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Trace elements in the pollen of *Ambrosia artemisiifolia*: What is the effect of soil concentrations?

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HIGHLIGHTS

- Concentrations of ragweed pollens: $\text{Zn} > \text{Mn} > \text{Ba} \approx \text{Cr} \approx \text{Cu} \approx \text{Ni} \approx \text{Pb} > \text{Cd} \gg \text{Ti}$.
- Mean pollen to root ratios always < 1 ; Cu, Ni and Zn have maximum ratios > 1 .
- Significant models of pollen concentrations for Cd, Ni and Pb, not for Cu and Zn.
- All models involve positive relations between trace elements in pollens and soils.
- Maximum estimate of monthly trace element inhale by humans through pollens < 50 ng.

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ABSTRACT

Concentrations of nine trace elements (Ba, Cd, Cr, Cu, Mn, Ni, Pb, Ti and Zn) were measured in a plant bearing allergenic pollens (ragweed) and their transfers from soils to the roots and then to the pollens were investigated. The soil, roots and pollens collected from flowers were sampled at 26 urban sites. Soil pH, soil organic carbon and total-recoverable trace elements (TE) in soil, roots and pollens were measured. The three biogeochemical compartments are well discriminated according to their TE concentrations. The concentrations (in $\mu\text{g g}^{-1}$) in pollens decreased as follow: $\text{Zn} (59.5\text{--}205) > \text{Mn} (19.4\text{--}117) > \text{Ba} \approx \text{Cr} \approx \text{Cu} \approx \text{Ni} \approx \text{Pb} (0.54\text{--}27.7) > \text{Cd} (0.06\text{--}0.77) \gg \text{Ti} (0.0015\text{--}0.0180)$. Mean elemental allocation within ragweed always favored roots over pollen but, at site level, inverse pattern is also observed mostly for Zn and slightly for Cu and Ni. Significant predictive models of TE concentrations in pollens were obtained using soil or root properties only for Cd, Ni and Pb. They all involved positive relationships between TE concentrations in pollens and in soil or roots. Estimates of short-term exposure of human to TE carried out by ragweed pollens indicate TE absorption of less than 50 ng, far below thresholds of air quality criteria. Investigating the TE chemistry of pollens is a required first step to validate the impact of TE in pollens on human health and on the prevalence and intensity of allergy symptoms and atopic diseases.

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1. Introduction

Trace elements (TE) can be toxic to organisms and ecological functions when present in available forms and at concentrations exceeding their background levels in undisturbed environments. In some areas, even the natural TE concentration can constitute a threat to the biosphere as a result of their accumulation in soils through biogenic, geogenic, and pedogenic processes (Garrett, 2000). Several examples of local soil contamination attributed to natural conditions are documented for TE such as arsenic (As), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) (Armiento et al.,

2011). When the available fraction of TE is considered, their toxicities generally follow a dose-time response relationship, where the intensity of the toxic effect increases with the amount absorbed and the duration of the contact between elements and organisms (Kabata-Pendias and Mukherjee, 2007). Industrialization has significantly increased TE concentrations in the various environmental compartments of the geosphere, especially in the atmosphere, where TE originate mostly from human activities (Kabata-Pendias and Mukherjee, 2007). Once in the atmosphere, TE associated to small particles (aerosols, dusts, pollen) can migrate over long distances. Aerial transport can therefore act as a source of TE for ecosystems located far from the source (Garrett, 2000). Moreover, atmospherically-bound TE can enter animal and human bodies directly through the respiratory system, notably when TE-containing particles are present in the air and act as carriers.

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Allergies and atopic diseases have become a public health concern in industrialized countries where increases in the prevalence were observed as well as the increase intensity of symptoms for sensitive populations (Ring et al., 2001; D'Amato et al., 2007). The reasons behind these trends are unknown, but one hypothesis suggests that the interaction between air pollutants (e.g. aerosols, particles, TE, SO₂, ozone) and airborne pollen grains could be involved (Ring et al., 2001; D'Amato et al., 2007). Two recent studies reported direct evidences of relationships between the presence of TE and pollen allergy. Aina et al. (2010) revealed that the pollen grains of the grass species *Poa annua* L. had a higher amount of allergens when grown on cadmium (Cd) contaminated soils than pollen of the same plant grown on a similar uncontaminated soil. Bellanger et al. (2012) further reported that a mean Pb concentration of 4.4 µg g⁻¹ in pollen grains was associated to an increase in the gene expression of a pollen allergic mediator (interleukin-5) in alveolar epithelial cells, and that the reaction followed a dose-time response. Three others allergic or inflammatory mediators considered in this study were not affected by Pb concentration. Although both studies point to a potential active role of TE on pollen allergy, the working hypothesis linking TE to the prevalence and the intensity of allergy symptoms still needs to be validated. Moreover, the mechanisms involved in the increased allergic reaction are poorly known.

One of the key constraints to achieving this goal is the lack of data on the TE chemistry of pollen grains. Moreover, the few published studies measuring TE concentrations in pollen grains all followed distinct methodologies (pollen collection in beehives vs in flowers) or experimental approaches (field study vs controlled artificial environment) and focused on different environments (urban vs non-urban) or trace elements (Kalbande et al., 2008; Lambert et al., 2012; Silva et al., 2012). For TE documented in more than one study, variations in pollen concentrations were often very large and could reach up to four orders of magnitude for Cu and seven orders of magnitude for Pb. For example, Lambert et al. (2012) sampled pollen of various plant origins directly in beehives of 18 sites of northwestern France surrounded by a diversity of land-use management and intensities of anthropogenic activities. The pollen samples contained highly variable (0.004–0.798 µg g⁻¹) Pb concentrations that was mainly attributed to temporal changes and spatial contrasts in land-use. Kalbande et al. (2008) conducted a study in a polluted urban area and measured similar, or slightly higher, Pb concentrations in pollen grains collected directly from flowers of *Cassia siamea*, *Cyperus rotundus* and *Kigelia pinnata*. When the pollen grains were exposed to the local polluted air, the concentrations of Pb and of other TE in pollens increased by a factor of up to 87-fold (Kalbande et al., 2008). It was suggested that the irregular external morphology of pollen grains favored the physical retention of TE-rich fine particles with a consequent increase in concentration (Ring et al., 2001). Evaluating the concentration range of TE in pollen and identifying the environmental factors controlling their allocation to pollen grains however remains a challenge.

Trace elements are not only attached to external pollen grains surfaces during atmospheric dispersion and following successive contacts with exposed soil surfaces, they are also sequestered in pollen grains as a consequence of root uptake from the soil. Moreover, pollen is the plant part considered to be the most sensitive to TE toxicity (Wolters and Martens, 1987). The sequestration of TE in pollen grains should therefore be anticipated to be low compared to other plant parts that are less sensitive to toxicity and not directly involved in plant reproduction. Such comparison between TE concentrations in pollen grains and other plant parts or soil materials was never performed. It follows that new data are needed for quantifying TE concentrations in pollen and for identifying the soil properties and processes

promoting the mobility of TE in the soil–plant system. This is a required first step to understand the potential role of TE on the prevalence and the intensity of allergy symptoms in human populations exposed to allergenic plants. Exposure to pollen grains contaminated by trace elements could also possibly affect non-atopic patients.

In this context, the transfer of TE from urban soils to a plant bearing allergenic pollen grains was investigated, emphasizing on the TE chemistry of pollen. The two specific objectives of the present work were: (1) to quantify the TE concentration of pollen grains sampled directly from plant flowers, prior to their release to the atmosphere and (2) to predict the concentration of TE in pollen grains using soil properties and TE concentrations in plant roots. The study was conducted in the Montréal metropolitan area (Canada), with common ragweed (*Ambrosia artemisiifolia*) as the model allergenic herbaceous plant species and focused on the TE barium (Ba), Cd, chromium (Cr), Cu, manganese (Mn), Ni, Pb, thallium (Tl) and Zn.

2. Material and methods

2.1. Description of field sites

A total of 26 urban sites were identified in the greater Montréal metropolitan area (Canada) based on the close proximity of human activities acting as sources of TE and on the abundance of *Ambrosia artemisiifolia* colonies (Fig. 1). This herbaceous plant is widespread in temperate regions and produces large amounts of allergenic pollen (D'Amato et al., 2007). The selected sites had *Ambrosia* colonies containing at least 20 individual plants and a mixture of young and mature plants. The variability of the dataset, notably for the TE concentrations in soils and plant tissues, was maximized by selecting sites covering a wide range of land use and located at various distances from potential TE sources, such as roads, highways or industrial sites. The land-use characteristics of the 26 samples and their basic soil properties are presented in Supplementary information file (SI 1).

2.2. Samples collection and preparation

The soil and plant samples were collected in August 2008, during the pollen season of *Ambrosia artemisiifolia*. At each site, the soil materials were sampled directly beneath the *Ambrosia artemisiifolia* plants from which roots and mature male inflorescences were collected. Three field composite samples of each environmental matrices (soil, root and pollen) were collected at each site in order to take into consideration the in site heterogeneity. For pollen, a mass of 500 mg of fresh pollen grains was targeted to perform chemical analyses. Therefore, a composite pollens sample was produced using between 15 and 30 inflorescences depending on their individual size and on the amount of pollens they contained. The composite samples of all matrices were well homogenized in the field. The pollen (~500 mg) and root (~50 g) samples were all transferred to the laboratory while only a representative fraction (~100 g) of the composite soil samples was sent to the laboratory. The soil samples were air-dried and then sieved at either 2 mm or 0.5 mm for subsequent chemical analyses. The roots sampled were carefully rinsed with deionized water to remove the attached soil materials and were dried at 60 °C. For each plant, the roots having a diameter of 1 mm or less and considered to be most active in elemental uptake were selected and ground. The mature male flowers were dried at 60 °C in paper bags and the pollens extracted by sieving using a 120 µm Nytex filter.

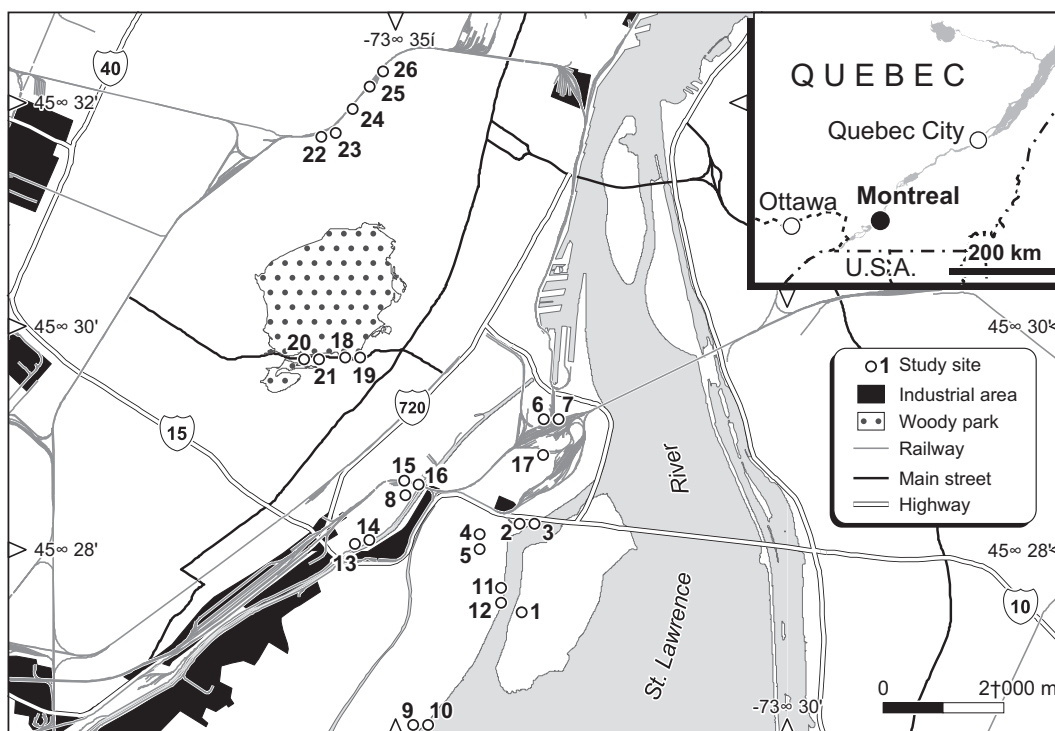


Fig. 1. Location of the 26 urban sites where soils and *Ambrosia artemisiifolia* roots and pollen grains were collected in Montréal (Canada) during the summer of 2008.

2.3. Chemical analyses

Chemical characterization of the soil materials was performed by measuring pH and organic carbon (SOC) content. The soil sub-fraction sieved at 2 mm was used to measure pH in deionised water at a soil:water ratio of 1:2 (Hendershot et al. 2008). The SOC content was determined by the dichromate redox method (Skjemstad and Baldock 2008) on soil materials sieved at 0.5 mm.

Measurements of the total-recoverable TE concentrations were performed on all samples, except for Ba, Cr, Mn and Tl that were measured only on pollen grains, using an acid digestion procedure (Benton Jones et al., 1990). Briefly, the samples, either 0.500 g soil and 0.200 g roots or pollen, were soaked overnight in 2 mL of concentrated nitric acid (trace metal grade HNO_3). Samples were then digested on a bloc digester at an average temperature of 120 °C for 5 h. Afterwards, the digest solution volume was adjusted with deionised water to 50 mL. The solutions were then slowly decanted for 24 h directly in tubes, at ambient temperature and on the laboratory bench, to retain only the supernatant. Decantation is safer to separate the solution from the solid residues compared to centrifugation that has a tendency to generate flocs due to the acidity of the solution with the associated risk of TE scavenging from the solution. The TE (Ba, Cd, Cr, Cu, Mn, Ni, Pb, Tl and Zn) concentrations were measured using ICP-mass spectrometry (Varian ICP-MS 820) by selecting the following isotopes for the total-recoverable TE measurement; ^{135}Ba , ^{111}Cd , ^{53}Cr , ^{65}Cu , ^{55}Mn , ^{62}Ni , ^{207}Pb , ^{205}Tl and ^{68}Zn . The detection limit of the method (DLM) was calculated independently for soils and *Ambrosia* tissues matrixes by multiplying by three the standard errors, obtained from multiple analyses of solutions with low solute concentrations (0.5 or 1 $\mu\text{g L}^{-1}$). The DLM are presented for all TE in Tables 1 and 2.

All analytical procedures in the laboratory were triplicated and were made with acid-washed (20% HNO_3) glassware and plastic materials. Details on the quality of the chemical analyses are provided in Supplementary information file (SI 2.1).

2.4. Statistical analyses

All the data treatments were performed using the R open-source software (R Core Team, 2013) and a significance level of up to $\alpha = 0.10$ was accepted. As a preliminary step, the normality of the distribution was evaluated for all variables using the Shapiro–Wilk test (R function *shapiro.test* from *stats* library). If necessary, a logarithmic transformation was applied to normalize the distribution. Multivariate data treatments, performed on standardized variables, were then applied to characterize and describe the dataset. A principal component analysis was first used to evaluate the patterns in TE concentrations in the pollen grains. As a second step, differentiation among the three environmental components sampled (soil, root and pollen) was assessed using a linear discriminant analysis based on the TE measured in the three components. Finally, the transfer of TE from soils to plants was described using multiple linear regressions. Details on the multivariate statistical analyses are available in Supplementary information file (SI 2.2).

3. Results

3.1. Trace element concentrations in soils, roots and pollen grains

Soils were sampled together with *Ambrosia* roots and pollen at 26 urban sites that had been disturbed at various levels of intensity and by different types of anthropogenic activities (Fig. 1 and SI 1). The sites covered a wide spectrum of soil properties, with pH ranging from 5.83 to 8.39 and SOC content varying between 11.1 and 82.2 g kg^{-1} (SI 1). The total-recoverable TE concentrations in soils followed the trend $\text{Zn} > \text{Pb} > \text{Cu} > \text{Ni} > \text{Cd}$ and soil contamination generally involved mixtures of two to four TE (Table 1). The variation in soil concentrations between sampling sites extended slightly over two orders of magnitude for Pb, was high for Cd, Cu and Zn but remained low for Ni. The lowest TE concentrations were always measured at site 1 (Table 1) whereas sites 15, 17 and 23

Table 1
Trace element concentrations ($\mu\text{g g}^{-1}$) in soils and in *Ambrosia artemisiifolia* roots and pollen grains collected in the Montréal (Canada) metropolitan area in August 2008.

Sites	Cd ^a			Cu ^a			Ni ^a			Pb ^a			Zn ^a		
	Soil	Root	Pollen	Soil	Root	Pollen	Soil	Root	Pollen	Soil	Root	Pollen	Soil	Root	Pollen
	$\mu\text{g g}^{-1}$														
1	0.13 (0.02)	0.84 (0.07)	0.22 (0.15)	9.33 (0.17)	59.2 (3.46)	16.4 (2.48)	12.6 (0.14)	6.15 (0.65)	1.63 (0.30)	6.07 (0.13)	20.7 (2.20)	1.00 (0.35)	26.8 (3.06)	187 (0.07)	77.9 (10.8)
2	1.27 (0.07)	0.54 (0.16)	0.12 (0.04)	101 (2.92)	36.2 (11.8)	19.9 (1.07)	32.7 (2.54)	3.11 (0.73)	2.82 (0.28)	129 (7.39)	11.0 (3.36)	2.08 (0.20)	525 (84.2)	260 (77.3)	113 (16.3)
3	0.51 (0.04)	0.45 (0.02)	0.10 (0.02)	37.2 (1.75)	73.2 (1.85)	15.7 (1.63)	26.8 (1.66)	5.86 (0.55)	2.25 (0.78)	71.7 (6.51)	12.6 (0.96)	2.40 (1.21)	145 (16.5)	63.1 (3.47)	80.5 (9.99)
4	0.62 (0.09)	0.38 (0.05)	0.17 (0.03)	103 (20.9)	130 (41.2)	27.7 (2.38)	41.0 (5.12)	6.84 (0.74)	12.6 (2.85)	52.6 (3.94)	9.45 (1.59)	6.85 (0.34)	328 (13.9)	184 (33.5)	167 (19.1)
5	0.54 (0.03)	0.44 (0.03)	0.07 (0.01)	46.3 (1.64)	68.6 (5.31)	20.9 (3.46)	27.6 (1.13)	8.26 (0.83)	5.24 (0.80)	68.3 (5.34)	15.5 (1.31)	2.47 (0.71)	138 (3.51)	109 (9.97)	95.9 (5.52)
6	0.44 (0.05)	0.42 (0.04)	0.15 (0.06)	38.7 (3.29)	139 (11.9)	17.6 (4.51)	26.2 (1.37)	6.81 (0.61)	3.41 (1.13)	237 (133)	35.8 (4.13)	3.26 (2.13)	213 (44.7)	160 (15.8)	95.1 (24.3)
7	0.54 (0.01)	0.24 (0.01)	0.06 (0.01)	37.5 (1.03)	31.9 (1.71)	17.7 (2.00)	24.1 (1.51)	4.65 (0.30)	4.52 (0.85)	134 (19.1)	20.2 (0.57)	1.59 (0.45)	256 (5.91)	63.4 (3.93)	92.2 (13.8)
8	1.29 (0.04)	0.71 (0.07)	0.20 (0.09)	76.8 (1.78)	53.9 (5.12)	14.3 (0.24)	33.6 (1.75)	3.97 (0.29)	1.72 (0.48)	443 (13.3)	38.2 (2.26)	7.87 (6.18)	406 (27.0)	129 (12.4)	75.5 (25.3)
9	1.88 (0.08)	0.70 (0.23)	0.11 (0.04)	40.9 (2.17)	68.8 (20.3)	16.4 (1.25)	19.6 (0.85)	5.15 (2.63)	1.56 (0.37)	104 (4.41)	20.5 (9.58)	1.50 (0.75)	331 (10.8)	76.3 (28.4)	65.6 (14.5)
10	0.80 (0.02)	0.73 (0.05)	0.11 (0.02)	44.5 (1.99)	70.3 (4.37)	15.6 (0.92)	27.3 (0.36)	5.70 (0.63)	2.96 (0.96)	121 (20.0)	23.8 (2.79)	2.95 (0.98)	173 (3.80)	81.4 (4.36)	71.0 (16.9)
11	0.91 (0.01)	0.78 (0.05)	0.13 (0.04)	33.8 (0.63)	36.5 (2.59)	12.7 (1.30)	26.0 (0.42)	4.26 (0.18)	1.53 (0.17)	119 (1.16)	17.7 (1.69)	1.89 (1.07)	190 (1.95)	64.2 (2.73)	59.5 (14.5)
12	0.68 (0.02)	0.59 (0.02)	0.08 (0.001)	34.9 (1.67)	119 (3.43)	13.3 (0.31)	26.9 (0.41)	5.56 (0.27)	2.23 (0.44)	104 (3.36)	21.0 (0.91)	2.07 (0.99)	131 (2.85)	69.2 (1.39)	66.6 (7.43)
13	0.64 (0.03)	0.29 (0.05)	0.09 (0.02)	97.5 (21.1)	98.4 (18.1)	13.5 (1.21)	31.3 (1.76)	4.68 (0.87)	2.26 (0.13)	106 (3.56)	15.6 (2.70)	2.24 (0.52)	213 (9.31)	90.9 (17.0)	205 (146)
14	0.63 (0.03)	0.29 (0.02)	0.08 (0.02)	87.9 (9.67)	80.6 (2.49)	16.2 (0.93)	34.8 (1.99)	4.52 (0.56)	2.69 (0.13)	76.7 (6.82)	9.27 (0.68)	2.33 (1.03)	236 (8.66)	124 (19.1)	126 (54.9)
15	3.23 (0.15)	0.41 (0.16)	0.34 (0.08)	273 (4.12)	75.3 (24.9)	15.8 (1.41)	105 (6.48)	4.50 (1.68)	2.81 (0.34)	211 (4.27)	9.90 (6.57)	2.30 (1.09)	1 475 (59.1)	57.1 (17.5)	82.4 (14.8)
16	1.17 (0.03)	1.68 (0.33)	0.13 (0.06)	202 (2.80)	123 (25.5)	16.2 (1.44)	41.9 (1.02)	4.60 (1.01)	2.29 (0.50)	454 (47.2)	44.8 (10.5)	2.58 (1.54)	315 (11.3)	136 (34.7)	76.0 (12.1)
17	7.06 (2.64)	2.60 (0.60)	0.25 (0.04)	321 (171)	100 (23.0)	18.3 (1.30)	46.2 (1.22)	4.44 (1.34)	2.73 (0.29)	255 (37.0)	26.7 (6.69)	5.81 (0.34)	973 (76.4)	301 (68.5)	128 (40.0)
18	0.55 (0.02)	0.54 (0.12)	0.14 (0.09)	44.5 (1.34)	84.7 (17.4)	20.5 (2.14)	19.5 (0.27)	4.21 (0.84)	2.27 (0.46)	73.4 (1.93)	70.8 (12.1)	2.83 (1.57)	151 (7.20)	169 (35.0)	83.5 (15.0)
19	4.36 (0.14)	4.04 (0.16)	0.77 (0.25)	76.9 (2.09)	22.8 (1.08)	23.1 (1.52)	19.8 (0.12)	2.32 (0.16)	2.58 (0.15)	114 (3.07)	8.38 (0.47)	2.60 (1.37)	261 (3.93)	80.0 (3.87)	82.7 (4.50)
20	0.48 (0.02)	0.60 (0.10)	0.15 (0.02)	38.9 (2.87)	25.2 (4.08)	19.9 (1.25)	16.7 (0.55)	3.04 (0.52)	3.11 (0.64)	73.4 (12.7)	23.3 (3.29)	4.04 (1.47)	127 (2.06)	58.8 (9.04)	77.4 (9.44)
21	2.31 (0.06)	1.23 (0.05)	0.22 (0.05)	127 (5.49)	105 (3.77)	19.4 (0.98)	40.0 (2.44)	5.70 (0.66)	1.95 (0.14)	391 (16.6)	28.2 (2.88)	5.10 (2.17)	508 (8.87)	77.0 (4.32)	74.4 (7.63)
22	0.56 (0.04)	0.59 (0.19)	0.23 (0.13)	71.7 (1.21)	93.8 (28.7)	20.3 (2.65)	33.5 (2.59)	8.07 (2.96)	4.11 (0.70)	108 (4.42)	17.4 (5.56)	3.65 (2.73)	306 (40.7)	163 (58.9)	96.8 (16.7)
23	5.56 (0.16)	1.01 (0.11)	0.26 (0.07)	303 (14.3)	111 (10.5)	19.4 (2.51)	74.2 (5.83)	5.07 (0.59)	4.55 (0.46)	887 (24.0)	33.5 (5.87)	5.21 (2.71)	1 174 (52.6)	146 (19.3)	115 (18.4)
24	1.85 (0.11)	0.48 (0.07)	0.12 (0.004)	265 (18.2)	152 (22.3)	19.5 (2.16)	66.0 (5.11)	5.59 (0.92)	2.14 (0.38)	431 (34.4)	34.2 (5.76)	2.86 (1.63)	530 (42.4)	115 (32.0)	69.7 (1.37)
25	0.49 (0.01)	0.44 (0.09)	0.12 (0.03)	19.0 (0.28)	32.4 (11.0)	14.9 (0.88)	14.2 (0.23)	1.76 (0.35)	1.13 (0.12)	51.9 (2.93)	12.5 (2.50)	1.66 (0.83)	119 (0.49)	281 (55.5)	81.6 (16.1)
26	0.42 (0.06)	0.31 (0.05)	0.11 (0.02)	25.3 (4.13)	47.1 (54.3)	18.5 (1.63)	15.1 (2.20)	2.44 (0.21)	1.99 (0.32)	69.5 (12.0)	9.73 (1.01)	3.05 (0.38)	352 (48.8)	113 (16.2)	85.2 (8.68)
Res/Ind ^b	10.0/22.0			63.0/ 91.0			50.0/50.0			140/600			200/360		
DLM ^c	0.012			0.09			0.09			0.07			0.22		

^a Data in parenthesis are the standard deviation obtained for three field replicates. For each trace element, significant differences in concentrations were observed between environmental compartments (soil, roots and pollen grains) according to the non-parametric Kruskal–Wallis test ($\alpha = 0.05$).

^b Soil criteria derived from the Canadian environmental quality guidelines (CCME, 2012) for residential (Res) and industrial (Ind) land uses.

^c DLM = detection limit of the method in $\mu\text{g L}^{-1}$.

had some of the highest TE concentrations. Moreover, significant positive Spearman correlations (data not shown; $r > 0.42$; $p < 0.05$) between soil total-recoverable TE concentrations and SOC content suggested that SOC partly regulated the retention of TE in these soils. According to the Canadian legislation (CCME, 2012), soils at several sites exceeded the concentration authorized for residential (13 sites for Cu, three for Ni, eight for Pb and 17 sites for Zn) or industrial (nine sites for Cu, three for Ni, one for Pb and seven sites for Zn) uses, although Cd concentrations always remained below the environmental thresholds (Table 1).

The total-recoverable TE concentrations in *Ambrosia artemisiifolia* roots and pollen grains are presented in Table 1 for Cd, Cu, Ni, Pb and Zn, similar to soil contents, while Table 2 documents TE (Ba, Cr, Mn and Ti) measured only in pollen grains. In the root compartment, the abundance of TE follows $\text{Zn} \approx \text{Cu} > \text{Pb} > \text{Ni} > \text{Cd}$ and concentrations varied by a factor of 5–15 between sites (Table 1). The trend (and ranges in $\mu\text{g g}^{-1}$) observed in pollen is Zn (59.5–205) $>$ Mn (19.4–117) $>$ Ba (4.76–22.6) $\approx$ Cr (0.54–22.3) $\approx$ Cu (12.7–27.7) $\approx$ Ni (1.13–12.6) $\approx$ Pb (1.00–7.87) $>$ Cd (0.06–0.77) $\gg$ Ti (0.0015–0.0180). All the pollen samples were below

Table 2

Concentrations of complementary trace elements in pollen grains of *Ambrosia artemisiifolia* collected in the Montréal (Canada) metropolitan area in August 2008.

Sites	Ba		Cr		Mn		Tl	
	Mean	(SD) ^a	Mean	(SD)	Mean	(SD)	Mean	(SD)
	$\mu\text{g g}^{-1}$							
1	5.20	(0.69)	0.54	(0.07)	24.0	(1.23)	0.0019	(0.00098)
2	17.7	(1.45)	1.86	(0.03)	37.0	(1.77)	0.0028	(0.00046)
3	12.0	(3.09)	1.59	(0.73)	30.1	(7.49)	0.0034	(0.00250)
4	22.6	(0.58)	22.3	(4.98)	117	(9.28)	0.0088	(0.00056)
5	9.90	(2.28)	6.33	(0.85)	43.4	(4.27)	0.0035	(0.00054)
6	10.3	(6.57)	2.56	(0.84)	36.4	(7.50)	0.0052	(0.00299)
7	4.76	(1.18)	1.70	(0.57)	25.2	(2.32)	0.0180	(0.00602)
8	18.8	(12.0)	1.53	(0.70)	28.3	(4.48)	0.0028	(0.00170)
9	10.8	(2.44)	0.87	(0.39)	26.8	(5.78)	0.0056	(0.00142)
10	8.32	(0.59)	1.54	(0.44)	35.1	(3.55)	0.0067	(0.00318)
11	13.6	(1.88)	1.04	(0.54)	31.3	(9.13)	0.0026	(0.00139)
12	11.1	(1.94)	0.99	(0.53)	37.0	(4.72)	0.0041	(0.00248)
13	17.5	(5.16)	0.82	(0.22)	23.4	(0.52)	0.0020	(0.00052)
14	13.5	(0.61)	1.01	(0.33)	29.9	(2.17)	0.0041	(0.00084)
15	14.0	(6.29)	2.39	(0.35)	23.5	(4.82)	0.0084	(0.00352)
16	11.2	(0.30)	2.03	(0.79)	26.7	(10.1)	0.0041	(0.00312)
17	21.7	(3.26)	2.58	(0.56)	34.4	(1.48)	0.0059	(0.00190)
18	9.81	(2.20)	4.31	(0.75)	41.6	(8.52)	0.0039	(0.00175)
19	10.0	(0.87)	3.49	(1.08)	36.6	(0.72)	0.0046	(0.00413)
20	13.4	(1.75)	3.93	(0.54)	39.8	(3.19)	0.0051	(0.00091)
21	16.8	(3.57)	3.03	(0.68)	36.9	(3.98)	0.0091	(0.00544)
22	16.6	(5.85)	2.37	(0.95)	31.5	(8.79)	0.0031	(0.00215)
23	20.2	(8.08)	2.38	(0.49)	29.4	(2.37)	0.0045	(0.00151)
24	13.5	(3.30)	1.53	(0.31)	19.4	(1.48)	0.0031	(0.00123)
25	15.1	(3.04)	1.70	(0.58)	29.0	(2.32)	0.0015	(0.00112)
26	11.6	(3.58)	1.98	(0.89)	39.4	(6.54)	0.0078	(0.00585)
DLM ^b	0.12		0.09		0.07		0.048	

^a SD = standard deviation obtained for three field replicates.

^b DLM = detection limit of the method in $\mu\text{g L}^{-1}$.

the environmental thresholds of the Canadian legislation (CCME, 2012) for solid materials, except for Zn where sample 13 exceeded the concentration authorized for residential use (Tables 1 and 2). Principal component analysis performed with pollen data revealed that the variability in TE concentrations followed three distinct patterns (SI 3). A first group of TE including Cr, Cu, Mn and Ni defined the first principal component axis. The second group was composed of Ba, Cd, Pb and Zn with Ba and Cd being associated to the second component axis while Pb and Zn had a slightly larger association with the first component axis. The Tl contributed almost equally to both principal components and appeared as an isolated element. Barium, Cd, Pb and Zn were not correlated to other TE, while Tl was slightly positively linked to the first group of TE (SI 3).

For the five TE measured in all environmental compartments (soil, roots and pollen), concentration ratios were calculated to describe the sequestration behavior of TE in *Ambrosia artemisiifolia* (Table 3). Both the root to soil and the pollen to soil concentration ratios were highly variable between sampling sites with coefficients of variation ranging from 52% to 220%, the lowest variation was recorded for Ni. For the root to soil concentration ratio, all TE except Cu had a mean ratio below one (Table 3), with enrichment trends as follows: $\text{Cu} > \text{Cd} > \text{Zn} > \text{Pb} > \text{Ni}$. However, maximum ratio values between 3.41 and 6.97 were recorded for all elements, except Ni. The mean pollen to soil concentration ratios were systematically below unity and followed the sequence $\text{Zn} > \text{Cu} > \text{Cd} > \text{Ni} > \text{Pb}$, with Cd, Cu and Zn having maximum ratio values exceeding unity. The patterns in TE allocation within *Ambrosia artemisiifolia* was further described by calculating a pollen to root concentration ratio and the TE sequence was $\text{Zn} > \text{Ni} > \text{Cu} \approx \text{Cd} > \text{Pb}$ (Table 3). Again, all the TE showed a mean enrichment factor below one although Cu, Ni and Zn displayed maximum pollen to root ratios higher than unity. Compared to

Table 3

Descriptive statistics for concentration ratios calculated with total-recoverable trace element concentrations in *Ambrosia artemisiifolia* plant parts and in soils.

Descriptive statistics	Cd	Cu	Ni	Pb	Zn
<i>Root-to-soil concentration ratio</i>					
Minimum	0.13	0.28	0.04	0.04	0.04
Maximum	6.21	6.34	0.49	3.41	6.97
Mean	0.91	1.42	0.18	0.30	0.74
Median	0.78	1.04	0.16	0.15	0.43
Standard deviation	1.13	1.32	0.09	0.66	1.35
<i>Pollen-to-soil concentration ratio</i>					
Minimum	0.04	0.06	0.03	0.01	0.06
Maximum	1.61	1.75	0.31	0.17	2.91
Mean	0.22	0.37	0.10	0.03	0.47
Median	0.14	0.33	0.08	0.02	0.34
Standard deviation	0.30	0.34	0.06	0.04	0.55
<i>Pollen-to-root concentration ratio</i>					
Minimum	0.08	0.11	0.26	0.04	0.29
Maximum	0.82	1.01	1.84	0.72	2.25
Mean	0.26	0.30	0.64	0.18	0.87
Median	0.25	0.22	0.53	0.16	0.87
Standard deviation	0.14	0.22	0.34	0.13	0.42

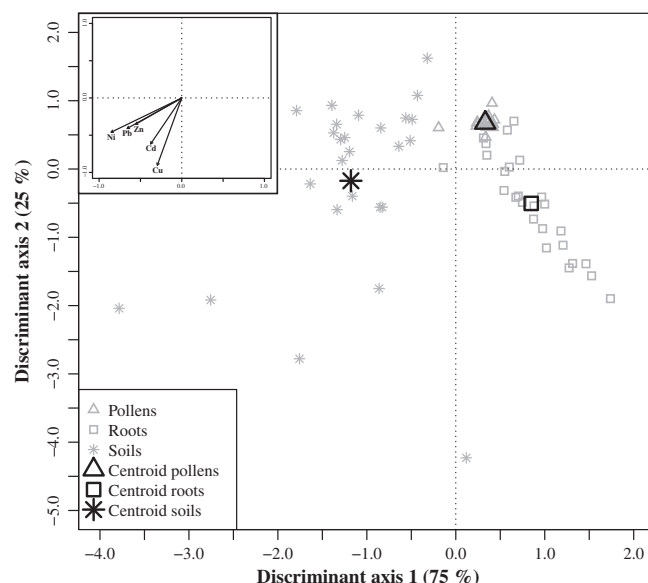


Fig. 2. Ordination plot of a linear discriminant analysis performed with total-recoverable trace element (Cd, Cu, Ni, Pb and Zn) concentrations in soils and in *Ambrosia artemisiifolia* roots and pollen grains. These three environmental compartments were sampled at 26 locations, disturbed by various types of human activities. The main biplot displays the distribution of the observations (grey) and the centroids of each environmental compartment (black) along the two-discriminant axes. Observations close to each other have similar trace element concentrations whereas observations far apart differ strongly. The small inset at the top left of the figure displays the correlations between the variables used in this analysis. The percentage associated to each axis refers to the percentage of the total variance explained by the given axis. An *a posteriori* cross-validation reclassification indicated that 87% of the observations were successfully reclassified by the calculated discriminant function.

the other ratios, the pollen to root concentration ratio showed a smaller variability in the dataset for all TE (Table 3). For the root to soil and the pollen to soil concentration ratios, the highest enrichment ratios were always reported for site 1 that also had the lowest TE concentrations in soils (Table 1), the only exception being the pollen to soil concentration ratio of Ni.

The concentrations of TE, as well as their variability between sampling sites, decreased following: soil $\gg$ roots $>$ pollen (Table 1). The contrast between these three compartments is clearly visible

Table 4
Best-fit multiple linear regression models of trace element (Cd, Cu, Ni, Pb and Zn) concentrations in the pollen grains of *Ambrosia artemisiifolia* against soil properties and trace element concentrations in soil or in the roots of *Ambrosia artemisiifolia*.

Trace element concentration in pollens (Y)	Model (X) ^a	Adjusted R ²	RSE ^b	RDF ^b	F	p
<i>Models obtained with soil properties as explanatory variables</i>						
Log Cd =	0.33 (±0.11)log Cd** – 0.83** (±0.04)	0.265	0.21	23	9.64	0.005
Cu =	No significant model	–	–	–	–	–
Log Ni =	0.37(±0.16)log Ni* – 0.24 (±0.16)log SOC ^{ns} + 0.24 ^{ns} (±0.25)	0.135	0.15	22	2.88	0.078
Log Pb =	0.32 (±0.07)log Pb** – 0.25 ^{ns} (±0.15)	0.442	0.15	23	20.0	<0.001
Zn =	No significant model	–	–	–	–	–
<i>Models obtained with trace element concentration in roots as explanatory variables</i>						
Cd =	0.14 (±0.02)Cd** + 0.06* (±0.02)	0.651	0.08	24	47.7	<0.001
Cu =	No significant model	–	–	–	–	–
Log Ni =	0.61 (±0.23)log Ni* + 0.02 ^{ns} (±0.16)	0.187	0.19	24	6.74	0.016
Pb =	No significant model	–	–	–	–	–
Zn =	No significant model	–	–	–	–	–

^a ^{ns} not significant at $\alpha = 0.05$; *Significant at $\alpha = 0.05$ and **significant at $\alpha = 0.01$. SOC = soil organic carbon.

^b RSE = residual standard error; RDF = residual degree of freedom, calculated from the difference between the number of samples and the number of explanatory variables integrated in the regression model – 1. All the regression models between pollens and soils were calculated from 25 samples whereas all the regression models between pollens and roots were calculated from 26 samples.

in the multivariate linear discriminant analysis presented in Fig. 2. The first discriminant axis explained 75% of the variance of the dataset, was defined by the elements Ni, Pb and Zn (inset of Fig. 2), and discriminated mainly the soil from the other two compartments (root and pollen). The second axis explained 25% of the variance, was correlated to Cd and Cu (inset of Fig. 2), and contrasted the pollen from the root compartments (Fig. 2). An *a posteriori* cross-validation reclassification of all observations, using the calculated discriminant functions, indicated that 87% of the 78 observations were successfully attributed to the appropriate environmental compartments (data not shown). The reclassification errors were associated to root ($n = 6$) and soil ($n = 4$) observations having low TE concentrations. These observations were classified as pollen grains eight times out of ten.

3.2. Relationships between trace elements in pollen grains and soil or root properties

Prediction models for TE concentrations in the pollen of *Ambrosia artemisiifolia* were determined by multiple linear regressions using the soil properties or the TE concentrations in *Ambrosia* roots as explanatory variables (Table 4). More significant models were obtained using soil properties (Cd, Ni and Pb models) than the root compartment (Cd and Ni models) as explanatory variables. Cadmium and Ni were the only TE showing significant pollen models using either the soil or the root compartments (Table 4). In these cases, better predictions were always achieved with root properties than with the soil compartment (Table 4). All the significant regression models showed the same structure where positive relationships existed between TE in pollen grains and the soil or root properties used as explanatory variables (Table 4). Among the significant pollen models, only the Ni regression involved a soil property, SOC, other than the soil total-recoverable TE concentrations. Yet, the apparent SOC link to pollen Ni concentrations was weak and not significant (Table 4). The regressions yielded no significant models for Cu and Zn using the available soil and root properties.

4. Discussion

4.1. Trace element transfers from the soil to pollen grains

The SOC content and the total-recoverable TE concentrations measured in soils fell within the range reported for Montréal (Sauvé et al., 1996) and other large cities of the world (Lorenz and Lal, 2009; Ajmone-Marsan and Biasioli, 2010; Vasenev et al., 2013). Anthropogenic activities, inducing the release of TE-rich

particulates into the atmosphere and their subsequent deposition on soil surfaces, clearly impacted on the soil TE concentrations measured in this study. Yet, identifying all TE sources in urban areas remains a challenge due to their abundance, diversity and heterogeneity in space and time (Ajmone-Marsan and Biasioli, 2010). The soil is however a sink for TE due to its elevated ion retention capacity induced mostly by organic matter and clay minerals. As such, the soil TE concentration measured at any given time combines both past and present TE emissions and depositions. Sites located closed to roads and highways (# 2–7, 13–17, 22–26) or at the vicinity of industrial sites (8, 13–17) were those having the higher TE concentrations, especially for Cu, Pb and Zn (Fig. 1, Table 1 and SI 1). Past and present atmospheric particulate emissions in the vicinity of transport lines were enriched in Pb, due to vehicle exhaust, as well as in Ba, Cu and Zn, due to non-exhaust emissions associated to oil leakage, brake pads, radiator and tire wear, and contributed to the TE burden of urban soils (Ajmone-Marsan and Biasioli, 2010; Karageorgos and Rapsomanikis, 2010; Apeagyei et al., 2011). Industrial activities (incinerators, petrochemical industry, repositories) in the Montréal urban area close to the sampling sites were also known to release TE mixtures into the environment (Ajmone-Marsan and Biasioli, 2010). These activities could explain the soil Cu, Ni and Zn peaks observed in sites closed to industrial areas (Fig. 1, Table 1 and SI 1).

Pollen grains represent a tiny fraction of plant biomass and are seldom analyzed, as indicated by the paucity of reports on the topic (Kalbande et al., 2008; Lambert et al., 2012; Silva et al., 2012). Clearly, this is the first study quantifying TE concentrations in the pollen grains of *Ambrosia artemisiifolia*. We also measured TE such as Ba, Cr and Tl that had never been analyzed in pollen grains of any plant (Table 2). The pollen concentrations were similar to previous studies for Ni and were up to one order of magnitude higher for Cd, Cu and Pb (Kalbande et al., 2008; Lambert et al., 2012). Contrary to most other studies, our measurements were performed on pollen grains sampled directly from the plant flower, prior to their dispersion in the atmosphere. In contaminated urban environments, Kalbande et al. (2008) demonstrated that Cu, Ni and Pb in pollens increased by 1.3 to 87-fold following their atmospheric dispersion because of the impaction and retention of particles containing TE (Ring et al., 2001). We therefore consider that our measurements correspond to a low limit for TE concentrations in pollen grains entering the human body via the respiratory system in contaminated environments such as cities. As for root materials, the TE concentrations in *Ambrosia artemisiifolia* were similar to or lower than values reported for the same plant specie at other contaminated sites (Huang and Cunningham, 1996; Pichtel et al.,

2000). The variability in *Ambrosia* root concentrations between studies was mainly associated to the level of soil contamination by TE. *Ambrosia artemisiifolia* has been viewed as a candidate plant for phytoremediation since it colonizes contaminated sites, but it cannot be considered a hyperaccumulator species based on root data and on its low ability to translocate TE to the above-ground biomass (Huang and Cunningham, 1996).

The root to soil and the pollen to soil concentration ratios indicated that the transfer of TE from the soil to *Ambrosia artemisiifolia* was highly variable between sampling sites. The ratios further revealed that Cd, Cu and Zn were more readily transferred to the plant than Ni and Pb (Table 3). The soil conditions at our sites, with near-neutral pH and high SOC content (SI 1), favored the formation of organic complexes in soils, limiting the mobility of TE and their availability to plants (Hinsinger and Courchesne, 2008). Under these physicochemical conditions, the transfer of TE less sensitive to complexation (Cd and Zn) or contributing to essential plant functions (Cu and Zn) is favored compared to non-essential elements or those likely to form organic complexes, such as Pb and Ni.

The mean pollen to root concentration ratios indicated that the roots were always richer in TE relative to pollen with Zn having the highest ratio (Table 3). However, some sampling sites showed the inverse trend, notably for Zn and to a lesser extent Cu and Ni. Similar pollen to roots ratios were reported in various Brazilian plant species for Cu, Pb and Zn (Silva et al., 2012); no such data have however been published for Cd and Ni. The limited Cd, Cu and Pb transfer from root to pollen could be due to the sensitivity of pollen grains to TE toxicity, pollen being considered a reliable indicator of atmospheric pollution by TE (Calzoni et al., 2007). According to *in vivo* studies, the inhibition of pollen germination was the main toxic effect of TE on pollen grains (Wolters and Martens, 1987); despite low TE concentrations in pollen ($<10^{-8}$ M for Cd) stimulate pollen germination (Xiong and Peng, 2001).

Cadmium, Ni and Pb in pollen, but not Cu and Zn, were predicted by soil or root properties (Table 4). Regressions analyses also showed that concentrations in soil or roots were positively linked to concentrations in pollen grains. The link between Cd, Ni and Pb in soil and pollen suggests that pollen grains did not attain their maximum sequestration capacity within the range of site conditions studied. It can further be hypothesized that Cd, Ni and Pb in pollen grains had no toxic effect because no plateau was reached in the relationship (SI 4) and because *Ambrosia* still allocated more Cd, Ni and Pb to pollen as concentration increased in soils. In the case of Cu and Zn, where no significant model was found, a maximum allocation to pollens appeared to have been reached at all sampling sites, even those with the lowest available soil Cu and Zn, without toxicity (SI 4). Copper and Zn concentrations in pollen grains were also higher than for other TE and inter-site variability was lower. Because both Cu and Zn fulfill essential functions in plants, it thus appears that the allocation of Cu and Zn to pollen slows down or stops during growth and that the Cu and Zn still taken up by roots are subsequently mostly stored in other plant parts.

Cadmium, Cu, Ni, Pb and Zn concentrations in pollen grains (Table 4) and in roots (data not shown) were not related to soil pH or SOC, except for Ni that was slightly but not significantly affected by SOC. This is surprising because pH and SOC often impact on the speciation and availability of TE in soils, and their uptake by plants (Hinsinger and Courchesne, 2008). The absence of links could be due to the limited pH range (2 of the 26 samples were out of the 7.31–8.39 range) (SI 1). Moreover, the pH and SOC conditions were unfavorable to TE mobility in soils. Alternately, *Ambrosia artemisiifolia* could rely on uptake strategies that are marginally related to pH or SOC, such as the release of specific root exudates or local changes in soil redox conditions (Hinsinger and Courchesne, 2008).

Table 5

Estimates^a of trace elements absorbed by humans through the inhalation of *Ambrosia artemisiifolia* pollen grains in the Montréal (Canada) metropolitan area.

Human inhalation scenarios ^b	Estimations (unit) ^c	Ba	Cd	Cr	Cu	Mn	Ni	Pb	Tl	Zn
Adult (rest)	MeDI (ng)	0.076	0.001	0.017	0.101	0.199	0.017	0.018	<0.001	0.532
	MaDI (ng)	0.342	0.004	0.075	0.453	0.892	0.077	0.080	<0.001	2.387
	MeSI (ng)	3.120	0.041	0.681	4.135	8.149	0.706	0.734	0.001	21.80
Adult (light work)	MeDI (ng)	0.127	0.002	0.028	0.168	0.332	0.029	0.030	<0.001	0.888
	MaDI (ng)	0.570	0.007	0.125	0.756	1.490	0.129	0.134	<0.001	3.987
	MeSI (ng)	5.210	0.068	1.138	6.907	13.61	1.178	1.225	0.002	36.42
Adult (hard work)	MeDI (ng)	0.166	0.002	0.036	0.219	0.432	0.037	0.039	<0.001	1.157
	MaDI (ng)	0.743	0.010	0.162	0.985	1.941	0.168	0.175	<0.001	5.193
	MeSI (ng)	6.787	0.088	1.482	8.996	17.73	1.535	1.596	0.003	47.43
Child	MeDI (ng)	0.038	<0.001	0.008	0.051	0.100	0.009	0.009	<0.001	0.269
	MaDI (ng)	0.173	0.002	0.038	0.229	0.229	0.039	0.041	<0.001	1.206
	MeSI (ng)	1.576	0.021	0.344	2.090	4.117	0.357	0.371	<0.001	11.02
Newborn	MeDI (ng)	0.008	<0.001	0.002	0.011	0.022	0.002	0.002	<0.001	0.058
	MaDI (ng)	0.037	<0.001	0.008	0.049	0.097	0.008	0.009	<0.001	0.259
	MeSI (ng)	0.338	0.004	0.074	0.045	0.884	0.077	0.080	<0.001	1.594
Air quality criteria ^d	(ng m ⁻³)	50	3.60	4.00	2 500	–	12.0	100	250	2 500
Concentration in air due to pollens ^c	(ng m ⁻³)	0.228	0.003	0.050	0.007	0.596	0.052	0.054	<0.001	0.039

^a Estimations were based on mean trace element concentrations in pollen grains of *Ambrosia artemisiifolia* presented in Tables 1 and 2 and on pollen atmospheric dispersion data collected in Montréal (Canada). Accordingly, the mass of an *Ambrosia artemisiifolia* pollen grains was set to 5.23 ng (Guérin, 1993), the mean length of the *Ambrosia artemisiifolia* pollen season to 41 d, and the mean and maximal *Ambrosia artemisiifolia* pollen concentrations in the air during the pollen season to 79.1 and 355 pollens m⁻³ d⁻¹, respectively (Comtois, 2006).

^b In the working adult scenarios, the adults were considered to work during 8 h and to rest during 16 h. Reference respiration capacities of 0.57; 1.72; 2.58; 0.29 and 0.06 m³ h⁻¹ were considered for the five scenarios, respectively (Diem and Lentner, 1972).

^c MeDI = mean daily inhalation of trace elements; MaDI = maximum daily inhalation of trace elements; MeSI = mean seasonal inhalation of trace elements.

^d The trace element air quality criteria and the trace element concentration in air due to pollen grains are documented for a period of 1 year of exposition (for trace elements in pollen grains it corresponds to one pollen season), except for Cu and Zn where an exposition period of 24 h is considered. The air quality criteria are from the Québec provincial government (MDDEP, 2010).

4.2. Are pollens a potential source of trace elements to humans?

Pollen grains are TE carriers and thus have the potential to act as an input pathway to humans through the respiratory systems. Trace element-bearing pollen grains are suspected to increase the prevalence of allergy and atopic diseases to humans and to be responsible for the increase intensity of symptoms for populations at risk (Ring et al., 2001; D'Amato et al., 2007). Recent works have strengthened this hypothesis since increased production of allergenic proteins in pollen grains and increased allergenicity in sensitive human population were reported for an allergenic herbaceous plant growing on Cd contaminated soils compared to same plant species grown on similar uncontaminated soils (Aina et al., 2010). These observations have not yet been explained, but could be due, among others, to a plant stress associated to TE or to interactions between TE and allergenic proteins within pollen grains. Such interactions were evidence by Bellanger et al. (2012) in an *in vivo* experiment conducted on cultured alveolar epithelial cells. They found a dose- and time-response relationships between Pb concentration in white birch pollen and the gene expression of an allergic mediator (interleukin-5) and stated that the increase allergenicity occurred at pollen Pb levels higher than $4.4 \mu\text{g g}^{-1}$. This threshold is exceeded at five of our 26 sampling sites suggesting that some of the sites exhibited pollen grains with sufficient TE to potentially increase the allergenicity of *Ambrosia artemisiifolia*. Clearly, new field data on the interactions between TE, pollen and allergic symptoms are needed to further test the effect of TE on pollen allergenicity.

Our pollen data provide a unique opportunity to estimate the amount of TE absorbed by humans via pollen inhalation and the level of human exposure, since *Ambrosia artemisiifolia* has been studied in the Montréal metropolitan area (Héguay et al., 2008). Indeed, 25 years of daily air sampling of pollen grains in Montréal allowed us to know the length of pollen season and the atmospheric pollen concentrations for this specie (Comtois, 2006). Therefore, to compute the estimates of Table 5, we used a mean pollen mass of 5.23 ng per grain (Guérin, 1993), a mean pollen season of 41 d as well as mean and maximum pollen air concentrations of 79.1 and $355 \text{ pollens m}^{-3} \text{ d}^{-1}$, respectively. In order to consider the effect of life stages, the sensitivity to TE toxicity and the intensity of daily activities, five scenarios were selected: adult at rest, adult doing light work, adult doing hard work, child and newborn (Diem and Lentner, 1972). For all scenarios, the mean daily (MeDI) or seasonal (MeSI) trace element absorption through pollen was low ($<50 \text{ ng}$) and followed the order $\text{Zn} > \text{Mn} > \text{Cu} \gg \text{Ba} > \text{Cr} \approx \text{Ni} \approx \text{Pb} \gg \text{Cd} \gg \text{Ti}$ (Table 5). Moreover, according to the air quality criteria established by the provincial Québec government (MDDEP, 2010), the TE content within *Ambrosia* pollen grains was below threshold by several orders of magnitude. Despite the fact that estimates are probably low because they do not consider the concentration increase in pollen grains due to transit in the urban atmosphere, our estimates suggest that *Ambrosia* pollen did not carry TE at levels high enough to generate TE toxicity to humans over a short-term (daily to seasonal) exposure. The cumulative impact of a long-term absorption of TE via pollen inhalation remains beyond the scope of this study but certainly deserves further attention.

5. Conclusions

This unique study investigated the transfer of TE from urban soils to the roots and then to the pollen grains of a plant bearing allergenic pollen, *Ambrosia artemisiifolia*. A series of nine TE were analyzed and showed moderate to high variability in pollen grains between sampling sites. The TE concentrations discriminated well

between the three biogeochemical compartments and generally decreased from soil material to root and to pollen. At some sampling sites, inverse trends existed, notably for Zn and to a lesser extent Cu and Ni, where the pollen compartment had higher concentration than the roots. Significant predictive linear models of TE concentrations in pollen grains were obtained for the non-essential Cd, Ni and Pb but the regression analyses yielded no significant model for the essential elements Cu and Zn. All the significant models involved positive relationships between TE in pollen and soil or root properties, in particular TE concentration in soil.

The presence of TE in pollen grains were considered in the literature as a potential explanation for the increase prevalence of allergenicity in industrialized countries as well as for the increased intensity of allergy symptoms in sensitive populations. In our study, the highest TE concentrations measured in pollen grains were well within the range of studies reporting an increased allergenicity of pollen due to the presence of TE. The inhalation of airborne pollen grains of *Ambrosia* could also favor the absorption of TE by humans. We therefore estimated the daily and pollen seasonal intake of TE by humans associated to *Ambrosia* pollen inhalation, using pollen dispersion data for the Montréal metropolitan area. The estimates remained below the air quality criteria. However, because the pollen analyzed were extracted directly from *Ambrosia* flowers and were never in contact with contaminated air particles, these estimates should be considered to represent low values.

The results from this study point to the need for new data to better understand the mechanisms involved in the transfer of TE from soil to pollen and to validate the impact of TE in pollen on human health, especially allergy symptoms and atopic diseases.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2013.09.113>.

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Supplementary information (SI)

SI 1: Site information and their generic soil properties.

SI 2: Methodological details.

SI 3: Ordination plot of a principal component analysis performed with trace element concentrations in the pollens of *Ambrosia artemisiifolia*. The percentage associated to each axis refers to the percentage of the total variance explained by the given axis. Variables close to each other are positively correlated whereas orthogonal variables are not correlated to each other.

SI 4: Concentrations of Pb (top) and Cu (bottom) in the pollen grains of *Ambrosia artemisiifolia* as a function of their total-recoverable concentrations in soil.

SI 1: Site information and their generic soil properties.

Site number	Site characteristic	pH (H₂O)^a	Soil organic carbon g kg⁻¹
1	Park and sport fields, previously a waste dump	8.24	11.1
2	Close to traffic, on a bridge	8.39	34.0
3		7.81	14.7
4	Close to traffic, on a large street	-	37.5
5		8.04	27.0
6	Railway track, close to traffic	7.56	38.3
7		7.89	20.3
8	Wasteland, house construction	7.89	32.1
9	Park close to cycling lane	7.70	75.3
10		7.67	55.0
11	Parking close to traffic	7.58	41.6
12		7.75	33.2
13	Industrial wasteland	7.77	52.6
14		7.79	58.9
15	Railway track, close to traffic	6.86	76.9
16		5.83	58.2
17	Wasteland used for parking, close to industries	7.82	44.3
18	Close to traffic on a large street	7.93	51.3
19		7.89	80.3
20	Park close to street	7.67	32.2
21		7.60	62.5
22	Wasteland close to railway track	8.02	29.0
23		7.41	62.7
24	Railway track close to an incinerator, large street	7.31	82.2
25	Cycling lane, close to railway track and incinerator	7.44	46.4
26	Parking, close to railway track and incinerator	7.63	30.1

a The soil pH is measured at a 1:2 soil:water ratio.

SI 2 : Methodological details

SI 2.1: Specification on the quality of chemical analyses

An internal control material was integrated in the analytical runs for the validation of soil pH and soil organic carbon measurements. For trace element measurements in soil materials and *Ambrosia artemisiifolia* tissues, external reference materials were used for validation. Reference materials from SCP Science (reference soil SS-2; <http://www.scpscience.com/>) and from the National Institute of Standards and Technology (NIST 1547; <http://www.nist.gov/srm/>) were selected for soil and plant tissues, respectively. The reference material NIST 1547 was used for both root and pollen grain tissues, because no reference material exists for pollen grains. From these controls and reference materials, the accuracy and precision of all the chemical analyses used in this study were calculated (Table SI 2) and provided acceptable results. Variability of accuracy for trace elements is explained by slight differences in the acid digestion procedure used in this study and in the certified laboratory, with both acid digestion procedures approved by the scientific community. Regarding the precision, the weak performance for Cd is explained by the data that are close to the detection limit of the method.

SI 2.2: Specification on multivariate statistical analyses

Principal component analysis (PCA): The PCA was performed with R function *dudi.pca* of the *ade4* library (Dray and Dufour, 2007. J. Stat. Soft. 22, 1-20) using a sub-fraction of the dataset containing only the total-recoverable trace element concentrations measured in pollen grains. A scaling-type 2 biplot of the first two principal components was produced to evaluate the links between trace elements.

Linear discriminant analysis (LDA): The LDA was performed with R function *discrimin* of the *ade4* library (Dray and Dufour, 2007. J. Stat. Soft. 22, 1-20). This multivariate approach creates the best discriminate functions to differentiate objects according to groups that are pre-defined by the user in order to maximize the inter-groups over the intra-groups variability in the dataset. The performance of the discrimination was determined using the cross-validation technique that compares *a priori* (real) and *a posteriori* (calculated from discriminant functions) classification of observations.

Multiple linear regression (MLR): The MLR were performed with R function *lm* from *stats* library. The prediction of total-recoverable trace element concentrations in pollens was performed using two types of explanatory variables, the soil properties (pH, soil organic carbon and total-recoverable trace element concentrations) or the total-recoverable trace element concentrations in roots. For the MLR using soil properties as explanatory variables, only the significant variables were kept in the predictive models. The significant explanatory variables were identified using the forward selection procedure (R function *step* from *stats* library) prior the production of the MLR.

Table SI 2: Accuracy and precision values^a for the chemical analyses performed in this study.

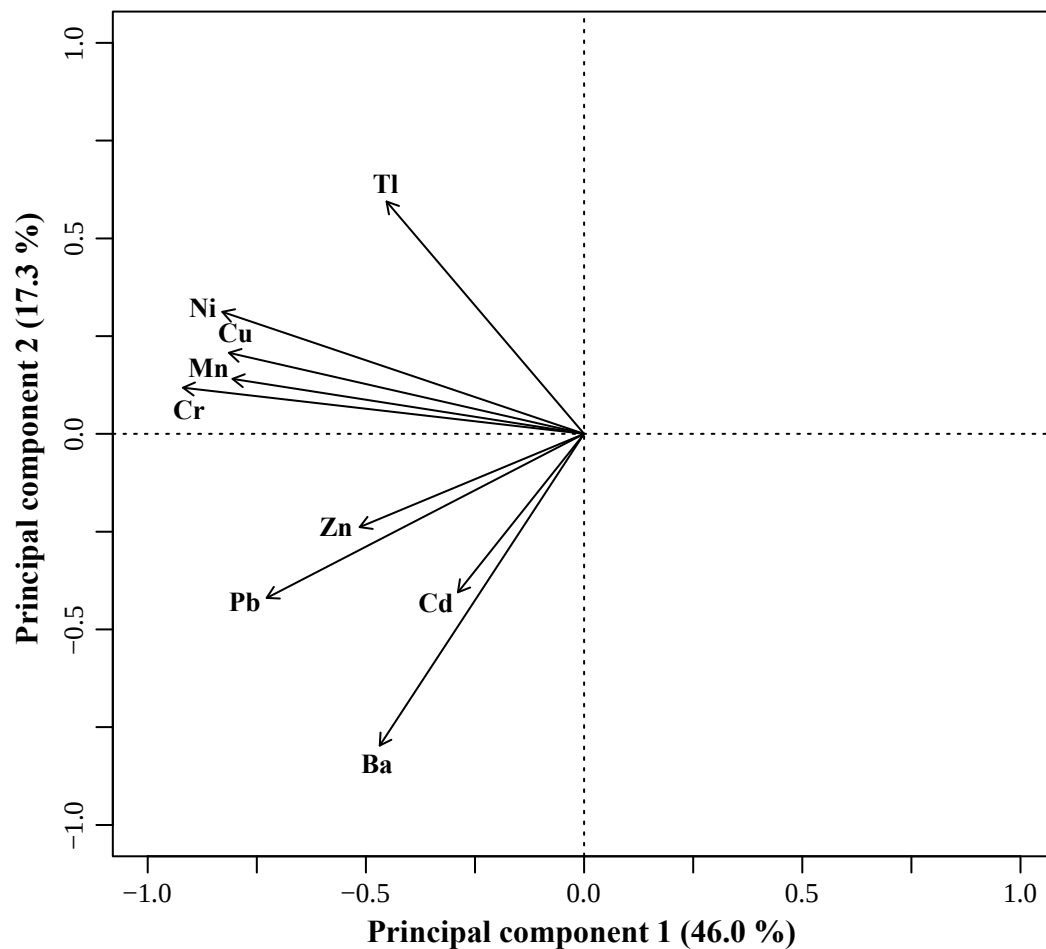
Variable	Reference material used	N ^b	Accuracy ^c ----- % -----	Precision ^d -----
pH (H₂O)	Internal soil sample	6	101	2.0
Soil organic carbon	Internal soil sample	4	102	10.0
Ba	NIST 1547	60	-	3.0
Cd	SS2	68	97.0	5.0
	NIST 1547	60	98.0	30
Cr	NIST 1547	60	76.0	6.0
Cu	SS2	68	108	4.0
	NIST 1547	60	101	13
Mn	NIST 1547	60	97.0	3.0
Ni	SS2	68	112	4.0
	NIST 1547	60	89.0	7.0
Pb	SS2	68	107	4.0
	NIST 1547	60	94.0	14
Tl	NIST 1547	60	-	6.0
Zn	SS2	68	108	4.0
	NIST 1547	60	96.0	7.0

a Accuracy “ is the agreement between an experimentally determined value and the accepted reference value ” and precision “ is the degree of agreement of repeated measurements of the same property ” (Anderson, 1987. *Practical Statistics for Analytical Chemists*, Van Nostrand Reinhold, New York).

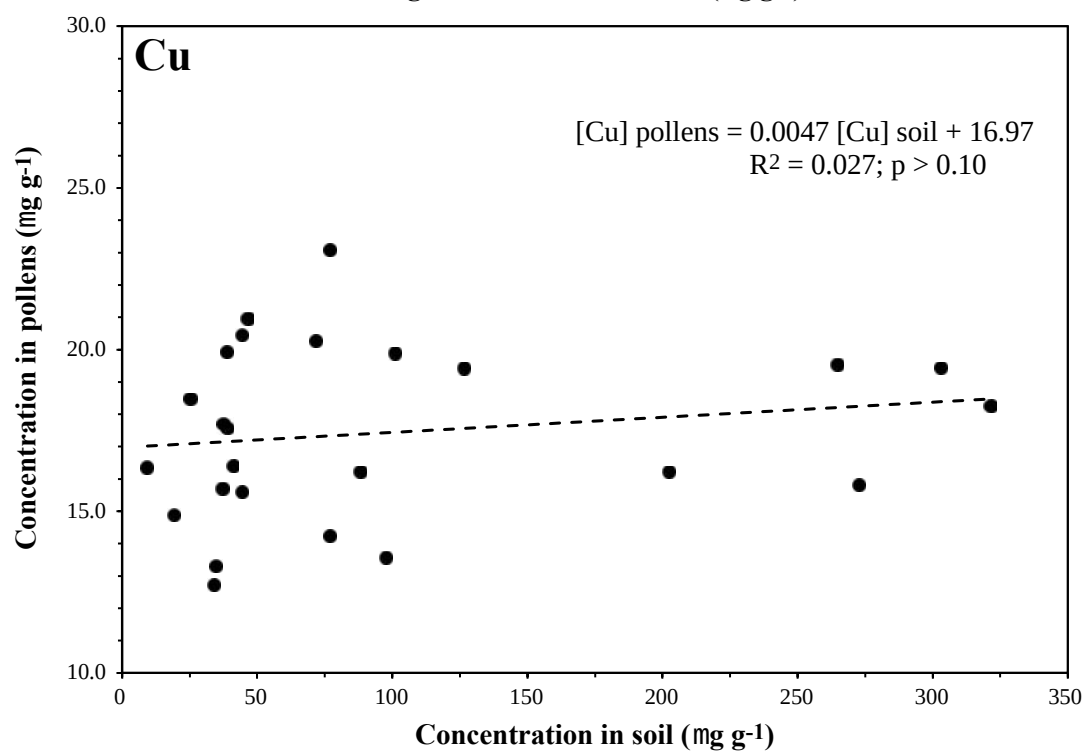
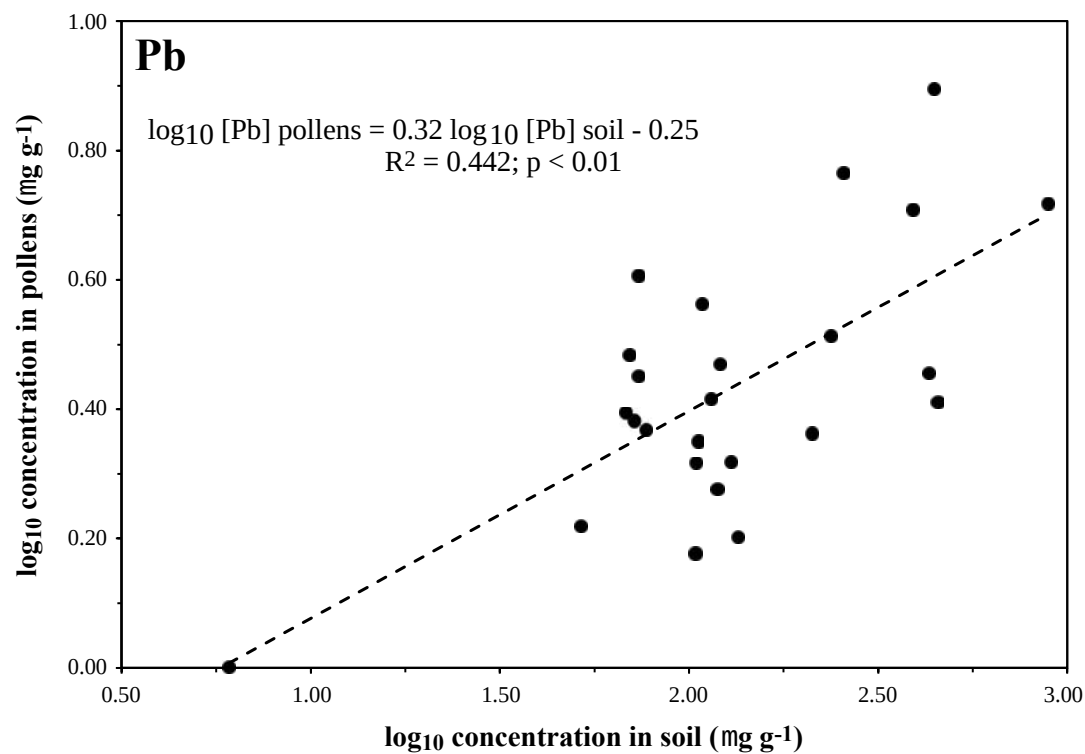
b Number of observations used to calculate accuracy and precision.

c $\text{Accuracy (\%)} = \frac{[|(X_m - X_{sd}) / (REF_m + REF_{sd})| + |(X_m + X_{sd}) / (REF_m - REF_{sd})|]}{2} * 100$
where X_m corresponds to the mean experimentally determined value, X_{sd} to the standard deviation experimentally determined value, REF_m to the mean accepted reference value and REF_{sd} to the standard deviation accepted reference value. The trace elements without accuracy value (Ba and Tl) are due to the absence of reference value provided with the reference material.

d $\text{Precision (\%)} = [X_{sd} / X_m] * 100$
where X_{sd} corresponds to the standard deviation value of the repeated measurements of the same property, X_m to the mean value of the repeated measurements of the same property.



SI 3: Ordination plot of a principal component analysis performed with trace element concentrations in the pollens of *Ambrosia artemisiifolia*. The percentage associated to each axis refers to the percentage of the total variance explained by the given axis. Variables close to each other are positively correlated whereas orthogonal variables are not correlated to each other.



SI 4: Concentrations of Pb (top) and Cu (bottom) in the pollen grains of *Ambrosia artemisiifolia* as a function of their total-recoverable concentrations in soil.