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RESEARCH ARTICLE

USING *Tilia tomentosa* IN HEAVY METAL POLLUTION MONITORING IN ANKARA PROVINCE, TURKEY

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ABSTRACT

Heavy metals, the concentration of which in the environment is constantly increasing and which can remain intact in nature for a long time, are a great threat to living things. In addition, due to the fact that it causes bioaccumulation in the structure of living things, the detection of heavy metal concentration is very important. Heavy metals constitute the largest part of air pollution. However, living things in areas where traffic is heavy are exposed to exhaust fumes, and this significantly negatively affects the health of living things. In our study, the areas where heavy traffic are concentrated in Ankara were determined and the possibilities of using linden trees, which were planted abundantly in the middle refuges, as a biomonitor for the change in heavy metal hunters were investigated. Changes of Pb, Fe, Cd, Cr and As elements in soil and plant samples were analyzed in ICP-MS device. SPSS 22 Statistical Package Program was used to evaluate the obtained data. The *Tilia tomentosa* Moench. parts (leaf, flower and branch) are evaluated, it is seen that the heavy metal amounts in the unwashed samples are higher than the washed samples for all heavy metals. The highest metal concentration among the stations was Fe (40681 $\mu g g^{-1}$) collected from Gazi station, the lowest concentration was Cd (4.9 $\mu g g^{-1}$) collected from the Mogan station on soils.

Keywords: Biomonitor, Heavy Metal, ICP-MS, *Tilia tomentosa* Moench

1. INTRODUCTION

The rapidly increasing world population causes air pollution to increase exponentially, especially due to the use of fossil fuels in large areas [1]. Due to diseases caused by air pollution, millions of people die every year around the world [2]. Heavy metals with an atomic number greater than 20 and with an atomic number of more than 5 g/cm³ in density, which are among the main causes of air pollution, are known as polluting metals. The main sources of heavy metals, which can be found in air, soil and water in different proportions and are released into the atmosphere from different sources, are mostly human-made factors such as urban wastes, chimney and exhaust gases, wastewater, mining and fertilizers [3].

Traffic-based pollution is one of the primary causes of air pollution in our country [4]. Human, plant and animal health are adversely affected by exposure to small amounts of toxic heavy metals such as cadmium (Cd) and lead (Pb) emitted from the exhaust of vehicles [5].

Heavy metals rising to the atmosphere can be mixed with soil and water through precipitation. Thus, it reaches the plants from the soil and the bodies of all living things in contact with the soil. In addition, heavy metals can contaminate surface waters such as streams and rivers, and it leaks from the soil and pollutes underground water resources. In this way, waters rich in heavy metals cause harmful effects for plants, animals and human bodies when used for agricultural areas [7].

As a result of research, the negative effects of heavy metals on human health have been clearly revealed [8]. From research Shaban et al. people who are exposed to high heavy metal concentrations have serious damage to the central nervous system, lungs, kidneys and liver, and this exposure even results in death. Koedrith et al. reported that at low concentrations of heavy metals, discomfort such as nose-throat

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irritation, cough, and shortness of breath were observed; Zeng et al. determined that it causes diseases such as asthma, cough, and respiratory distress in children [9-10-11].

The use of plants as biomonitors is important for monitoring heavy metal pollution. Lichen, moss, flowering plants and epiphytic plants are used regionally and locally [13-14-15-1-8-17]. For this purpose, lichens and mosses are preferred plants because they can accumulate many elements in much greater quantities than their needs [18]. However, since it is not known how long these plants are exposed to heavy metals, especially higher plants have been used in recent years [19-20]. Examples of higher plants are trees, and shrubs. These plants are very popular for this subject due to their ability to accumulate trace elements from soil, water and air [13].

There are differences between deciduous and evergreen species in the calculation of heavy metal accumulation in higher plants. Annual heavy metal accumulation can be calculated in deciduous higher plant species, while long-term heavy metal accumulation can be calculated in evergreen species. In the calculation of heavy metal pollution, samples taken from different organs of the plant are brought to the laboratory environment and analyzed in different devices [21].

Tilia tomentosa (Linden tree) species was used in this study. The reason for using this species is that there are plenty of linden trees in areas with heavy traffic in Ankara, and people want to collect the leaves and flowers of this tree and use it for healing purposes.

For this purpose, the potential for use of the species as a bioindicator will be determined by measuring the heavy metal concentrations in the linden trees in the region and the harms of the results in terms of human health will be evaluated.

3. MATERIAL AND METHOD

3.1. Study Area

In our study, linden samples were collected from six different regions in Ankara: Karapürçek (city center), Samsun Road (main road), Gazi District (main road), Pursaklar (secondary road), Protocol Road (main road), and Mogan (control). Samples are divided three in to branches, leaves, and flowers. Some of the samples were washed, and some were analyzed without washing. In addition, soil samples from which the specimens grew have been collected for analysis (Figure 1).

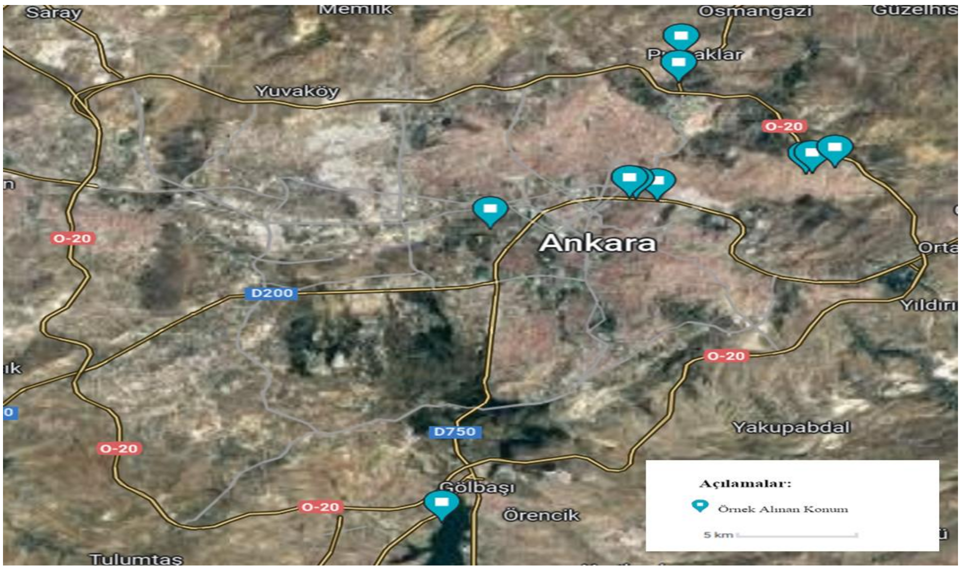

Figure 1. Study area

3.2. Sample Preparation

The plant samples collected from the study area were brought to the laboratory environment and half of them were washed with double distilled water to save them from surface contamination. All linden samples were dried in an oven at 80 ^{0}C for 24 hours. Samples that got rid of moisture were ground in a mortar and transferred to bags so that they become homogeneous. Soil samples were taken from a 10 cm area after the debris was cleaned from the surface, and from a depth of 15 cm around the tree root using a steel auger. Soil samples were placed in nylon bags to prevent contamination. The samples brought to the laboratory were dried for 1 day, air-dried, and then stored in labelled bags [22].

3.3. Digestion and Metal Determination

The samples were dissolved in a microwave instrument using 10 ml of pure HNO3. After dissolving, the volume of the samples was made up to 10 ml with double distilled water. Element values in the samples were determined using the ICP-MS device. Standard solutions and control samples were used to identify any contamination that could contaminate the samples from the outside. All trials were done in 3 repetitions. Care was taken to ensure that all chemicals used were analytical. (Merck, Darmstadt, Germany) [22].

Soil samples brought to the laboratory were dried in an oven at 80 °C for 24 hours and homogenized using a 2 mm sieve. The samples were dissolved in a microwave instrument using 10 ml of pure HNO_3/HCl mixture. After dissolving, the volume of the samples was made up to 10 ml with double distilled water. Element values in the samples were determined using the ICP-MS device [23].

3.4. Biotransfer Factor (BTF)

Biotransfer factor in plants; It was determined from the samples was calculate to determine the heavy metal transfer capability and yield of the plants depending on the concentration of metals in the soil.

The following formula was used for BTF calculation: TF = Cv/Cs, where Cv is the metal concentration detected in the parts of *Tilia tomentosa* and Cs is the metal concentration in the soil [24].

3.5. Statistical Analysis

For each sample, the mean, standard deviation, minimum and maximum values of the readings with 3 replicates were calculated. The $p \leq 0.05$ value was considered significant in the statistical comparison of the means. In addition, in order to facilitate the evaluation of the data obtained, ANOVA test with a confidence interval of 95% using the SPSS 20 program and Duncan tests for determining the difference in multiple comparisons were applied for the necessary groups and parameters. Means and differences between groups were interpreted by comparing them by means of these tests.

4. RESULTS AND DISCUSSION

The most important heavy metals are Fe, Cu, V, Mn, Zn, Ni, Cr, Mo, Co, Be, Cd, Pb, TI, Sb, Ag, As, Se, Hg, Sn, and Al elements. Among these, elements such as Zn, Mn, Fe, Cu, Ni, and Mo are vital micronutrients for plants and animals, and their high concentrations can cause harmful effects. Others are much more dangerous [25-26].

However, heavy metals are not easily destroyed in nature and tend to bioaccumulation in living things. Factors causing metal accumulation; mineral fertilizers, mining, biocides, wastewater, exhaust and flue gases, and sewage [27]. It can also be emitted into the atmosphere as volatile compounds [28-30]. Heavy metals from industrial sources and carcinogens; They are As, V, Ni, Cd, Zn, Cr and Pb elements [31]. Considering these properties of heavy metals and the dangers they may pose for living things, it is very

important to determine heavy metal concentrations, to find areas that may pose a risk, and to find the risk level in these areas [32].
In our study, the concentrations of Cd, Cr, As, Fe and Pb elements in *Tilia tomentosa* parts (leaf, flower and branch) and soil were investigated (Table 1-3).

Lead is not an absolute requirement for plants, but is very dangerous for health when it exceeds 300 ppm [33]. It significantly affects the plant water regime by negatively affecting cell wall stability and cell turgor. It also affects nutrient uptake as it is retained by the roots of the plant by reducing root growth [34] (Table 1). The highest Pb concentration among the stations was collected from Samsun Road station (43,72 μgg^{-1}) that of linden branch, the lowest concentration was (0,95 μgg^{-1}) collected from the Mogan station that of linden flowers (Table 1).

Although iron toxicity is not very common, in plants, it secretes root secretions that lower the soil pH [35]. It also causes burns on the leaves and stunting on the root and stem whereas negatively affects amino acid binding and protein synthesis in the plant [36-37]. The highest Fe concentration has been found in linden leaves (4637.78 μgg^{-1}) at Pursaklar station whereas the lowest concentration has been found in linden flower (100.9 μgg^{-1}) at Mogan station (Table 1).

Cadmium changes nitrogen and carbohydrate metabolisms in plants in many ways. By inhibiting photosynthesis, it causes the growth of stomata and the consequences of water loss, thus deteriorating the working conditions of chlorophyll [38]. The highest Cd concentration among the stations was collected at Karapürçek station (12,41 μgg^{-1}) on leaves and the lowest concentration was (0,12 μgg^{-1}) collected at Mogan station on flowers (Table 1).
Chromium, which adversely affects the plant body, negatively affects root development in the plant. This reduces the amount of nutrients and water taken from the soil and significantly reduces plant yield and quality [39]. Plants exposed to this stress produce reactive oxygen species to defend themselves, causing many damage to the plant [40]. The highest Cr concentration among the stations was collected from Karapürçek station (151,1 μgg^{-1}) on leaf, the lowest concentration was (37,6 μgg^{-1}) collected from the Mogan station on leafs (Table 2).

Arsenic has a toxic effect, restricting germination in some plants it whereas increasing arsenic concentration causes significant reductions in plant height, grain yield, number of full grains, grain weight, and root biomass [41]. The highest Cr concentration among the stations was collected at Karapürçek station (44,4 μgg^{-1}) on leaf, and the lowest concentration was (0,16 μgg^{-1}) collected at Mogan station on flowers (Table 2).
High concentrations of heavy metals were detected in the unwashed plant parts collected from the stations. This was thought to be due to heavy traffic, in which high level metals are transported. A similar result was achieved by Leblebici et al. (2020) in their studies conducted in Nevsehir, where they determined that there was a higher level of accumulation of Pb and As compared with the other metals that were found [23].

When the linden parts (leaves, flower and branch) are evaluated, it is seen that the heavy metal concentration in the unwashed samples are higher than the washed samples for all heavy metals. The results are similar to the study of Leblebici and Kar (2018) [22]. When the plant parts are evaluated according to the ANOVA test, it is seen that the heavy metal concentrations in flower samples are lower than those in branch and leaves samples (Table 1, 2).

Table 1. Comparison of accumulation of Lead (Pb); Iron (Fe); Cadmium (Cd) in different parts of *Tilia tomentosa;* leaves, flower and branch with washed and unwashed samples (µg g^{-1}).[a]

Heavy Metals	Parts	Stations	Unwashed Samples	Washed Samples
		Karapürçek	30,08±3,12[a]	6,65±0,12[ab]
		Samsun Road	25,66±2,85[ab]	8,91±0,32[ab]
	Leaves	Gazi	25,78±2,63[ab]	15,91±1,14[a]
		Pursaklar	18,21±1,79[b]	10,04±1,06[ab]
		Protokol	13,2±1,54[b]	8,85±0,45[ab]
		Mogan	12,88±0,95[c]	5,38±0,12[b]
		Karapürçek	3,57±0,15[b]	2,24±0,14[b]
		Samsun Road	7,2±0,74[a]	4,47±0,53[a]
Pb	Flower	Gazi	5,62±0,23[ab]	3,09±0,25[ab]
		Pursaklar	3,48±0,11[b]	2,23±0,18[b]
		Protokol	5,43±0,21[ab]	3,99±0,74[ab]
		Mogan	1,82±0,02[c]	0,95±0,08[c]
		Karapürçek	29,56±3,41[ab]	16,55±2,35[ab]
		Samsun Road	43,72±5,15[a]	22,89±5,41[a]
	Branch	Gazi	35,66±4,21[ab]	16,11±2,61[ab]
		Pursaklar	15,8±2,13[b]	8,14±1,42[b]
		Protokol	18,38±2,75[b]	8,87±1,78[b]
		Mogan	11,12±1,02[c]	4,16±0,94[c]
		Karapürçek	3363,16±12,06[b]	1636,93±6,54[ab]
		Samsun Road	3397,03±11,04[b]	1415,52±5,41[b]
	Leaves	Gazi	4291,57±15,84[ab]	2337,55±7,25[a]
		Pursaklar	4637,78±13,42[a]	2092,44±6,25[ab]
		Protokol	2695,74±9,01[b]	1409,64±4,12[b]
		Mogan	424,35±1,54[c]	178,14±1,36[c]
		Karapürçek	312,74±4,10[b]	197,56±2,15[b]
		Samsun Road	813,49±5,03[a]	627,2±5,16[a]
Fe	Flower	Gazi	325,18±3,64[b]	212,67±1,78[b]
		Pursaklar	511,91±5,01[ab]	410,71±2,62[ab]
		Protokol	346,76±2,39[b]	321,14±2,30[b]
		Mogan	193,77±1,03[c]	100,9±1,03[c]
		Karapürçek	1105,42±6,01[a]	1041,3±9,35[a]
		Samsun Road	414,38±3,24[b]	374,6±2,18[b]
	Branch	Gazi	1070,55±4,12[ab]	947,1±8,14[ab]
		Pursaklar	281,45±1,32[b]	187,49±2,74[b]
		Protokol	590,07±2,14[b]	326,07±5,14[b]
		Mogan	137,97±1,02[c]	153,48±1,48[c]
		Karapürçek	12,41±1,02[a]	3,17±1,25[a]
		Samsun Road	5,9±0,62[ab]	1,59±0,89[ab]
	Leaves	Gazi	3,28±0,12[b]	1,92±0,79[b]
		Pursaklar	2,36±0,08[b]	1,05±0,64[b]
		Protokol	1,25±0,04[b]	0,87±0,14[b]
		Mogan	0,69±0,01[c]	0,44±0,09[c]
		Karapürçek	0,96±0,05[b]	0,36±0,07[b]
		Samsun Road	1,01±0,08[ab]	0,67±0,08[a]
Cd	Flower	Gazi	1,36±0,09[a]	0,38±0,04[b]
		Pursaklar	0,74±0,03[b]	0,4±0,05[ab]
		Protokol	0,83±0,04[b]	0,51±0,06[ab]
		Mogan	0,33±0,01[c]	0,12±0,02[c]
		Karapürçek	3,38±0,12[a]	1,92±0,08[a]
		Samsun Road	1,93±0,10[b]	1,66±0,05[ab]
	Branch	Gazi	1,71±0,09[b]	0,77±0,03[b]
		Pursaklar	1,5±0,06[b]	0,78±0,04[b]
		Protokol	1,52±0,07[b]	1,02±0,08[b]
		Mogan	1,21±0,03[c]	0,98±0,02[c]

[a] For a given station, mean concentrations followed by the same letter are not significantly different ($p<0.05$).

Table 2. Comparison of accumulation of Chromium (Cr) and Arsenic (As) in different parts of *Tilia tomentosa;* leaves, flower and branch with washed and unwashed samples (μg g^{-1}).[a]

Heavy Metals	Parts	Stations	Unwashed Samples	Washed Samples
		Karapürçek	151,1±2,03[a]	85,4±3,05[ab]
		Samsun Road	102,2±1,98[b]	71,6±2,75[ab]
	Leaves	Gazi	121,3±2,14[b]	78,5±2,92[ab]
		Pursaklar	98,4±1,42[b]	57,1±1,74[b]
		Protokol	161,4±3,25[ab]	88,6±3,01[a]
		Mogan	45,8±0,95[c]	37,6±0,95[c]
		Karapürçek	124,5±3,14[ab]	114,6±5,41[a]
		Samsun Road	105,6±2,51[b]	102,8±4,12[ab]
Cr	Flower	Gazi	113,4±2,87[b]	107,9±3,25[ab]
		Pursaklar	135,4±3,11[a]	104,7±3,10[ab]
		Protokol	128,3±2,56[ab]	102,7±3,06[ab]
		Mogan	95,2±0,93[c]	76,8±2,01[b]
		Karapürçek	72,8±3,14[a]	68,1±2,36[a]
		Samsun Road	65,7±2,64[ab]	60,3±1,85[ab]
	Branch	Gazi	69,7±2,73[ab]	63,6±1,83[ab]
		Pursaklar	52,8±2,10[ab]	49,1±1,02[b]
		Protokol	63,1±2,16[ab]	61,5±1,87[ab]
		Mogan	60,4±1,23[b]	46,4±0,86[b]
		Karapürçek	44,4±1,12[a]	8,95±0,13[a]
		Samsun Road	24,71±1,01[ab]	6,78±0,11[b]
	Leaves	Gazi	15,13±1,03[ab]	6,47±0,10[b]
		Pursaklar	14,21±0,96[ab]	8,51±0,12[ab]
		Protokol	9,25±0,41[b]	5,8±0,09[b]
		Mogan	9,09±0,12[b]	4,62±0,04[c]
		Karapürçek	0,78±0,05[b]	0,64±0,04[ab]
		Samsun Road	1,98±0,16[ab]	0,81±0,09[ab]
As	Flower	Gazi	2,4±0,41[a]	1,09±0,12[a]
		Pursaklar	1±0,12[ab]	0,75±0,08[ab]
		Protokol	1,6±0,31[ab]	0,52±0,04[b]
		Mogan	0,42±0,04[c]	0,16±0,01[c]
		Karapürçek	12,54±2,01[a]	3,53±0,41[ab]
		Samsun Road	6,99±1,32[ab]	5,29±0,67[a]
	Branch	Gazi	7,13±1,14[ab]	2,56±0,35[b]
		Pursaklar	5,5±1,03[b]	2,59±0,37[b]
		Protokol	5,95±1,06[b]	1,53±0,13[b]
		Mogan	3,07±0,92[c]	0,86±0,10[c]

[a] For a given station, mean concentrations followed by the same letter are not significantly different ($p<0.05$).

*Permissible limits (mg/kg) were adopted from WHO (2000) and The Soil Pollution Control Regulation (2001) on plant and soil [42, 43].The highest metal concentration among the stations was Fe (40681 μgg^{-1}) collected from Gazi station, and the lowest concentration was Cd (4.9 μgg^{-1}) collected from the Mogan station on soils (Table 3). According to the ANOVA test in lead, metal concentration in all stations is different from each other on soil samples ($p<0.05$) (Table 3).

The reason for high levels of heavy metal concentrations in the soil and plant samples can be result of being increased exposure to dissolved chemicals due to heavy traffic and decreased quality of soil [44].

Table 3 Heavy metal concentration (mean ±SD, $\mu g g^{-1}$, n=5 per location) in soils in Ankara Province [a].

Soil	Cd	Cr	As	Pb	Fe
Karapürçek	20.31±2.01[a]	400.61±5.04[ab]	45.68±0.21[b]	168.58±2.14[bc]	16901.2±12.54[b]
Samsun Road	9.54±1.03[b]	653.02±7.12[a]	60.77±0.34[ab]	371.22±2.41[a]	12871.7±8.41[b]
Gazi	9.53±1.42[b]	230.98±3.01[b]	61.59±0.58[ab]	324.86±2.05[ab]	40681.06±24.15[a]
Pursaklar	7.5±2.05[b]	434.56±6.02[ab]	67.08±0.87[a]	125.22±1.52[c]	14845.7±9.74[b]
Protokol	16.46±.15[ab]	226.93±2.98[b]	57.53±0.45[ab]	292.8±2.56[b]	18894.1±10.31[b]
Mogan	4.9±0.12[c]	102.75±1.05[c]	25.16±0.12[c]	103.78±1.03[d]	10627.9±5.14[c]
Permissible Limits*	3	100	20	50	300

For a given metal, mean concentrations followed by the same letter are not significantly different ($p<0.05$).

The correlations of heavy metal, transfer factors and correlations were detected in *Tilia tomentosa* parts and soil samples collected from six different stations.

When the correlation between the concentration of metals in washed and unwashed flower, stem and leaf samples was investigated, the strongest and most positive correlation was determined as Cr, which was found to be the highest in linden flowers and leafs. The correlation was statistically significant ($p<0.01$) (Table 4).

Table 4 Correlation coefficients for heavy metal concentrations between plant parts ($p<0.01$**) ($p<0.05$*).

	Cr	As	Pb	Fe	Cd
Leaves	0,883**	0,416	-0,13	0,644*	0,224
Branch	0,609*	0,491	-0,151	0,576*	-0,270
Flower	0,937**	0,520*	-0,171	0,909**	0,371

Permisible limits in plants Pb (0.2 $\mu g\ g^{-1}$), Fe (30 $\mu g\ g^{-1}$), Cd (0.1 $\mu g\ g^{-1}$), Cr (3 $\mu g\ g^{-1}$), As (0.1 $\mu g\ g^{-1}$) [42].
According to the transfer factors from soil to plant parts, the highest transfer factor was determined at 0,656 for As in leaves while the lowest transfer factor was found to be in Pb and Fe (0,019) in flowers (Table 5).
Transport of heavy metals from soil to plant is one of the main components of human exposure to heavy metals through the food chain. This study determined that the BTF values between sampling sites and plant parts were significantly different. The BTF values of all metals were found to be low in terms of transfer factors. This may be because the metal concentration in the soil is higher than the concentrations in plant parts. Similarly, Leblebici et al. reported that there was no linear increase in BTF values of plants as a result of heavy metal concentration in the soil [23] (Table 5).

Table 5. Transfer factors of metals from soil to parts of *Tilia tomentosa* in Ankara province, Turkey

	As	Cd	Pb	Cr	Fe
Leaves	0,656	0,615	0,081	0,231	0,113
Branch	0,187	0,166	0,117	0.111	0,027
Flower	0,035	0,066	0,019	0,207	0,019

5. CONCLUSION

In this study, heavy metal accumulation in *Tilia tomentosa* parts (leaves, flower and branch) and soil due to heavy traffic was investigated in the province of Ankara. In addition, according to the criteria published by international organizations, it was determined that the metal accumulation at the stations was at a very critical level. Consequently, measures may need to be taken to prevent heavy metal contamination in soil and reduce heavy metal translocation from soil to food.

As *Tilia tomentosa* is used as a beverage, heavy metal monitoring in soil and plant is important. It is important to reduce metal concentrations in the study area and to investigate their effects on human health. By continuing these studies in areas with heavy traffic, acute and chronic health problems and metal exposure can be prevented.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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