



# Assessment of lettuces grown in urban areas for human consumption and as bioindicators of atmospheric pollution

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## ARTICLE INFO

Edited by: Muhammad Zia-ur-Rehman

### Keywords:

Air quality  
Bioaccessibility  
Phytomonitoring  
PM emissions  
Trace elements  
Urban agriculture

## ABSTRACT

This study proposes using the network of urban gardens to grow vegetables and to monitor air quality, and it also evaluates whether food grown on a clean substrate in an urban environment is safe for consumption. For this purpose, lettuces were exposed to different degrees of air pollution in five locations in the city of Copenhagen, plus a reference site. Six specimens were placed at each site and, after the exposure period, half of each sample was washed. Subsamples were then digested by a total extraction method and a bioaccessible extraction method, and the concentration of 23 elements subsequently measured by ICP-MS. The results showed that exposed samples in areas of higher atmospheric pollution accumulated a larger amount of trace elements associated with typical urban sources. They also highlighted the importance of washing food to remove particles that adhere to their surface. However, bioaccessibility testing demonstrated the importance of including bioaccessibility in risk analyses and how this factor varies depending on the type of matrix. In this case, bioaccessibility was higher for plant tissue than for particulate matter. Lastly, metal concentrations in lettuce were compared with legal values and an analysis of daily intake showed that the levels in Copenhagen were within limits for the protection of human health.

## 1. Introduction

In its latest report on air pollution, the World Health Organization states that approximately 90% of the world's population is being exposed to levels of particulate matter (PM) pollution above the safe limits of air quality guidelines for human health (WHO, 2021). However, most countries are not taking measures to reduce air pollution (e.g. by promoting greener energy generation, cleaner transport, a circular economy and energy efficiency or limiting greenhouse gas emissions) or, if they are, it is at a slow pace. Anthropogenic airborne particles commonly carry trace elements, organic compounds and salts (nitrates and sulphates) adsorbed on their surface. PM is therefore one of the air pollutants of most concern, especially particles of small diameter (less than 10, 2.5 or 1 micrometres, i.e. PM<sub>10</sub>, PM<sub>2.5</sub> and PM<sub>1</sub> fractions), because they can penetrate deep into the respiratory tract, from there enter the bloodstream and ultimately reach the various organs of the body, causing a variety of health effects such as heart and respiratory diseases (USEPA, 2003), and eventually premature death. As a result,

PM has been classified as carcinogenic for humans (Group 1) by the International Agency for Research on Cancer and is considered the most widespread environmental carcinogen (IARC, 2013).

Monitoring the concentration and composition of ambient PM is essential for assessing whether a geographical region is meeting regulatory levels to protect public health, determine the different sources of atmospheric pollution in urban areas, and design effective actions to improve air quality (USEPA, 2020). Continuous monitoring systems using physicochemical methods are commonly used for this purpose, providing accurate single-point data, but there is an absence of information on the cumulative effects on living organisms of long-term exposure to pollutants. These techniques have the advantage of providing quantitative data that can be associated with specific emission sources, but they are very costly and labour intensive (Hoodaji et al., 2012). There are also passive devices, such as deposit gauges, that do not require an electrical power supply, but these do not provide real-time data.

Biological methods, however, have the advantage of incorporating

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<https://doi.org/10.1016/j.ecoenv.2023.114883>

Received 1 July 2022; Received in revised form 31 March 2023; Accepted 5 April 2023

Available online 10 April 2023

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real exposure and interactions of certain complex mixtures of chemicals with additive, synergistic or antagonistic effects. Even at low pollutant concentrations, the bioaccumulation capacity of some species of lichens, mosses or vascular plants allows the detection of substances that would otherwise be present in concentrations below analytical detection limits or that are emitted sporadically. In addition, the adverse effects of exposure to environmental contamination can be visually observed through changes in organisms. Lastly, biomonitoring methods do not require expensive equipment and skilled personnel, and consequently are very useful for large measurement networks and for developing countries (De Temmerman et al., 2004). They do have some limitations, however, such as the complexity of identifying the exact origin of the pollution and the possible death of the specimens under study.

In addition to the use of lichens and mosses, plants have also been shown to be effective for biomonitoring air quality, especially in urban and industrial areas where anthropogenic pressure may result in a scarcity or even absence of the most sensitive indicator species (Markert et al., 2011; Ram et al., 2015). An earlier study using rapeseed and kale has already shown a relationship between the exposure points where plants were grown and the concentration of several trace elements of anthropogenic or natural origin that were related to the ambient conditions of the environment (Izquierdo-Díaz et al., 2019). Similarly, lettuce can be used as a potential bioindicator since: a) it is a leaf vegetable, the part of the plant that is commonly consumed is the leaf, and it is generally consumed directly raw (it cannot be peeled and is rarely cooked, at most it is washed); b) it is a globally consumed food, with an annual world production of 27,660,187 tonnes (FAO, 2019a) and is also one of the typical crops for urban gardens due to its ease of cultivation; c) it is a hardy annual plant (some species can even grow in cold climates) and therefore, unlike fruit, can be used as a biomonitor at different times of the year; d) the growth period of lettuce for harvest is around 65–130 days from sowing or 30–70 days after transplanting (Wikifarmer, 2021), which is an appropriate exposure duration; and e) it has a relatively large leaf surface area, which increases the particle interception efficiency (Kulshreshtha et al., 2009; Ram et al., 2014) and thus, the amount of accumulated toxic substances. Previous studies have demonstrated that lettuce can be used as a bioindicator of roadside air pollution (Hassan and Basahi, 2013) and of emissions from a lead recycling plant (Uzu et al., 2010).

The morphological features of leaves (anatomy and structure) and wettability are important factors in determining the efficiency to capture and retain airborne particles. The interception is primarily influenced by the cuticle and epidermal foliar layer (stomata, trichomes and wax), as well as by surface area, geometry and roughness (Blanusa et al., 2015; Ram et al., 2015). Trichomes, which are small hair-like structures that cover the surface of lettuce leaves, can trap PM particles by acting as physical barriers, preventing them from entering internal leaf tissues. Stomata, which are small openings on the leaf surface, also play a role in PM trapping, as particles can be trapped in the stomatal pores and subsequently transported to the internal leaf tissues. The presence of polar functional groups, such as carboxyl, hydroxyl, and amine groups, can aid in the adsorption and sequestration of PM through chemical bonds with particles. Several studies have shown the efficiency of numerous herbaceous and plant species to intercept particulate matter (Adhikari et al., 2023; Li et al., 2019; Lyu et al., 2023). Due to the high surface area to volume ratio of lettuce foliage, their leaves can be expected to be efficient filters of airborne particles.

In addition to the issue of urban atmospheric contamination, global population growth, especially in urban areas, coupled with land degradation and the corresponding decrease in the availability of fertile soil are leading to an increase in people suffering from hunger, with an estimated 750 million people (approximately 10% of the world population) facing food insecurity (UN, 2019). Therefore, the Food and Agriculture Organization of the United Nations (FAO), together with other organisations, is promoting urban agriculture with the aim of providing enough nutritious food to eradicate hunger and malnutrition

through agricultural production systems that help boost productivity, but are also resilient and sustainable from a socioeconomic and environmental point of view (FAO, 2019b).

However, one of the main obstacles to the development of urban agriculture is concern about the possible contamination of the sites where they are located. In recent years, the number of scientific publications on this topic has multiplied, reflecting the growing interest in and awareness of this issue and the need to assess risk levels and establish preventive or corrective measures to ensure it is a safe option for society. In a study investigating the potential health risk of urban gardening (Warming et al., 2015), elemental concentrations in crops were found not to reflect soil concentrations and did not exceed legal limits for Cd and Pb in food. However, lettuce frequently showed the highest concentrations of trace metals, indicating that this crop is sensitive to air pollution. Another study analysing vegetables grown in a historical glasswork production region concluded that the heavy metal concentrations in the crops were within the legal values of commercial food and that the risk from consumption of the vegetables was within safe limits (Augustsson et al., 2015). However, in heavily contaminated sites, the risk associated with the intake of urban crops is significantly higher, and the influence of other exposure routes (i.e. drinking water, soil ingestion or dust inhalation) is expected to increase analogously. In urban gardens near mining and metallurgical industries, leafy species such as lettuce could bioaccumulate excessive amounts of heavy metals, but the phytoavailability of these contaminants can be limited by appropriate fertilisation practices that promote their immobilisation in the soil (Kicińska and Wikar, 2021).

In line with the two challenges facing humanity (i.e. controlling air quality and combating global food insecurity), the objective of this study was to assess whether lettuce grown in urban orchards could serve as a food source as well as a tool for monitoring atmospheric contamination. The specific objectives were: a) to evaluate whether lettuce could be used as an effective bioindicator of atmospheric pollution in an urban environment, b) to test whether washing lettuce leaves significantly reduces the load of pollutants deposited on them, c) to verify the extent to which the trace element content of lettuces grown in cities on a clean substrate is safe for human consumption, and d) to estimate the bioaccessibility of these pollutants and the implication of this for an assessment of risk to human health through food intake.

## 2. Materials and methods

### 2.1. Plant material and growth conditions

In this study, lettuce (*Lactuca sativa*) seeds were planted in seedbeds (to ensure germination), and transplanted one week later into plastic pots using a commercial substrate of sphagnum peat moss (Pindstrup Mosebrug, Fertilizer No.2), which contains the nutrients necessary for plant growth during the experiment. These elements are mainly N, P, K, Ca and Mg, along with other micronutrients such as boron, copper, iron, manganese, molybdenum and zinc. The details of the substrate composition, containing the aforementioned essential elements, have been reported previously by Izquierdo-Díaz et al. (2019). The influence of soil on the trace element concentration of the samples can therefore be excluded since the samples at all sites were planted on the same clean substrate and it was assumed that any differences in accumulation were due solely to the atmospheric environment to which they were exposed. The initial growth period, until transfer to the various study sites, was in a greenhouse at the University of Copenhagen.

To reduce the frequency of irrigation, a pseudo-hydroponic system was specifically designed in which plastic boxes were fitted with six holes in the lid so that the pots were suspended in them. Three textile wicks were inserted into the lower part of the box so that water (Milli-Q®) from the reservoir at the bottom was absorbed by capillary and osmotic gradients (Supplementary Material Fig. A.1).

## 2.2. Exposure sites

The study was conducted in the city of Copenhagen (Denmark), which has a population of almost 800,000, and with approximately 2500,000 people in the whole metropolitan area. Although Copenhagen is rated as the most bicycle-friendly city in the world (Copenhagenize Design Co, 2019), in 2010 34% of passenger journeys to work or study were made by car (motor vehicles accounted for 377,000 tonnes of CO<sub>2</sub> emissions). It is expected that by 2025 this figure will still be close to 25% (Technical and Environmental Administration of City of Copenhagen, 2012). However, total emissions of PM<sub>2.5</sub> and PM<sub>10</sub> in Denmark in 2019 were 13.02 kt and 23.20 kt respectively, and those of some trace elements were As 0.23 t, Cd 0.71 t, Cr 1.63 t, Cu 43.34 t, Hg 0.23 t, Ni 2.62 t, Pb 12.01 t, Se 0.44 t and Zn 60.57 t (Danish Centre for Environment and Energy, 2021).

Five exposure sites were selected for this study (Table 1), two in the city centre and three in Naerum, a suburban residential district located approximately 18 km north of Copenhagen city centre (Fig. 1A). A box with six pots was installed at each exposure site. The concentrations of particulate air pollutants in Copenhagen (PM<sub>2.5</sub> = 10.6–11.9 ug/m<sup>3</sup> and PM<sub>10</sub> = 13.0–16.7 ug/m<sup>3</sup>) are slightly higher than in Naerum (PM<sub>2.5</sub> = 9.4–10.5 ug/m<sup>3</sup> and PM<sub>10</sub> = 11.5–12.2 ug/m<sup>3</sup>) (DCE, 2019) (Fig. 1B).

An additional box was kept in a climate chamber with temperature, humidity and particle filter control in order to obtain a base level for the concentration of each element. Six seedlings were sampled shortly before the rest of the specimens were taken outside to the exposure sites to determine the initial concentrations in the seedling tissue.

## 2.3. Sampling, digestion and analysis

After 45 days at their respective locations (from 2 May to 16 June), the boxes were collected and transported to the laboratory. During this period, the cumulative precipitation in the city of Copenhagen was 36.42 mm. Each lettuce was cut through the stem with a steel knife (discarding dead leaves or those in contact with the soil) and stored in paper bags. For each site, half of the samples were washed three times with deionised water and the other half left unwashed in order to examine possible differences between the amount of contaminants absorbed and deposited (Bonanno, 2014). Therefore, in total, three replicates were obtained for each site and washing treatment (for a total of 36 samples). Samples were first dried in an oven at about 60 °C for 48 h in paper bags until constant weight (Khan et al., 2020), and then ground into powder using a vertical shaker in individual plastic containers with zirconium balls inside.

For the extraction of the analytes of interest (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Se, V and Zn), two methods were used: complete digestion (López-Rayó et al., 2016) and the simplified bioaccessibility extraction test (Izquierdo et al., 2015).

**Table 1**  
Description and location of the biomonitoring exposure sites.

Site	Description	Coordinates
“Control” (C)	Climate chamber with controlled test conditions.	
“Park” (P)	City centre: next to a busy arterial road.	55°41'10.94"N 12°32'39.89"E
“Road” (R)	City centre: close to a little-travelled street from which it is separated by a 2 m plant barrier.	55°40'54.59"N 12°32'26.88"E
“Urban orchard” (U)	Naerum: urban community orchard, 1.1 km from a motorway and more than 100 m from a little-travelled street. Half of its perimeter is surrounded by a forest.	55°49'46.50"N 12°31'54.53"E
“House garden” (V)	Naerum: kitchen garden in an area of single-family housing 400 m from a motorway.	55°49'43.81"N 12°32'38.87"E
“Motorway” (W)	Naerum: beside the motorway.	55°49'37.50"N 12°31'33.69"E

The latter was chosen to evaluate the fraction of the total content that is soluble in the gastric environment and available for human absorption. For the total content, around 100 mg of sample material were weighed, mixed with 2.5 mL of HNO<sub>3</sub> (70%) and 1 mL of H<sub>2</sub>O<sub>2</sub> (15%) (ACS reagent grades), and digested using a microwave oven (Multiwave 3000, Anton Paar, GmbH, Graz, Austria). Lastly, the solutions were made up to 50 mL with Milli-Q® water and stored in polypropylene tubes. The bioaccessible extraction consisted of a solution that simulates gastric juices, using a 0.4 M glycine (Sigma-Aldrich) solution adjusted to pH 1.5 with concentrated hydrochloric acid. In an Erlenmeyer flask, 50 mL of this solution was added to 0.5 g of sample and then agitated in an air bath thermostatic shaker at 37 °C for 1 h. The mixture was subsequently centrifuged at 10,000 rpm for seven minutes to achieve proper separation, and filtered through quantitative filter paper. Finally, trace element concentrations were measured in both types of solution by inductively coupled plasma mass spectrometry (7900 ICP-MS, Agilent), although Hg and Sb were subsequently excluded from the database because almost all the samples were below their detection limit.

A blank and a certified reference material (Apple leaves, SRM 1515, NIST) were included in each batch of samples and, for quality control purposes, processed in the same way as in all the preparation and analysis methods (adding the same volume of reagents and taking an identical mass of CRM as for the samples). For the calibration curves, in addition to a reagent blank, 12 standards of increasing concentrations were prepared from a multi-elemental solution (PlasmaCal, SCPScience). To determine the precision, a sample was analysed in quintuplicate, obtaining a relative standard deviation of less than 20% for all elements except P (20.3%) and S (41.1%) (Table A.1). In terms of the method's accuracy, recovery factors ranged between 88% and 116%, except for B (132%), Rb (77%) and As, Na and Se, which were significantly higher (Table A.1). Therefore, for the last three elements, only a qualitative discussion is provided, while a quantitative comparison with legal values should take this uncertainty into account.

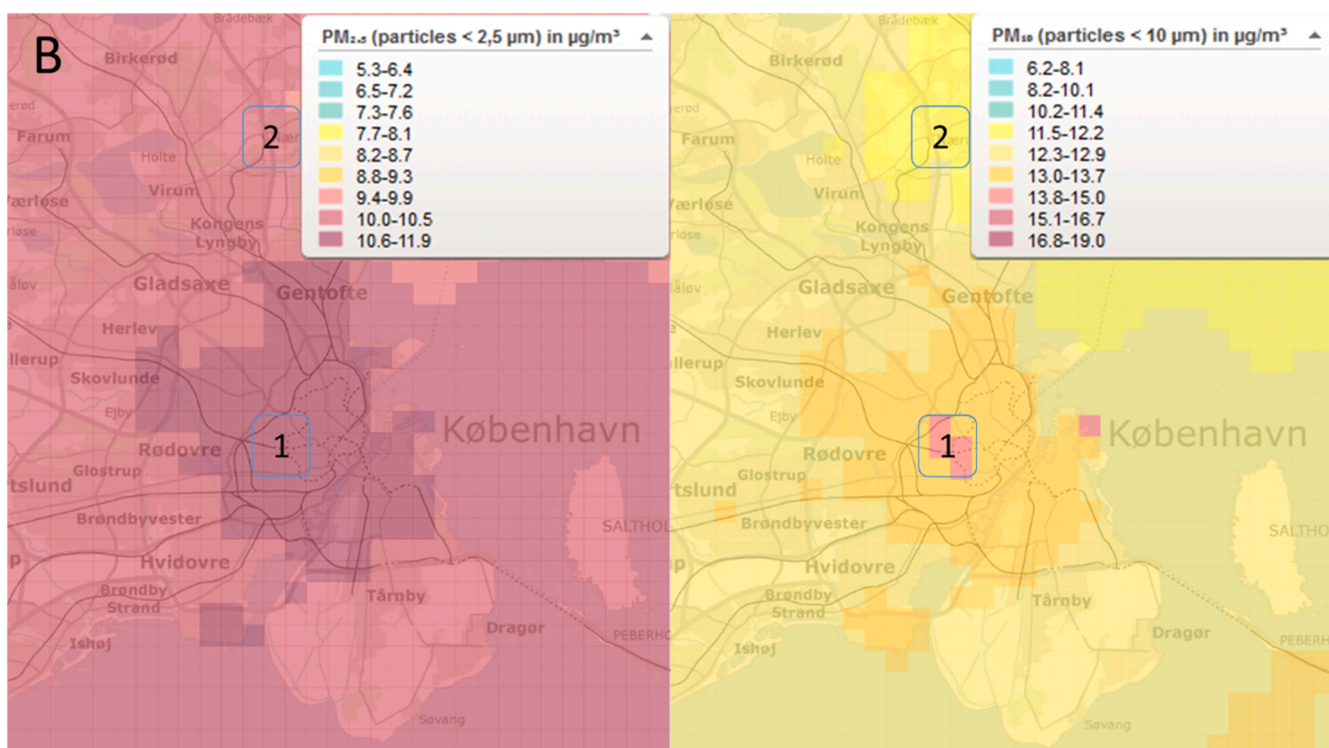
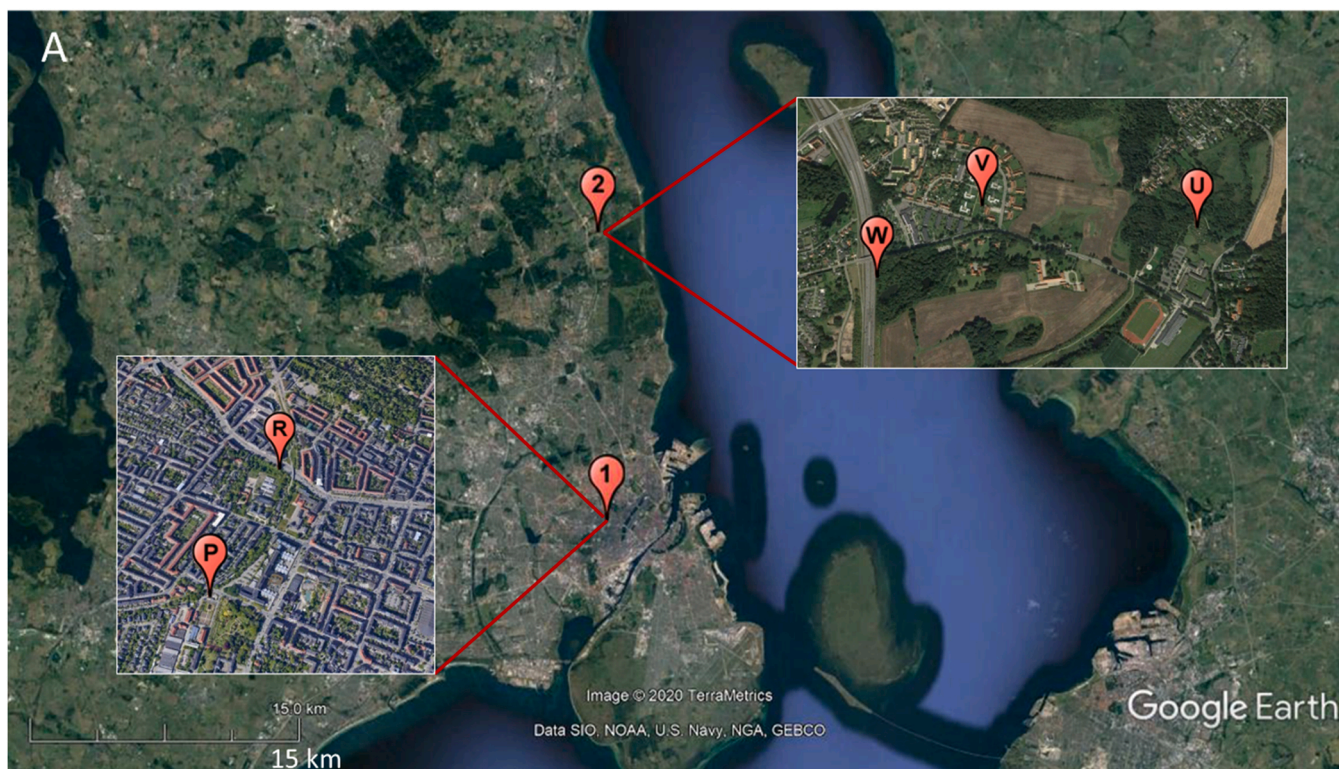
## 2.4. Data processing

Statistical analyses were based on dry-weight concentrations and were performed using Microsoft Excel 2016, R programming language 3.6.2, StatGraphics 18 and ProUCL 5.1. To check whether there were significant differences in elemental concentrations between the different exposure sites, a multiple means comparison Tukey's HSD (honest significant difference) test was performed, assuming as null hypothesis that the means of two data samples are equal and rejecting it with a confidence level of 95.0%, i.e. considering all differences with a p-value < 0.05 as significant. Similarly, a Kruskal-Wallis median comparison test was performed, which also compares samples from two populations, but using a non-parametric method based on ranks (also with a p-value < 0.05 as significant), which does not assume a normal distribution. To explore whether the elements could be correlated and grouped together, a cluster analysis was also carried out using Ward's minimum variance hierarchical clustering method (appropriate for quantitative variables) for linkage and the squared Euclidean distance.

To help interpret the results, a series of indices were calculated: pollution index (PI, Eq. 1) to compare the degree of contamination between the different exposure points (Qingjie et al., 2008), and the washout factor (WF, Eq. 2) to check the decrease in the concentration of contaminants after washing the leaves:

$$PI = \sqrt[n]{\prod_i C_i} \quad C_i = \text{concentration of the } i \text{ element (mg/kg)} \quad (1)$$

$$WF(\%) = \frac{\bar{C}_u}{\bar{C}_w} \cdot 100 \quad \begin{array}{l} \bar{C}_u = \text{average concentrations of unwashed samples} \\ \bar{C}_w = \text{average concentrations of washed samples} \end{array} \quad (2)$$



**Fig. 1.** A) Location of biomonitoring exposure points. 1: Copenhagen city centre (R = “Road”, P = “Park”) and 2: Naerum (U = “Urban orchard”, V = “House garden”, W = “Motorway”). B) Spatial representation of PM<sub>2.5</sub> and PM<sub>10</sub> concentrations.

For the human health risk assessment, dry-weight concentrations were transformed into wet values (Eq. 3), and the average daily dose of each element due to lettuce consumption was calculated (Eq. 4):

$$C_{ww} = C_{dw} \frac{100 - M}{100} \quad C_{ww} = \text{wet - weight concentration (mg/kg)} \quad (3)$$

$C_{dw}$  = dry - weight concentration (mg/kg)  
 $M$  = moisture content(%)

$$DI = C_i \cdot IR \cdot CF$$

$$DI = \text{dietary intake (mg/d)}$$

$$C_i = \text{element concentration (mg/kg)}$$

$$IR = \text{ingestion rate (g/d)}$$

$$CF = \text{conversion factor (10}^{-3}\text{ kg/g)}$$
(4)

### 3. Results and Discussion

#### 3.1. Atmospheric pollution levels

Trace element concentrations for each sample can be found in the [Supplementary Material Tables A.2 and A.3](#). [Fig. 2](#) shows notable differences in pollution indices (PI) between locations. According to the total concentrations in unwashed samples, the degree of pollution was higher in the city centre and near the motorway than in the less contaminated suburban sites (in descending order: “Road” >> “Motorway” > “Park” >> Initial > “House” > Control > “Orchard”) ([Fig. 2a](#)). The “Initial” PI, which corresponds to the seedlings, was higher than that of adult samples at some sites after the exposure period. As other studies have pointed out ([Marković et al., 2009](#); [Schlegel et al., 2016](#)), most minerals have a relatively high concentration in plant tissues during the initial stage of plant development, which they are required for successful growth, with a gradual dilution occurring as the plant matures, and consequently decreasing the element concentration per unit of biomass unless there is a greater external input. For washed samples, the previous sequence of decreasing concentrations was broadly the same (“Motorway” ≈ “Road” > “Park” > “Orchard” ≈ Control ≈ “House”) but for the most contaminated site (i.e. “Road”) the PI was reduced by a factor of three, while for the “Control” and the “Orchard” it remained practically unchanged. This indicates, first, that the highest contaminant contents are associated with the deposition of particles on the leaves (which are removed by washing) and, second, that a fraction of these contaminants is, to some extent, absorbed by the lettuce and incorporated into the vegetable tissue (through foliar uptake or to a lesser extent by enrichment of the substrate by particle deposition and subsequent translocation through the roots).

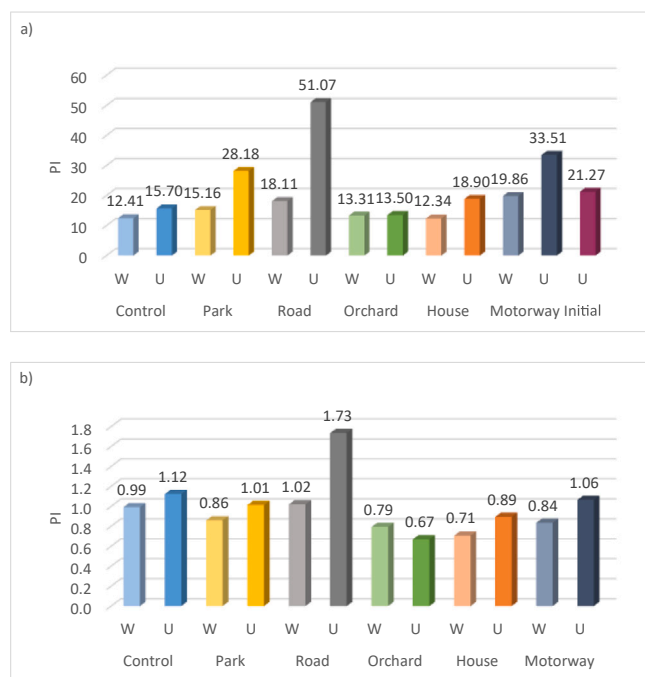
It is not easy to identify whether the accumulation of pollutants in internal plant tissues comes from the soil by root cell absorption or from

the atmosphere through the leaf surface, because both types of uptake pathways can occur simultaneously. Suggested mechanisms of foliar uptake of trace elements involve adsorption of particles on the surface wax and subsequent diffusion through fissures present on the leaf cuticle, and penetration via stomatal openings. In addition, under conditions of high humidity or rainfall events, trace elements can solubilise and favour penetration through aqueous pores. Once inside, metal(loid)s can be transported to other parts of the organism via the phloem vascular system, similar to photosynthates, with less than 1% estimated to reach root tissues. The uptake of trace elements by roots is governed by passive diffusion processes and has been extensively studied, being the main route of incorporation of nutrients into the plant. Metal(loid) translocation from roots to the aerial parts (approximately 5%) is via the xylem stream in the form of chelates and is induced by evapotranspiration. For both mechanisms (foliar or root uptake), the absorbed dose is proportional to the trace element concentration in contact with the surface layer of the respective plant tissues ([Shahid et al., 2017](#)). PIs were consistently lower for bioaccessible concentrations than for total contents, with reduction factors ranging from 12.5 (“Control” washed) to 31.5 (“Motorway” unwashed), and generally higher for unwashed samples ([Fig. 2b](#)). The degree of contamination based on bioaccessible PI followed almost exactly the same sequence as for total contents, except that in the “Control” samples it was higher than at the other exposure points, with the exception of the “Road” location. Regarding unwashed samples, the elevated bioaccessible “Control” PI was due to the fact that having grown in the climatic chamber, the deposition of particles was insignificant and the bioaccessibility of the elements in the plant tissue was higher than those that adhered to the surface of the particulate matter (as discussed in [Section 3.3](#)). Moreover, it should also be noted that the bioaccessible PI of unwashed samples at the “Orchard” site was lower before washing than after. A possible explanation is that the particles deposited at this site, unlike the other outdoor locations, were mostly of natural origin. In this case although with total extraction the different elements would be released, using bioaccessible digestion those contained in the mineral structure were not extracted, with lower relative concentrations in unwashed orchard plants than after washing (natural particles contribute to sample mass, but not to pollutant mass).

#### 3.2. Spatial distribution of atmospheric trace elements

The results of the comparison of the mean concentrations at each site depending on the extraction protocol (total/bioaccessible) and the washing procedure (without/with) by means of a Tukey test are given in the [Supplementary Material Tables A.4, A.5, A.6 and A.7](#). The lettuce exposed at the “Road” site presented the highest concentrations of all the elements analysed (except for those associated with the peat substrate, i.e. Ca, Cd, Mg and Mn) in unwashed samples ([Table A.4](#)). After washing the samples, the disparities in total concentration between the different sampling sites disappeared for quite a few elements, indicating that the major contribution of contaminants was due to particles adhering to the surface of the lettuce, which were removed by washing ([Table A.5](#)). However, the content was still higher at the “Road” site than at most other sites for B, Cu, K, Mo, S and Se, as well as for Na, P and Zn at the “Road” and “Motorway” sites. This indicates that a high percentage of contaminants were eliminated after washing, but that during the exposure time the plant would also absorb part of the elements, either through its tissue or by deposition of particles at the base of the plant, their dissolution and subsequent absorption by the roots. Sulfur enrichment is also associated with SO<sub>x</sub> absorption in gaseous form from coal-fired power station emissions, as observed in the washed samples from the “Road” site, which is located in the city centre and without a vegetation barrier.

When comparing the concentrations of the samples digested by glycine (i.e. bioaccessible) samples ([Tables A.6 and A.7](#)), the pattern observed was similar to that of the total contents, but the differences were smaller (e.g. “Road” was no longer higher in all cases, and



**Fig. 2.** Pollution index (PI) at each location (W = washed, U = unwashed) for a) total and b) bioaccessible concentrations.

“Motorway” and “Park” showed differences for fewer elements). Compared with the total extraction, the bioaccessible concentrations of certain elements (i.e. As, Ca, Mg and Mo) in the control samples were no longer lower than those at other exposure points. This apparent contradiction could be explained by the fact that bioaccessible extraction manages to remove a smaller percentage of contaminants associated with particles (more tightly retained on its surface by means of inner and outer sphere complexation) than those that are incorporated into plant matter. Thus, in these reference samples where there was no particle deposition and it was only plant tissue, the bioaccessible concentration could be higher for some elements than in the lettuce samples exposed outdoors. Xiong et al. (2014) obtained similar results with bioaccessibilities of Cd, Pb and Zn in spinach and cabbage above 75%, while for particulate matter bioaccessibility decreased for all three elements below 10%, and down to 0.7% for Pb.

The median comparison test also showed that there were differences between the concentrations of the different exposure points for the unwashed samples, especially for “Road”, which still showed higher concentrations for almost all elements than the “Orchard” and “Control” sites (Tables A.4, A.5, A.6 and A.7). However, for washed samples, there were fewer significant differences (especially for bioaccessible extraction). This confirms that the accumulation of contaminants mainly came from particle deposition.

An exploratory analysis of the data to identify the grouping of the variables according to their similarity showed that the elements could be separated into two main clusters (Fig. 3). The first cluster was elements typically of anthropogenic and urban origin (Al, As, Co, Cr, Cu, Fe, Mo, Ni, Pb, Se, V and Zn). These pollutants are generally associated with present and past emissions from urban and industrial sources, including traffic (most of these presented their highest concentrations in samples from the “Road” and “Motorway” sites) and fuel combustion. The enrichment of Pb at the “Road” and “Park” sites was very likely the consequence of the historical accumulation of this metal in urban soil particles and their subsequent relocation, which is still a legacy from its widespread use in the past until it was banned in the manufacture of many products (e.g. petrol or paints).

The other cluster was composed of elements that are mainly of natural origin (B, Ca, Cd, K, Mg, Mn, Na, P, Rb, and S), macronutrients that are contained in the peat substrate from plant growth, but also come from sea spray and salt that is spread on roads and pavements to lower the melting point of water and prevent ice formation (Skorbiłowicz and Skorbiłowicz, 2019). In a subsequent step, these elements were divided into two subgroups, with Ca, Cd, Mg and Mn exhibiting higher concentrations in the control samples than at some of the exposure sites with a lower degree of contamination. These last elements would be preferentially absorbed from the substrate and accumulated in the vegetation tissue. Consequently, for the reference samples (which do not have particles deposited on their surface), the quantity of elements in

relation to their weight was higher than that from the other exposure sites (particles + plant mass). The absorption of Cd is explained by its Ca-like characteristics in terms of the same ionic charge (+2) and similar ionic radius. Therefore, the uptake of Cd by plants is closely related to the movement of Ca, being antagonistic ions competing when they co-occur in soils (Eller and Brix, 2016; Hayakaway et al., 2011). Furthermore, the higher relative mobility and phytoaccumulation of Cd in crops compared with other metals has been well documented (Khan et al., 2015).

### 3.3. Effect of vegetable washing

Table 2a shows the washout factor calculated according to Eq. 2 for the results of the total extractions. One group of sampling sites (i.e., “Park”, “Road” and “Motorway”) exhibited statistically significant differences between the concentrations of the samples before and after washing for a set of elements typically associated with urban pollution. However, for the other sampling points, there were no relevant differences for any element with a p-value < 0.05 (although the concentrations in unwashed samples were generally higher). Remarkably, in the case of “Orchard”, for most of the elements the WF was even lower than unity, indicating that the concentration after washing (i.e. just in the plant tissue after removing the surface dust) was higher. This indicates that the particles deposited at this exposure point had a different source, being of natural origin from the surrounding environment and not anthropogenic, probably clays and hydroxide minerals because of the higher Al and Fe content, which increases the sample mass, but not the global trace element content.

Likewise, there was also a division between elements showing higher concentrations in the unwashed samples and those whose concentrations were similar upon washing. In particular, the WF was statistically significant at the “Road”, “Park” and “Motorway” sites for Al, Co, Fe, Se and V (at the three sites), Cr and Zn (except at the “Park” site), Ni and Pb (excluding the “Motorway” site) and As, Cu and Mn (just for the “Road” site). All these elements are associated with emissions from road traffic, heating and other urban sources (Alloway, 2004; Charlesworth et al., 2011). This shows, first, that sites with greater atmospheric pollution accumulate a greater amount of pollutants, so lettuce can be used as a bioindicator of air quality and, second, that washing vegetables is an effective measure for reducing the intake of toxic elements. In particular, for elements such as Al, Cr or V, this reduction could reach a factor of 20, which also indicates that these particles are weakly adhered to the surface of the leaves after deposition and can easily be washed off.

Looking at the bioaccessible fraction (Table 2b), the trend is similar, with fewer elements showing significant differences in WF, which also had lower values (the maximum in this case was 8.54 times for Al at the “Road” site). This was largely due to the fact that the contents extracted by glycine were significantly lower, and in particular for the mineral fraction (i.e. particles on the surface of the leaves), as discussed in the following section. Specifically, WF was significant for Al, Cr and V (“Road”, “Park” and “Motorway” sites), Zn (“Road” and “Motorway” sites) and Cu, Ni, Pb and Se (only at the “Road” site).

### 3.4. Bioaccessibility

Fig. 4 shows the bioaccessibility of the different elements, calculated using the slope of the linear regression of the bioaccessible concentrations versus the total concentrations, forcing the line to intersect the origin (Izquierdo et al., 2015). For the unwashed samples, the coefficients of determination were very high for most elements, i.e.  $R^2 > 0.80$  (except for As, Co, Fe, Mo, Ni, for which the adjustment was lower due to the existence of some outliers), while for washed samples this correlation was slightly weaker.

As can be seen, the bioaccessibility of washed samples (corresponding primarily to plant tissue) was greater than that of unwashed samples (which included both plant matter and particles adhered to the

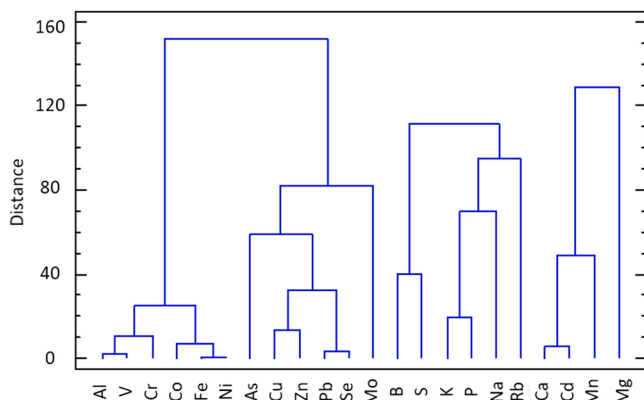


Fig. 3. Trace element cluster diagram.

**Table 2a**  
Washout reduction factor for trace element total contents. Numbers in bold and underlined indicate significantly different mean concentration values between washed and unwashed samples (p-value < 0.05).

Type	Al	As	B	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	Rb	S	Se	V	Zn
Control	1.83	1.00	1.02	1.22	1.26	1.43	3.92	1.00	2.35	1.17	1.26	1.31	1.21	1.01	1.92	1.18	0.21	1.15	1.20	1.20	1.96	1.26
"Park"	<b>8.48</b>	1.39	0.97	1.05	0.98	<b>2.50</b>	7.07	1.12	<b>3.76</b>	1.07	1.01	0.98	1.72	0.89	<b>4.39</b>	1.07	<b>4.37</b>	1.18	0.89	<b>2.37</b>	<b>8.96</b>	1.28
"Road"	<b>20.65</b>	<b>2.38</b>	1.08	1.11	1.06	<b>4.88</b>	<b>20.32</b>	<b>2.42</b>	<b>8.28</b>	1.02	1.01	1.18	<b>2.12</b>	1.02	<b>7.48</b>	1.11	<b>13.57</b>	1.19	1.00	<b>3.85</b>	<b>29.14</b>	<b>1.63</b>
"Orchard"	3.49	0.73	0.92	0.73	0.81	1.02	<u>1.38</u>	0.59	1.42	0.79	0.79	0.77	0.70	0.97	<u>1.21</u>	0.87	<u>0.87</u>	0.87	0.80	1.10	<u>4.19</u>	0.85
"House"	3.69	1.19	1.11	1.20	1.27	1.65	<u>2.97</u>	1.01	2.17	1.10	1.19	1.34	1.34	1.13	1.91	1.16	2.26	1.19	1.08	1.62	3.89	1.38
"Motorway"	<b>4.65</b>	1.55	1.14	1.03	1.04	<b>2.15</b>	<b>4.71</b>	1.21	<b>2.89</b>	1.01	1.11	1.00	1.57	1.18	2.82	1.10	3.16	0.99	1.04	<b>1.99</b>	<b>5.74</b>	<b>1.33</b>

**Table 2b**  
Washout reduction factor for trace element bioaccessible contents.

Type	Al	As	B	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	Rb	S	Se	V	Zn
Control	0.81	1.17	0.93	1.17	1.19	1.13	1.17	1.01	2.06	1.08	1.18	1.25	<b>2.10</b>	0.91	1.30	1.07	0.57	1.09	1.31	1.03	1.06	1.21
"Park"	<b>2.54</b>	1.04	0.95	1.05	0.98	1.14	<b>1.45</b>	1.01	1.31	1.05	0.99	0.99	1.21	0.88	1.24	1.07	1.72	1.10	0.93	1.24	<b>1.64</b>	1.33
"Road"	<b>8.54</b>	1.14	1.02	1.06	1.00	1.86	<b>3.30</b>	<b>1.86</b>	2.70	0.99	0.95	0.98	1.82	0.99	<b>2.46</b>	1.06	<b>6.12</b>	1.08	1.20	<b>1.84</b>	<b>4.04</b>	<b>1.48</b>
"Orchard"	<b>1.07</b>	0.74	0.92	0.73	0.82	0.90	<u>1.00</u>	0.71	1.19	0.75	0.80	0.77	0.65	0.93	0.85	0.83	<u>0.89</u>	0.82	0.70	0.85	1.03	0.86
"House"	1.79	1.12	1.05	1.16	1.23	1.41	1.21	1.04	1.54	1.04	1.14	1.30	2.10	1.06	1.32	1.09	1.72	1.14	1.16	1.32	1.20	1.22
"Motorway"	<b>3.09</b>	1.05	1.11	1.03	1.04	1.31	<b>1.63</b>	1.16	1.27	1.01	1.09	0.99	1.41	1.18	1.17	1.10	1.89	0.96	1.30	1.22	<b>1.77</b>	<b>1.42</b>

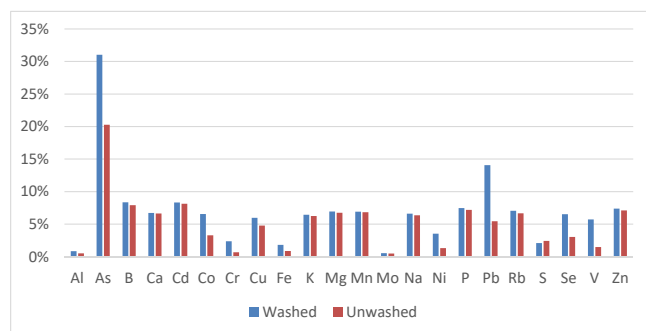


Fig. 4. Bioaccessibility of trace element samples depending on the washing treatment.

exposed surfaces), with the sole exception of S. This would indicate that the elements contained in plant tissues were more bioaccessible than those of inorganic particles, the latter being strongly retained in the inner mineral structure or adsorbed on the surface by means of inner or outer-sphere complex mechanisms. However, in some cases, the difference between washed and unwashed was not significant (B, Ca, Cd, K, Mg, Mn, Mo, Na, Rb, Zn), which may indicate that most of these elements were also incorporated by the plant from the substrate. However, for other elements, the ratio of washed to unwashed was 1.5 times (Al, As), or even more than double (Co, Fe, Ni, Pb, Se) or triple (Cr, V), which would suggest a notable external contribution, coming from the deposition of atmospheric particles, which supports the discussion in the previous sections.

Bioaccessibility values were generally below 10% (except for As and Pb, which were up to 31% and 14.1% respectively) and lower than those of other studies on bioaccessibility in dust or soil particles (Darko et al., 2017; De Miguel et al., 2012; Madrid et al., 2008; Xiong et al., 2014). However, depending on the type of matrix, source of pollution and the in vitro extraction method, bioaccessibility values have a wide range of variation and exhibit values of the same order or even lower than those obtained in this study (Ng et al., 2015). This implies that, when conducting a human health risk assessment, the risk through lettuce intake would be overestimated by more than one order of magnitude (up to two for Al and Mo, as well as for Cr and Fe for unwashed samples) if the total content is considered instead of the bioaccessible fraction, which would particularly apply for those substances having a toxicity value derived from studies in which the analyte was administered dissolved in an aqueous matrix (e.g. As).

### 3.5. Food safety for human consumption

Dry concentrations were converted to a wet basis (Eq. 3) to compare

Table 3

Elements intake from consumption of lettuce grown in urban areas, 95% upper confidence limit (UCL95) of the mean total concentrations, and legal and toxicological reference values.

		B	Cd	Cu	Fe	Mg	Mn	Mo	Ni	P	Pb	V	Zn	
<b>UCL95 (mg/kg ww)</b>		1.69	0.02	0.51	28.99	230.93	11.20	0.01	0.11	287.67	0.06	0.04	3.28	
<b>Health thresholds</b>	MLCCF <sup>1</sup> (mg/kg)		0.2								0.3			
	UL <sup>2</sup> (mg/d)		Adult 20 Child 6	0.03 <sup>3,5</sup> 0.01 <sup>3,5</sup>	10 3	45 40	350 110	11 3	2 0.6	1 0.3	4000 3000	0.29 <sup>4,5</sup> 0.11 <sup>4,5</sup>	1.8 12	40 12
<b>Dietary Intake (mg/d)</b>	Per capita	Adult	0.04	0.0004	0.011	0.61	4.90	0.24	0.0003	0.0024	6.10	0.0013	0.0009	0.07
		Child	0.01	0.0001	0.003	0.18	1.41	0.07	0.0001	0.0007	1.75	0.0004	0.0003	0.02
	Consumer only	Adult	0.06	0.0006	0.018	1.02	8.13	0.39	0.0005	0.0040	10.13	0.0022	0.0015	0.12
		Child	0.03	0.0003	0.008	0.45	3.60	0.17	0.0002	0.0018	4.49	0.0010	0.0007	0.05

<sup>1</sup> Maximum levels for certain contaminants in foodstuffs (Communities, 2008): leaf vegetables

<sup>2</sup> Tolerable Upper Intake Level (IOM, 2006). Adult: > 18 y, Child: 4–8 y

<sup>3</sup> (EFSA, 2011)

<sup>4</sup> (EFSA, 2010), although the panel discussed that it would no longer be entirely appropriate as there is no evidence for a threshold with no effects

<sup>5</sup> Original data were presented in µg/kgBW-wk; data were converted to mg/d by multiplying by a factor of 80 kg for adults and 31.8 kg for children and dividing by 1000 µg/mg and 7 d/wk

them with legal values and to calculate daily intakes, since lettuce is almost always consumed fresh. For this purpose, a moisture content of 95.1% was used (USEPA, 2011). Of the metals analysed and included in Commission Regulation No. 1881/2006 (EC, 2006), which sets maximum levels for certain contaminants in foodstuffs (Table 3), neither Cd (mean content = 0.016 mg/kg and maximum = 0.024 mg/kg) nor Pb (mean content = 0.024 mg/kg and maximum = 0.145 mg/kg) exceeded the permissible values for leaf vegetables in any of the samples. In fact, except in one case, the concentrations were one order of magnitude lower.

However, a simple risk analysis was performed to assess the possible risk of intake of elements other than Cd and Pb. Dietary intake (DI, Eq. 4) was estimated and compared with tolerable upper intake levels (UL) (IOM, 2006), defined as “the maximum level of daily nutrient intake that is likely to pose no risk of adverse effects”. For this purpose, data from the five outdoor locations (i.e. “Motorway”, “House”, “Orchard”, “Park” and “Road”) were selected, and an average lettuce intake in terms of washing and a degree of exposure to contamination were assumed (i.e. using the data from all samples for each element). On this basis, the 95% upper confidence limit (UCL95) of the mean total concentration was calculated for each element. For the lettuce intake rate, several studies included in the Exposure Factors Handbook (USEPA, 2011) were consulted, with the aim of calculating a final average value. A child (6–12 y) and an adult (20–49 y) were considered as potential receptors, and consumer-only intake (just including values for the population that consumed lettuce during the food survey period) and per capita intake (also incorporating individuals who stated no consumption) were assumed.

Table 3 shows that the DI does not exceed its corresponding UL for any of the receptors or elements. The element that could be considered closer to the health safety threshold is Mn, and the DI for the child consumer-only is 17 times less than its respective UL. For other elements such as B, Cu, Mo, Ni, P, Pb, V and Zn, the differences are even greater, up to more than two orders of magnitude. These results were based on total concentrations; if only the bioaccessible fraction were considered, the difference between the actual intake of elemental contaminants and their permissible values would be even greater.

Therefore, lettuce grown in an urban environment in Copenhagen on a clean substrate could initially be considered safe for consumption by the general population. However, it should be noted that only contaminants from lettuce were considered. Therefore, it would be advisable to add the contribution of other foodstuffs in a typical diet to obtain a comprehensive diagnosis.

## 4. Conclusions

The results of this study suggest that lettuce can be used as a bio-indicator of air quality since there is an enrichment in several of the

trace elements analysed that corresponds to the exposure environment in which lettuce has grown (i.e. urban area, near roads with traffic and next to a forest, and all under the influence of sea spray because they are close to the coast), especially if samples are not washed. Therefore, the use of lettuce would seem able to indicate sites that are exposed to higher levels of air pollution. It also shows that not only local atmospheric activities, but also the contribution of the background levels of the growth medium may be important in exposure to pollutants such as Pb. Thus, it can serve as an affordable complementary system to chemical-analytical method measurements and conventional air quality networks.

Secondly, for most elements, and in particular at the most polluted sites (i.e. "Road", "Motorway" and "Park"), after washing the samples, there was a clear decrease in the concentration of elements that are commonly associated with traffic and other industrial activities. This indicates that contaminants are mainly associated with particles adhering to the leaf surface and that their absorption into the plant tissue is limited. Therefore, it is highly recommended that produce grown in urban gardens is washed to reduce the potential ingestion of contaminants in surface-attached particles.

However, the content of elements extracted with bioaccessible digestion (which would correspond more closely to reality) was much lower than the total content in the samples (up to two orders of magnitude in some cases). Therefore, this parameter should be considered in human health risk assessment equations. Furthermore, bioaccessibility values are different depending on the matrix, being higher for plant tissues than for mineral particles, and should therefore be estimated separately for the food and soil ingestion pathways. Studies that have established the toxicity value should also be reviewed to verify the type of matrix used to administer the contaminants.

Lastly, according to the results of the study, the individual health risk from the consumption of lettuce grown in urban gardens appears to be negligible. This statement is only valid on the assumption that clean soil is used during planting and that the lettuce is adequately washed before consumption. It is also recommended that sites away from roads and with green barriers are selected in which to grow crops in urban areas. However, further evaluations should be carried out considering the additive contribution of contaminants from other agricultural products to verify that safety thresholds are still not exceeded. It is also necessary to check whether food grown in cities with worse atmospheric pollution conditions is suitable for human consumption.

#### CRedit authorship contribution statement

**Miguel Izquierdo-Díaz:** Conceptualization, Methodology, Validation, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Veronika Hansen:** Validation, Investigation, Writing – review & editing. **Fernando Barrio-Parra:** Formal analysis, Visualization, Writing – review & editing. **Eduardo De Miguel:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration. **Yawen You:** Formal analysis, Investigation. **Jakob Magid:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Project administration.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data Availability

Data will be made available on request.

#### Acknowledgments

M. Izquierdo is grateful to Universidad Politécnica de Madrid for the PhD research stay fellowship (Programa propio 2017: Convocatoria de ayudas al personal docente e investigador para estancias breves en el extranjero) and to the CARESOIL-CM (S2018/EMT-4317) research grant of the Regional Government of Madrid (Comunidad de Madrid, Spain).

#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.114883.

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