acid composition of plant leaves according to Le Guédard et al. (2008, 2012a,b), see Section 2.3 for details. Actually, six plants for each condition were dedicated to fatty acid composition analysis. A second experiment (E₂) focusing on foliar uptake was then performed: 30 plants of each condition (not exposed and exposed to atmospheric fallouts) were dedicated both to fatty acid composition and pollutant concentrations analysis as indicated in Fig. 1. Metal concentration in soil is provided elsewhere (Schreck et al., 2012b). Moreover, it’s important to notice that during the whole of the second week of this foliar exposure experiment, the smelter activity was stopped (public holidays); PM was not emitted by the factory during this period.

2.3. Fatty acid extraction, analysis and identification

Both for E₁ and E₂ experiments, sampling consisted of fresh foliar tissue (between 20 and 200 mg, as reported by Le Guédard et al., 2012a,b) taken from the different lettuces grown on the industrial landfill or on the control area. Leaf samples were immediately placed in screw-capped tubes containing 1 ml analytical grade methanol acidified with 2.5% (v/v) H₂SO₄ (Le Guédard et al., 2008; Le Guédard et al., 2012a,b). Samples were stored at 4 °C before analysis.
Fatty acid analysis and identification was then performed according to Le Gueudard et al. (2008, 2012a,b). Tubes containing the leaf samples in acidified methanol were heated to 80 °C for 1 h and then cooled before adding 1.5 ml H2O and 0.75 ml hexane. 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 and helium as carrier gas. The initial temperature of 160 °C was held for 1 min, followed by a 20 °C min⁻¹ ramp to 190 °C and a second ramp of 5 °C min⁻¹ to 210 °C, and held for 6 min. FAMES were identified by comparing their retention times with standards (Sigma Chemical, St. Louis, MO, USA).

2.4. Metal concentration analysis

For the E2 experiment, lettuce tissues were washed according to home-washing processes usually performed before consumption (Birbaum et al., 2010; Uto et al., 2010; Schreck et al., 2012b) in order to focus on sanitary risk induced by ingestion of polluted vegetables. Each week, six samples of plant leaves were 72 h oven-dried within 97°C. The accuracy of measurements was checked using reference materials: Pb, Cd, Cu, Zn, As and Sb concentrations were measured by inductively coupled plasma-mass spectrometry ICP-MS (X Series II, Thermo Electron) according to the method described by using the three-level calibration curve (Alltech) with flame ionization detection and helium as carrier gas. The initial temperature of 160 °C was held for 1 min, followed by a 20 °C min⁻¹ ramp to 190 °C and a second ramp of 5 °C min⁻¹ to 210 °C, and held for 6 min. FAMES were identified by comparing their retention times with standards (Sigma Chemical, St. Louis, MO, USA).

3. Results and discussion

3.1. Fatty acid composition in lettuce leaves across treatments

Fatty acid compositions in lettuce leaves following the E1 experiment are shown in Table 1. It firstly appears that after two weeks in the green-house (under monitored conditions), lettuces...
having grown on the contaminated soil did not display a significant lower C18:3/C18:2 + C18:1 + C18:0) ratio (2.61 ± 0.1) than seedlings having grown on the control soil (2.70 ± 0.5). In addition, it also appears that after an additional (from 1 to 4 week) exposure, this ratio did not significantly decrease in the presence of soil and/ or air contaminants. This indicates that in comparison with our previous studies (Le Guédard et al., 2008; AFNOR, 2012; Le Guédard et al., 2012a,b), following the E1 experiment, the effect of contaminants did not induce a huge oxidative stress. Hence, in comparison with these previous studies, a more thorough statistical analysis of the results was required to determine whether the lettuce fatty acid composition was significantly affected by the soil and/or air field exposures to inorganic pollutants.

3.2. Statistical determination of a parameter to predict the impact on lipid composition of plant exposure to metals and metalloids

Fatty acid composition is variable across treatments, as confirmed by the results of the 3-way MANOVA (Table 2). Isolated factors (Duration, Air and Soil) have significant effects on fatty acid compositions. No interaction term is significant. The consequences of the latter assertion are that: (1) both air and soil metal contaminations have a significant effect on fatty acid composition and (2) soil and air pathways do not interact; they have non-synergetic effects. Fatty acid concentrations are log-transformed, as indicated by the results of the Box–Cox procedure (95% confidence intervals of λ for all 6 fatty acid variables are included within [−0.25, 0.25]), before being provided to the LDA. The proportions of explained variances are 82.32, 90.3, 32.22, 30.15, 0.63, 0.86% on each discriminant. Eigenvectors are provided in Table 3. The projection of data samples on the first two eigenvectors is illustrated in Fig. 2.

As illustrated in Fig. 2, the LDA efficiently discriminates treatment. More specifically, the first component substantially discriminates duration of exposure (Y1 increases with the duration of exposure) and the second component discriminates contamination pathway (Control > Soil > Air > Soil & Air). Such assessments are substantiated by the results of 3-way ANOVAs on LDA outputs. Significance of isolated factors (Duration, Air and Soil) as well as two-factor and three-factor interactions is provided in Table 2. Results of normality (Shapiro–Wilk) and homoscedasticity (Brown–Forsythe) tests are also provided. Results show that residual variance is equal across treatments. Residual variance is normally distributed for variables Y2–Y6. Isolated factors (Duration, Air and Soil) have significant effects on variables Y1–Y6 (depending on the variable) and no interaction is significant. ANOVA results also show that Y1 substantially discriminates duration of exposure better than the other LDA output variables and that Y2 better discriminates contamination pathway. Given that we are more interested in discriminating pathway than duration of exposure, we now focus on the following on the second discriminant:

\[ Y_2 = -11.97\log_{10}(C16:0) + 12.03\log_{10}(C18:1) - 7.12\log_{10}(C18:0) + 3.02\log_{10}(C18:3) - 2.76\log_{10}(C18:2) + 6.85\log_{10}(C13:0) \]

Which is approximately equal to 12 log10 (Z) with:

\[ Z = \frac{(C16:1) - (C18:3)}{(C16:0) \times (C18:3) 0.57 \times (C18:1) 0.23} \]

Table 2

P-values of the tests of the 3-way MANOVA (first column) and the 3-way ANOVAs (remaining columns) which connects fatty acid compositions to treatment factors. Factors are air, soil, duration, and interactions and dependent variables of the ANOVAs are LDA linear combinations of fatty acid contents (C16:0, C16:1, C18:0, C18:1, C18:2, C18:3) which are denoted Y1–Y6. P-values of tests for normality (Shapiro–Wilk) and homoscedasticity (Brown–Forsythe) are also provided (Significance codes: < 0.001 ***; < 0.01 **; < 0.05 *; < 0.05 **).
Z values are of equal spread for all treatments, no matter the duration and the contamination pathway. Values of Z across treatments are illustrated in Fig. 3. The Z quantity has the three following compelling properties. Z is the product of three fatty acid concentration ratios (C16:1/C16:0, C18:3/C18:0, and C18:1/C18:2) with different weights (1, 0.57, and 0.23 respectively). This property will be used later when exploring the relationships between uptake and phytotoxicity. Z is a dimensionless quantity which values are in our experiments comprised between 0 and 1. The latter property will be of interest in order to compare Z values across treatments and across experiments. Finally, Z is normally distributed and of equal variance across the 18 groups (Shapiro–Wilk: \( p = 0.35 \); Brown–Forsythe: \( p = 0.58 \)). Consequences are facilitated subsequent statistical parametric testing. Three-way ANOVA results show that isolated factors (Air, Soil, and Duration) have significant effects on Z, and no interaction is significant (Table 4). Values of the Z quantity across treatments are illustrated in Fig. 3. Values of the three ratios C16:1/C16:0, C18:3/C18:0, and C18:1/C18:2 are illustrated in Supplementary Material S2 and significance of ANOVA testings with respect to treatment factors are provided in Table 4.

### Table 3

<table>
<thead>
<tr>
<th>Discriminant</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
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<tr>
<td>( \log_{10}(C16:0) )</td>
<td>-0.28</td>
<td>-11.97</td>
<td>-6.85</td>
<td>8.44</td>
<td>19.75</td>
<td>-2.50</td>
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<td>( \log_{10}(C16:1) )</td>
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<td>12.03</td>
<td>-1.42</td>
<td>-2.62</td>
<td>-4.96</td>
<td>-5.78</td>
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<tr>
<td>( \log_{10}(C18:0) )</td>
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<td>2.48</td>
<td>-9.26</td>
<td>-4.22</td>
<td>-4.72</td>
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<tr>
<td>( \log_{10}(C18:1) )</td>
<td>-3.05</td>
<td>3.02</td>
<td>3.84</td>
<td>9.52</td>
<td>-2.77</td>
<td>-1.27</td>
</tr>
<tr>
<td>( \log_{10}(C18:2) )</td>
<td>5.62</td>
<td>-2.76</td>
<td>8.99</td>
<td>-6.53</td>
<td>10.07</td>
<td>9.35</td>
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<tr>
<td>( \log_{10}(C18:3) )</td>
<td>-6.49</td>
<td>6.85</td>
<td>-6.23</td>
<td>-1.22</td>
<td>-16.97</td>
<td>4.04</td>
</tr>
<tr>
<td>Proportion (%)</td>
<td>82.32</td>
<td>9.03</td>
<td>3.22</td>
<td>3.02</td>
<td>1.55</td>
<td>0.86</td>
</tr>
</tbody>
</table>

3.3. Interactions between metals uptake and fatty acids of Lactuca sativa L. leaf membranes: a new way to explore relationship between uptake and phytotoxicity

We mention above that oxidative stress very likely did not occur in plants during \( E_1 \) experiment. In agreement, the fatty acid ratio with the most important weight in Z values is the C16:1/C16:0 ratio, and it is generally admitted that mono-unsaturated fatty acids are not targets of oxidative stress as reported by Cipak et al. (2006, 2008). C16:1 fatty acid in leaves is (almost) exclusively associated with thylakoid phosphatidylglycerol (PG), and the absence of this unsaturated fatty acid associated with this phospholipid may impact photosynthesis activity (Ivanov et al., 2012). Hence, Z parameter formula suggests that process particles, carrying a mixture of metal(loid)s, initially induce an early alteration on chloroplast membranes, whatever the way of uptake involved (air-leaf or soil-root pathway), and could lead later to the disruption of photosynthetic function, finally involving phytotoxicity (Kobayashi et al., 2007; Aronsson et al., 2008).

C16:1 fatty acid in leaves is synthesized from C16:0 (associated with PG) by a plastidial fatty acid desaturase. Hence, it appears that the simplest explanation for the weight of the C16:1/C16:0 ratio in Z values is to assume that this desaturase is inhibited by metal uptake. Actually we focused on week 1 and week 2 (highest differences in Z values), and we considered, in the absence of contaminants, that for 1000 C16:0 synthesized molecules, 32 molecules of C16:1 were synthesized, and that 76.5% of the remaining C16:0 molecules were elongated to synthesize C18:0. We also considered that 97.6%; 97.7% and 77% of C18:0, C18:1; C18:2 were desaturated to form C18:1, C18:2 and C18:3 respectively. These assumptions lead to the following fatty acid composition: 22.7%; 3.2%; 1.8%; 1.7%; 16.2% and 54.4% of C16:0; C16:1; C18:0; C18:1, C18:2 and C18:3 respectively (supplementary material S3), closed to the

Fig. 2. Projection of samples on the first two LDA eigenvectors for \( E_1 \) experiment.
experimental values shown in Table 1 for weeks 1 and 2 in the absence of pollutants. By assuming that both air and soil contaminants inhibited by 25% the desaturation of C16:0 molecules, and that there was an additive effect of soil and air contaminants, we obtained 0.59; 0.43; 0.43 and 0.32 for Z values in the absence and in the presence of soil, air, and both soil and air contaminants respectively. This illustrates the heavy weight of the C16:1/C16:0 ratio in Z value.

The appearance of C18:3/C18:0 and C18:1/C18:2 in Z calculations could be explained by a very slight effect of air pollution (only) on the C18:0 desaturation (96.3% instead of 96.7% of C18:0 desaturated), and by a very slight effect of soil pollution (only) on the C18:2 desaturation (78.5% instead of 77% of C18:2 desaturated). This leads to 0.59; 0.45; 0.41 and 0.31 for Z values in the absence and in the presence of soil, air, and both soil and air contaminants respectively, and the resulting fatty acid compositions indicated are closed to the experimental values shown in Table 1 for week 1 and 2 in the absence and in the presence of pollutants (Supplementary Material S3).

All of these data explain why both air and soil metal contaminations have various significant (Table 4) effects on the various fatty acid ratios, while they have non-synergistic and non-antagonist effects.

3.4. Leaf fatty acid composition in lettuce shoots following foliar E2 exposure

The soundness of the potential use of the Z parameter to evidence air pollution is assessed by using data from the second experiment (E2). Fatty acid composition across treatments is provided in Table 5 and Z values are illustrated in Fig. 4. Results of normality, homoscedasticity, and ANOVA tests show that Z is still normally distributed (p = 0.304335) and of equal variance across treatments (p = 0.078323). Both Air and Duration have a significant effect on Z (Air, p = 0.009904**; Duration, p = 0.834278; Air × Duration, p = 0.000045***).

Differences between control and exposed are not significant at week 1 (p = 0.9999) and week 2 (p = 0.1958). Differences are significant at week 3 (p = 0.01663*) and week 4 (p = 0.0032**) and, similar to what has been found regarding the E1 experiment, Z is lower for contaminated plants. In addition, it can be noted that looking at the control experiments (i.e. in the absence of contaminants) the changes in the fatty acid composition as a function of time (a decrease in 18:3) observed following the E1 experiment were not observed in E2. This is likely because light, temperature and/or humidity were not the same, but we are unable to demonstrate it (it was not the aim of the present study, and the experiments were not designed to study this point). Nevertheless, this observation strengthens the Z because despite these differences, this parameter made it possible to evidence adverse effects of pollutants following both E1 and E2 experiments.

Schreck et al. (2012b) have shown a high foliar uptake of metals (reissued in Supplementary material S1). As mentioned above, in the present experiment, emissions from plant was stopped during week number 2 with a subsequent reduction of PM fallouts, and the stop in smelter activity was therefore also observed as a break in metal uptake by plant. Hence following the E2 experiment, it appears that the Z which has been defined earlier is efficient in

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**Table 4**

<table>
<thead>
<tr>
<th>Z</th>
<th>C16:1/C16:0</th>
<th>C18:3/C18:0</th>
<th>C18:1/C18:2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air × Soil</td>
<td>0.352496</td>
<td>0.007205 **</td>
<td>0.262648</td>
</tr>
<tr>
<td>Brown–Forsythe</td>
<td>0.580434</td>
<td>0.232108</td>
<td>0.470564</td>
</tr>
<tr>
<td>Air</td>
<td>0.000000 ***</td>
<td>0.001366 **</td>
<td>0.00532 ***</td>
</tr>
<tr>
<td>Soil</td>
<td>0.000064 ***</td>
<td>0.000131 ***</td>
<td>0.319733</td>
</tr>
<tr>
<td>Duration</td>
<td>0.000185 ***</td>
<td>0.000000 ***</td>
<td>0.000004 ***</td>
</tr>
<tr>
<td>Air × Duration</td>
<td>0.533569</td>
<td>0.420212</td>
<td>0.810316</td>
</tr>
<tr>
<td>Air × Soil</td>
<td>0.201538</td>
<td>0.274224</td>
<td>0.751036</td>
</tr>
<tr>
<td>Soil × Duration</td>
<td>0.061689</td>
<td>0.756811</td>
<td>0.212121</td>
</tr>
<tr>
<td>Air × Soil × Duration</td>
<td>0.182960</td>
<td>0.478196</td>
<td>0.694727</td>
</tr>
</tbody>
</table>

---

Fig. 3. Box-and-whisker plots of Z parameter across treatments for E1 experiment.
highlighting differences in air quality near a factory, and, the fatty acid composition seems to be a sensitive but also a reversible parameter. Nevertheless, no significant correlation could be highlighted between metal concentrations in plant leaves and Z values (results not shown); Z parameter is only a qualitative marker of effect of metal pollution.

4. Conclusions and perspectives

This study opens up the answers to the three main issues which were raised in the introduction part. We evidenced that, as root transfer, foliar transfer also impacts fatty acid composition, that the synergistic combination of statistical and ecotoxicological approaches provide new insights for understanding the mechanisms involved in metal(loid) uptake by plants and phytoxicity. Thus, further studies such as microscopy, spectroscopy and isotope fractionation are actually in progress to explore the additional implication of biotic (e.g. plant species) and abiotic (exposure duration, pedoclimatic conditions, metal speciation) factors on uptake and phytoxicity.

References


Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2013.04.024.

Table 5

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration (week)</th>
<th>C16:0 (%)</th>
<th>C16:1 (%)</th>
<th>C18:0 (%)</th>
<th>C18:1 (%)</th>
<th>C18:2 (%)</th>
<th>C18:3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>20.7 ± 0.9</td>
<td>1.7 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.8 ± 0.3</td>
<td>22.1 ± 1.3</td>
<td>52.1 ± 1.5</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>23.8 ± 1.9</td>
<td>1.6 ± 0.2</td>
<td>2.2 ± 0.8</td>
<td>1.6 ± 0.2</td>
<td>17.5 ± 1.8</td>
<td>53.3 ± 2.2</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>22.1 ± 1.3</td>
<td>2.1 ± 0.1</td>
<td>2.5 ± 1.2</td>
<td>3.3 ± 0.6</td>
<td>18.2 ± 1.5</td>
<td>51.8 ± 2.7</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>22.8 ± 1.6</td>
<td>2.3 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>2.3 ± 0.3</td>
<td>19.1 ± 3.0</td>
<td>51.7 ± 1.7</td>
</tr>
<tr>
<td>Air</td>
<td>1</td>
<td>20.5 ± 1.9</td>
<td>1.7 ± 0.3</td>
<td>2.3 ± 1.0</td>
<td>2.1 ± 0.8</td>
<td>20.1 ± 1.1</td>
<td>53.3 ± 3.6</td>
</tr>
<tr>
<td>Air</td>
<td>2</td>
<td>21.2 ± 0.6</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.6</td>
<td>1.9 ± 0.5</td>
<td>19.0 ± 0.8</td>
<td>54.4 ± 1.1</td>
</tr>
<tr>
<td>Air</td>
<td>3</td>
<td>24.2 ± 1.3</td>
<td>1.6 ± 0.3</td>
<td>3.3 ± 1.7</td>
<td>3.1 ± 1.5</td>
<td>15.8 ± 1.7</td>
<td>51.9 ± 3.2</td>
</tr>
<tr>
<td>Air</td>
<td>4</td>
<td>26.7 ± 1.5</td>
<td>1.6 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>13.2 ± 0.9</td>
<td>55.4 ± 1.2</td>
</tr>
</tbody>
</table>

Fig. 4. Box-and-whisker plots of Z across treatments for air-plant exposure in E2 experiment.

Acknowledgements

We gratefully acknowledge ADEME, the French Agency of Environment and Energy for funding the DIMENSION project, as well as STCM, Chemical Metal Treatments Company for its technical help in experimental set-up and financial support.


