

# Assessing Roadside Conditions and Vehicular Emissions Using Roadside Lettuce Plants

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## Abstract

Effects of the roadside atmosphere were investigated by placing pots of lettuce plants (*Lactuca sativa* L. cv Romaine) along a transect representing different levels of urbanization in Jeddah city, Saudi Arabia. Concentrations of elements in soil samples, foliage dust, and leaves of lettuce collected from rural, urban, suburban, residential, and industrial areas were analyzed for Ni, Pb, Co, Zn, V, Sb, As, Cu, Cr, K, Mg, Ca, Fe, Al, and Na. We found higher concentrations of these elements in the urban and industrial areas than in the other areas. These high concentrations could be attributed to traffic sources and depend on traffic and urbanization levels. Lettuce plants can be used as bioindicators to determine the effects of air pollution in the atmosphere of the city. It is necessary to monitor toxic metals for plants used for human consumption.

**Keywords:** roadside dust, foliage dust, traffic-related elements, lettuce (*Lactuca sativa* L. cv Romaine), biomonitoring

## Introduction

Motor vehicle emissions are a major source of air pollution in the world [1-4]. Bell [5] stated that urban areas in arid and semiarid regions experience high concentrations of pollutants associated with motor vehicle emissions in the form of fine particulates (PM<sub>10</sub> and PM<sub>2.5</sub>), ozone (O<sub>3</sub>) of nitrogen dioxide (NO<sub>2</sub>), and nitric oxide (NO).

There is an increasing awareness to identify hot spots caused by traffic road dust around the globe [3]. Governments are starting to reduce, regulate, and set up limits on levels of heavy metals that should not be exceeded [6] because they are ubiquitous.

Increasing industrialization, urbanization, and vehicular traffic in Jeddah could increase levels of heavy metals in air and soil, which leads to a high pollution pressure on the biota and eventually would pose a threat to food safety and human health [7].

Plants proved to be very powerful tools in assessing environmental pollution because of their wide distribution [1, 4]. Plants growing near roadsides show poor growth and reduced photosynthetic rates, and suffer from an accelerated degradation of epicuticular waxes [8].

Alfani [9] assessed concentrations of toxic elements in ambient air in Italy through elemental analyses of foliar dust and leaf tissues, and this was supported recently with a similar study in Vienna [3].

It is assumed that the concentrations of heavy metals and air pollutants are lower in the rural areas than urban ones due to higher industrial activities and increased traffic [3-13]. Although this subject was extensively studied in developed countries [10-13], to the best of our knowledge, nothing is known about the responses of vegetation in developing countries, especially in arid and semiarid regions. There is a dearth of information on effects of vehicle exhausts on plants in the region. This study was undertaken to fill the above-mentioned gap of knowledge aimed at evaluating the concentrations of airborne heavy

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metal in leaves of lettuce plants (*Lactuca sativa* L. cv Romaine) and topsoil in Jeddah Province, Saudi Arabia.

## Materials and Methods

### Study Area

Sampling sites were located in and around the city of Jeddah (on the western coast of Saudi Arabia) at N 21°67', E 39°15', 15 meter above sea level. Its population of around 3.5 million represents one of the largest cities by population and industry in KSA. It has well-established traffic networks because it is the port of the kingdom, resulting in high population density and a dense urban and infrastructural network.

Five sampling locations (urban, suburban, rural, residential, and industrial) were chosen along a gradient representing different levels of urbanization in order to quantify the hazardous effects of air pollution caused by traffic. The gradient extended over a distance of approximately 23 km within the boundaries of the city.

### Plant Material and Growth Conditions

Seeds of Lettuce (*Lactuca sativa* L. cv Romaine) plants were obtained from the Department of Agronomy and Crop Sciences, Faculty of Agriculture, Egypt. Seeds were washed with distilled water to remove excess pesticides or herbicides and to break dormancy. They were soaked in distilled water at 30°C overnight, and then surface-sterilized with 10% NaClO for 15 min. Sterilized seeds were thoroughly washed with tap water, sown in 5L plastic pots containing washed sand and multipurpose compost (3:2) in a controlled glasshouse under 30/25°C day/night temperature, 300  $\mu\text{mol m}^{-2}\text{s}^{-1}$  photon flux density, 12-h photoperiod and 70% relative humidity on 3 January 2012. There were 120 pots, each containing 10 seeds. Each pot was supplied twice with 50 mL tap water weekly. When the seedlings emerged, 7 days after sowing, they were thinned to five seedlings/pot. Twenty pots were distributed in each site within the investigated areas and exposed to ambient conditions for 75 days.

Lettuce plants were chosen because of their compact and convenient sizes as well as rapid growth and their nutritional value.

Twenty pots were kept inside the greenhouse to serve as control.

### Soil, Leaves, and Foliage Dust Collection and Preparation

Plants and soil samples (0-10 cm depth) were collected from each pot at each site on 25 December 2011, packed into labeled polythene bags and brought to the laboratory to be analyzed for elemental composition to detect the effects of traffic and ambient air pollution chemical analysis.

Half of the leaves were collected and pooled according to sampling sites within the investigated areas in labeled plastic bags and stored at +4°C in the dark before preparation for analysis [14-16]. They were dried and homogenized with an electric mixer for elemental analysis [16, 17].

The remaining leaves were washed with deionized water to wash down the foliar dust. Then they were stored in a 500 ml plastic box containing 250 ml of deionized water. These boxes were shaken for 20 min at room temperature. The dust containing suspension was filtered and dried in a microwave to determine the dry weight of dust [16].

Soil samples were oven dried, gently ground, and sieved to 2 mm mesh size, homogenized, and used for analyses [18, 19].

All chemicals used were of analytical reagent grade and were utilized as received [3].

Plants and soil samples from pots grown in the greenhouse were also collected and used as control throughout this study.

### Elemental Analysis

Elemental analysis was performed by inductively coupled plasma optical emission spectrometry (ICP-OES) using an IRIS Intrepid II XSP instrument. A sixpoint calibration procedure was applied with multi-element calibration solution (Merck ICP multi-element standard solution IV).

### Statistical Analysis

Data were subjected to one way ANOVA using the SATATGRAPHICS statistical software package. Least Significant Difference (LSD) Test was applied to assess the significant differences among the mean values of different attributes. The values are means of 10 replications. The relationships between the concentrations of heavy metals in soils and elemental concentrations in the leaves and dust foliage were assessed using correlation analysis. There were 24 replicates.

## Results

### Metal Contents in Soil Samples

Table 1 shows mean concentrations of the investigated metals in different experimental sites. In all control samples, concentrations of V, Sb, As, Cu, and Cr were found to be below detection level ( $\text{BDL} \leq 0.1 \text{ mg}\cdot\text{kg}^{-1}$ ), while concentrations of Ni, Pb, Co, Zn, Mg, Ca, Fe, Al, and Na were 1.93, 0.76, 0.34, 2.01, 187.9, 21.7, 1012, 603, 312, and 459  $\text{mg}\cdot\text{kg}^{-1}$  dry weight, respectively.

There were significant differences ( $P \leq 0.05$ ) in concentrations of metals collected from different soils along an urbanization gradient. The lowest concentrations were recorded in the rural site, followed by the residential one, while the highest concentrations were recorded in the urban and industrial sites (Table 1).

Table 1. Analytical data (mg·kg<sup>-1</sup>) of the tested metal ions in different experimental sites.

Element	Soil samples (mean±SE)					
	Control	Rural	Urban	Suburban	Residential	Industrial
Ni	1.93±0.05 <sup>a</sup>	17.89±1.72 <sup>b</sup>	70.54±4.81 <sup>c</sup>	57.86±6.32 <sup>d</sup>	39.54±4.83 <sup>c</sup>	76.87±8.21 <sup>c</sup>
Pb	0.76±0.03 <sup>a</sup>	16.65±1.58 <sup>b</sup>	120.65±9.39	89.87±8.44 <sup>d</sup>	40.65±5.28 <sup>c</sup>	112.53±11.5
Co	0.34±0.04 <sup>a</sup>	1.27±0.09 <sup>b</sup>	5.17±0.17	3.54±0.23	2.96±0.58 <sup>c</sup>	3.25±0.6
Zn	2.01±0.06 <sup>a</sup>	28.61±2.12 <sup>b</sup>	497.7±23.8	217.87±11.2 <sup>d</sup>	161.65±15.6 <sup>c</sup>	326.71±21.4
V	BDL	6.57±0.07 <sup>b</sup>	136.9±11.3	110.23±9.7 <sup>c</sup>	105.69±12.9 <sup>c</sup>	143.21±13.6
Sb	BDL	0.37±0.01 <sup>b</sup>	3.87±0.07	1.39±0.05 <sup>c</sup>	1.28±0.09 <sup>c</sup>	2.74±0.8 <sup>d</sup>
As	BDL	3.96±0.01 <sup>b</sup>	20.6±1.37	18.23±1.33 <sup>c</sup>	16.08±1.23 <sup>c</sup>	21.36±2.31
Cu	BDL	9.45±0.07 <sup>b</sup>	40.54±2.83	24.65±1.81 <sup>d</sup>	19.73±1.58 <sup>c</sup>	43.54±4.69
Cr	BDL	4.02±0.03 <sup>b</sup>	123±8.86	91.72±7.72	77.80±5.17 <sup>c</sup>	164.7±14.7
K	187.9±10.2 <sup>a</sup>	8,411±192 <sup>b</sup>	14,356±1507	11,512±983	10,983±1021 <sup>c</sup>	15,432±1,492
Mg	21.7±1.08 <sup>a</sup>	8,764±298 <sup>b</sup>	31,398±418	22,919±194	12,135±172 <sup>c</sup>	39,185±472
Ca	1012±135 <sup>a</sup>	31,315±2,015 <sup>b</sup>	36,918±893	34,114±1,023	25,853±1,982 <sup>c</sup>	50,432±3,937
Fe	603±20.6 <sup>a</sup>	3,245±212	5,437±498	8,326±928	2,891±218 <sup>b</sup>	92361±918
Al	312±21.3 <sup>a</sup>	578±41.8 <sup>b</sup>	1,096±73.8	895±84.9 <sup>c</sup>	842±84.1 <sup>c</sup>	1,157±127
Na	459±19.6 <sup>a</sup>	9,465±218 <sup>b</sup>	14,535±783	11,375±828 <sup>c</sup>	9,129±421 <sup>b</sup>	16,752±2,017

Values (means±SE, N=10) in the same row followed by different letter(s) are significantly different at P<0.05. BDL – below detection level.

Concentrations of Ni ranged from 17.89 to 76.87 mg·kg<sup>-1</sup> in soils collected from rural and industrial locations, respectively (Table 1). Similarly, Pb content increased 2, 2, 7, and 8-fold, while Zn contents increased by 6, 7, 12, and 17-fold in soils collected from residential, suburban, urban, and industrial areas, respectively, when compared with concentrations collected from rural areas (Table 1). Moreover, other elements analyzed in the present study showed the same pattern, where rural soils have the lowest concentrations of heavy metals, while soils collected from urban and industrial sites contain the highest levels of these metals.

#### Elemental Concentrations in Leaves and Foliage Dust

Elemental concentrations in leaves and foliage dust of Lettuce (*Lactuca sativa* L. cv Romaine) plants are shown in Table 2. Concentrations of V, Sb, As, Cu, and Cr were BDL in the control samples.

We found significant differences in concentrations of the measured elements ( $p \leq 0.05$ ), where the concentrations of heavy metals in leaves and foliage dust collected from urban and industrial sites were significantly ( $P < 0.05$ ) higher than those collected from rural, suburban, and residential areas (Table 2).

The average highest concentrations of Ni (61.76 and 56.67 mg·kg<sup>-1</sup>) were detected in leaves and foliage dust

samples collected from industrial sites, respectively, while the lowest (0.92 and 0.27 mg·kg<sup>-1</sup>) were measured in samples collected from control sites for the same samples, respectively. The second highest values (47.78 and 51.72 mg·kg<sup>-1</sup>) were found in leaves and foliage dust collected from urban sites, respectively (Table 2).

Zinc was found to be the fourth highest level (min. 1.23±0.07, max. 322.7±43.7 and 0.95±0.05 and 498.41±53.91 mg·kg<sup>-1</sup> in leaves and foliage dust, respectively) after Fe, Al, Na in the present investigation (Table 2).

Iron was found to be at high levels in all samples from all locations, and higher (31,863±2,798) at industrial area and lowest (41.8±4.23) at the control site (Table 2). Moreover, Fe concentrations were higher in leaf samples than soil specimens (Tables 1 and 2). Furthermore, chemical analysis of other elements in leaves and foliage dust followed the same pattern, where control samples have the lowest concentrations of heavy metals and elements followed by the samples collected from the rural site, while the samples collected from urban and industrial sites contain the highest levels of these elements (Table 2).

A least-squares linear regression analysis was obtained for all metals between the concentrations of the element in soils and in the leaves and dust foliage (Table 3). The results show that the correlation coefficients (r) for these metals were significant at  $p < 0.001$ .

Table 2. Elemental concentrations in leaves and dust wash (mg·kg<sup>-1</sup> dry weight).

Element	Leaf samples					
	Control	Rural	Urban	Suburban	Residential	Industrial
Ni	0.92±0.03 <sup>a</sup>	19.45±1.64 <sup>b</sup>	47.78±6.25 <sup>d</sup>	31.11±4.95 <sup>c</sup>	16.94±2.01 <sup>b</sup>	61.76±6.84 <sup>e</sup>
Pb	0.42±0.05 <sup>a</sup>	5.26±0.74 <sup>b</sup>	127.87±11.9 <sup>e</sup>	95.23±11.32 <sup>d</sup>	42.37±4.39 <sup>c</sup>	143.85±17.53 <sup>f</sup>
Co	0.11±0.01 <sup>a</sup>	0.78±0.08 <sup>b</sup>	4.23±0.91 <sup>e</sup>	2.67±0.36 <sup>d</sup>	1.96±0.04 <sup>c</sup>	3.79±0.84 <sup>e</sup>
Zn	1.23±0.07 <sup>a</sup>	24.8±3.45 <sup>b</sup>	409.5±21.43 <sup>e</sup>	178.4±15.92 <sup>d</sup>	140.1±17.3 <sup>c</sup>	322.7±43.7 <sup>e</sup>
V	BDL	2.56±0.07 <sup>b</sup>	100.5±12.84 <sup>e</sup>	78.2±10.82 <sup>d</sup>	67.9±7.36 <sup>c</sup>	125.9±16.56 <sup>f</sup>
Sb	BDL	0.45±0.005 <sup>b</sup>	3.98±0.68 <sup>c</sup>	2.01±0.58 <sup>d</sup>	1.56±0.06 <sup>c</sup>	4.17±0.51 <sup>f</sup>
As	BDL	2.98±0.06 <sup>b</sup>	19.7±2.03 <sup>d</sup>	15.8±1.39 <sup>c</sup>	14.5±1.02 <sup>c</sup>	25.7±3.56 <sup>e</sup>
Cu	BDL	8.56±1.02 <sup>b</sup>	38.1±4.03 <sup>c</sup>	19.6±2.74 <sup>b</sup>	13.8±1.12 <sup>c</sup>	50.3±6.19 <sup>d</sup>
Cr	BDL	2.98±0.37 <sup>b</sup>	99.4±11.63 <sup>e</sup>	87.9±9.36 <sup>d</sup>	56.8±6.38 <sup>c</sup>	135.5±17.93 <sup>f</sup>
K	101.6±6.7 <sup>a</sup>	1569±127 <sup>b</sup>	4906±297 <sup>d</sup>	2679±302 <sup>c</sup>	3037±372 <sup>c</sup>	5901±567 <sup>e</sup>
Mg	6.5±0.21 <sup>a</sup>	6751±84.9 <sup>b</sup>	4002±310	3115±296	2742±317	1004±197 <sup>b</sup>
Ca	529±10.44 <sup>a</sup>	26983±2012 <sup>b</sup>	29938±2567 <sup>d</sup>	23910±1921 <sup>c</sup>	19836±2198 <sup>b</sup>	34981±3374 <sup>e</sup>
Fe	106±7.43 <sup>a</sup>	38271±4734 <sup>b</sup>	5692±391 <sup>d</sup>	4589±527 <sup>c</sup>	3005±327 <sup>b</sup>	31863±2798 <sup>e</sup>
Al	107±9.82 <sup>a</sup>	467±51.7 <sup>b</sup>	927±37.4 <sup>c</sup>	603±72.8 <sup>b</sup>	593±63.8 <sup>b</sup>	1983±281 <sup>d</sup>
Na	354±18.47 <sup>a</sup>	7839±405 <sup>b</sup>	11094±967 <sup>e</sup>	8931±783 <sup>c</sup>	8095±635 <sup>d</sup>	17047±1927 <sup>f</sup>
	Foliage dust samples					
Ni	0.27±0.02 <sup>a</sup>	19.45±2.32 <sup>b</sup>	51.73±4.03 <sup>d</sup>	29.45±3.64 <sup>c</sup>	14.16±2.15 <sup>b</sup>	56.67±5.47 <sup>d</sup>
Pb	0.11±0.01 <sup>a</sup>	5.98±1.05 <sup>b</sup>	163.8±21.2 <sup>e</sup>	100.57±12.3 <sup>d</sup>	61.04±7.03 <sup>c</sup>	167.36±23.08 <sup>e</sup>
Co	BDL	1.07±0.06 <sup>b</sup>	4.07±0.29 <sup>d</sup>	3.55±0.24 <sup>c</sup>	3.01±0.31 <sup>c</sup>	4.17±0.41 <sup>d</sup>
Zn	0.95±0.05 <sup>a</sup>	25.9±3.06 <sup>b</sup>	608.5±73.9 <sup>f</sup>	311.34±41.4 <sup>c</sup>	100.25±12.6 <sup>d</sup>	498.41±53.91 <sup>e</sup>
V	BDL	2.98±0.06 <sup>b</sup>	98.25±10.01 <sup>e</sup>	86.9±7.09 <sup>d</sup>	70.2±7.11 <sup>c</sup>	141.4±18.28 <sup>f</sup>
Sb	BDL	0.51±0.04 <sup>b</sup>	4.56±0.56 <sup>c</sup>	2.81±0.11 <sup>d</sup>	1.23±0.08 <sup>c</sup>	5.91±0.73 <sup>f</sup>
As	BDL	3.01±0.08 <sup>b</sup>	21.00±2.09 <sup>d</sup>	14.5±1.04 <sup>c</sup>	12.04±1.23 <sup>c</sup>	32.7±2.46 <sup>e</sup>
Cu	BDL	9.05±1.37 <sup>b</sup>	41.94±4.28 <sup>c</sup>	21.04±2.37 <sup>d</sup>	15.9±2.12 <sup>c</sup>	62.9±7.93 <sup>f</sup>
Cr	BDL	3.01±0.69 <sup>b</sup>	108.6±11.68 <sup>d</sup>	99.43±8.97 <sup>d</sup>	57.2±7.16 <sup>c</sup>	170.3±24.91 <sup>e</sup>
K	110.3±0.6 <sup>a</sup>	1,890±175 <sup>b</sup>	7,910±819 <sup>c</sup>	2,981±327 <sup>d</sup>	3,092±297 <sup>d</sup>	6,281±569 <sup>e</sup>
Mg	2.1±0.08 <sup>a</sup>	7,190±839	3,991±412 <sup>c</sup>	3,636±326 <sup>b</sup>	3,297±315 <sup>b</sup>	1,294±178 <sup>d</sup>
Ca	45.6±3.07 <sup>a</sup>	27,981±2,893 <sup>b</sup>	30,192±3,101 <sup>d</sup>	24,187±2383 <sup>b</sup>	19,901±2018 <sup>c</sup>	35,917±4,382 <sup>d</sup>
Fe	41.8±4.23 <sup>a</sup>	40,912±3,289 <sup>f</sup>	7,815±819 <sup>d</sup>	6,751±738 <sup>c</sup>	4,893±417 <sup>b</sup>	27,983±3,195 <sup>e</sup>
Al	25.7±3.14 <sup>a</sup>	598±63.9 <sup>b</sup>	1,085±119 <sup>c</sup>	862±76.9 <sup>d</sup>	701±83.2 <sup>c</sup>	21,963±2,836 <sup>f</sup>
Na	57.3±4.85 <sup>a</sup>	8729±792 <sup>b</sup>	12,848±1,327 <sup>d</sup>	10,935±1037 <sup>c</sup>	10,018±1002 <sup>bc</sup>	1,8937±2,176 <sup>c</sup>

Values (means±SE = 10) followed by different letter(s) are significantly different at P≤0.05.

## Discussion

The chemical analysis of soils, leaves, and foliage dust revealed a significant difference in concentrations of heavy metals and elements collected from different sites in Jeddah. This can be attributed to the different traffic densi-

ty between the six sites. This is in agreement with results of Kadi [3], who showed that soils of Jeddah have high concentrations of Zn, V, Sb, Pb, Ni, Cr, Co, As, and K, and he attributed these elevated levels to the strong influence of traffic and industrial activities in urban and industrial environments.

Elevated concentrations of nickel in soils, and plant samples collected in this study could be attributed to emissions from motor vehicles that use nickel gasoline and by abrasion and corrosion of nickel from vehicle parts. Recently, Aissa and Kéloufi [20] found similar results in Algeria. The results further revealed that the sources of nickel in Jeddah are emissions from motor vehicles running on petroleum and diesel fuel. The elevated concentrations of Zn could be attributed to tire products in the collected specimens.

Lead reaches plants through soil and aerosol sources. Higher Pb concentrations in soil and plant samples collected near roads in urban and industrial areas in the present study than those from rural ones confirm the suggestion that Pb is widespread in urban road dust [29, 30]. Pb comes from industrial and domestic wastewater and air pollution resulting from vehicle exhaust output and incineration of fossil fuels into the environment [31].

Jeddah has congested roads and many residential areas in proximity to these roads and around industrial zones; this would worsen air quality in the city. Moreover, this would pose a threat to people living nearby who breathe contaminated air or by eat food containing Pb [32-34].

Zinc is an essential element in all living organisms and plays a vital role in the biosynthesis of proteins (hormones and enzymes). It was found to be the fourth highest levels in all samples after Fe, Al, and Na in the present investigation. These high levels of zinc would reduce productivity [35, 36].

Cu is very important for chlorophyll normal functioning and any excessive supply would cause chlorosis, leading to loss in nutritional quality [36, 37]. The principle sources of Cu are home tools production, metal manipulating, road traffic, and ash [20].

Fe is an essential element used by photosynthetic and respiratory enzymes. The high concentrations of Fe detected in this study may be partly due to the absorption from soil by the roots of plants. Moreover, elevated levels in leaves collected from rural and industrial areas compared to controls indicate the prevalence of Fe in the environment of Jeddah.

The significant difference in elemental concentrations of soils, foliage dust, and leaves between urban, industrial, residential, rural, and control areas give some confidence that industrial activities and traffic are major sources of pollution in urban areas. This is in general agreement with similar results in Jeddah (KSA) [3]; Austria [16]; Algeria [20]; Spain, Slovenia, and Italy [21]; Hong Kong [22]; Nigeria [23]; Turkey [24, 25]; and Egypt [26]. Moreover, heavy metal contents in Macedonian medicinal plants grown in polluted areas were found to exceed WHO guidelines, while those collected from the unpolluted areas were below these guidelines [27]. Recently, Çelik et al. [36] found concentrations of elements at high levels in industrial areas in Turkey in the order of Fe>Zn>Pb>Cu>Cd. Nevertheless, metal concentration in samples is higher in the present study than in Europe [16, 21, 28], Africa [20, 23], and Asia [22, 24, 25]. There is a strong correlation between heavy metal con-

Table 3. Relationships between heavy metal concentrations in soil and elemental concentrations in leaves and dust wash of lettuce.

Element	r	
	leaves	foliage dust
Ni	0.645*	0.674**
Pb	0.834**	0.754**
Co	0.514*	0.524*
Zn	0.673**	0.710**
V	0.503*	0.547*
Sb	0.697**	0.817**
As	0.467*	0.868**
Cu	0.762**	0.789**
Cr	0.789**	0.847**
K	0.896**	0.847**
Mg	0.818**	0.649*
Ca	0.517*	0.638**
Fe	0.847**	0.859**
Al	0.846**	0.874**
Na	0.451*	0.668*

r – correlation coefficient, \*p< 0.01, \*\*p< 0.001, n=24

centration in leaves and degree of urbanization. This clearly indicates that the origin of metal contamination in the investigated area is related to vehicular traffic [3, 38].

## Conclusion

The main reason for high concentrations of heavy metals in plants located in industrial areas and in urban roadsides are the industrial activity and the density of the traffic. The positive correlation between elemental concentrations in soils and in leaves and foliage dust indicate that the origin of investigated elements in the present study is related to road traffic. We strongly recommend not consuming any food and vegetables from urban roadsides. Lettuce plants proved to be suitable for use in environmental studies as a bioindicator.

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