

Comparison of Three Tree Leaves as Biomonitors of Heavy Metals Contamination in Dust, A Case Study of Isfahan

^{*1}Alireza Aghaei, ²Hosein Khademi, ³Saeid Eslamian

¹MSc Student, Department of Soil Science, College of Agriculture, Isfahan University of Technology, Isfahan, Iran, ²Professor, Department of Soil Science, College of Agriculture, Isfahan University of Technology, Isfahan, Iran, ³ Professor, Department of Water Engineering, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

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Abstract

The major challenges facing atmospheric pollution assessment are the difficulties and the high costs associated with direct sampling. Instead of dust samples, tree leaves have been used as biomonitors to overcome these difficulties. In this study, leaves were collected from the three tree species of *Platanus orientalis L*, *Ulmus boissieri*, and *Morus alba* at 22 sites to monitor pollution in the city of Isfahan. Sampling was carried out on a monthly basis over a period of 7 months (May to November 2014). The difference between concentrations of such heavy metals as Zn, Cu, Ni, Mn, Fe, Co, and Pb on washed and unwashed leaf samples was determined to assess the capability of the leaves in monitoring atmospheric dust. The difference between the dust accumulated each month and that of the previous month was found to increase. From a certain point in time onwards, this increasing trend was observed to shift to a decreasing one because of a heavy rainfall event. Maximum differential concentration of heavy metals between washed and unwashed leaves in seven consecutive months was observed in the leaves of *Platanus orientalis L*. On other hand, a significant correlation was found between the concentrations of heavy metals collected in dust traps and those in the dust accumulated on the leaves of *Platanus orientalis L*. This indicates that *Platanus orientalis L* leaves offer a better potential for tracing atmospheric heavy metals and can, thus, be used as a cheap and available tool for monitoring atmospheric heavy metals.

Keywords: Heavy metals, Atmospheric dust, Tree leaves, Biomonitors

Introduction

The increasingly intensified droughts in recent years due to the climate change and desertification in the Middle East have led to increasing dust pollution in the region. This is complicated by the increasing desertification that has dried more soil and released tiny particles, known as dust (Sing and Sing, 2010), to form aerosols joining the airflow by wind currents or other physical processes. The main concern about air pollution in cosmopolitan areas is huge dust emission, or aerosols, produced in a short time (Wang et al., 2016). Depending on the ecological conditions, aerosols containing heavy metals can

remain in suspension in the air for only a limited time before they are quickly deposited on soil, water, or other surfaces and accumulate in the form of dry or wet deposits on the surfaces of moss, lichen, or tree trunks (Fujiwara et al., 2011).

One of the most recent and reliable methods used for assessing the amounts and types of environmental pollutants, especially air pollutants, is the use of natural biomonitors. Many plants are able to absorb and store most pollutants through their aerial tissues while they also absorb those in the soil and water. This makes them an especially useful proxy for instruments to estimate and monitor atmospheric pollutants (Celik et al., 2005; Madejon et al., 2004). Since trees are fixed and do not move, they serve as the best means of measuring dust pollution in the air (Ram et al., 2012). It has also been observed that tree branches and leaves are very efficient in trapping atmospheric particles, especially in urban areas (Nowak et al., 2006; Qiu et al., 2009). Over the last few decades, tree leaves have been increasingly used as biomonitors of heavy metals in the environment (Al-Khashman., 2004). Heavy metal concentrations, particularly in urban trees, depend on the uptake of their roots or the amount of dry and wet deposits on their aerial tissues (Lehndorff and Schwark. 2010). Several studies have been conducted on the deposition of urban atmospheric heavy metals on tree leaves. In all of these studies, trees have been introduced as cheap and accessible means for assessing atmospheric heavy metals.

Korori et al (2010) reported that the increased number of motor vehicles as a result of the growing population in Tehran has led to elevated heavy metal concentrations in the herbal fibers of plants growing in the city. In another study of atmospheric heavy metals, Al-Khashman et al. (2011) used *Phoenix dactylifera* leaves as the best and cheapest biomonitor for assessing air-borne heavy metals in arid and semi-arid areas, particularly in crowded industrial cities in arid areas having high loads of dust. The authors found that increased heavy metal concentrations in leaf samples had a positive and significant correlation with increasing industrial activities in the area.

In assessing the temporal trend and aggregation of some heavy metals using *Quercus robur* and *Tilia*

cordata in Belgrade, Aničić et al. (2011) found that atmospheric subsidence had a stronger correlation with heavy metal concentrations in *Quercus robur* leaves than with those in *Tilia cordata* leaves. The authors also observed that the temporal trend of Zn, Cu, Ni, Mn, Fe, Co, and Pb accumulation could be better traced in *Quercus robur* leaves than in those of *Tilia cordata*. Deljanin et al. (2014) studied the deposition of Pb isotopes in the four tree species of *Tilia cordata*, *Aesculus hippocastanum*, *Acer platanoides*, and *Betula pendula* in Belgrade, Serbia, to find *Betula pendula* leaves as the best biomonitor for identifying the source of Pb. The study also revealed that *Betula pendula* leaves were capable of storing the highest amount of Pb in the cortical layer. Madejón et al. (2006) studied absorption of heavy metals in the leaves and fruits of *Olea europaea* and *Quercus robur* in Spain. They found that *Quercus robur* leaves were imbued with heavy metals from atmospheric subsidence and that *Quercus robur* served as a better bio-aggregator than *Olea europaea*. Studying urban heavy metals in the leaves of tree species in Istanbul, Turkey, Baycu et al. (2006) reported the two species of *Fraxinus excelsior* and *Ailanthus glandulosa* played important roles in trapping such heavy metals as Ni and Pb so that *Fraxinus excelsior* leaves absorbed a large amount of Ni from the atmosphere while *Ailanthus glandulosa* absorbed the greatest amount of atmospheric Pb on its leaf surfaces. The present study focuses on tracing the sources of heavy metals in atmospheric dust using the three tree species commonly grown in Isfahan. In order to discriminate between the heavy metals absorbed from the soil and those accumulated on tree leaves from the atmosphere, the tree leaf samples were divided into washed and unwashed.

Experimental

The sampling area

The present study took place in the urban area of Isfahan. The city is one of the largest and most crowded in Iran with a population of 1,967,796 people and covers an area of 482Km² located between the eastern longitudes from 51° 32' to 51° 47' and northern latitudes from 32° 38' to 32° 46'. Ranked as the second in terms of industrial activities in Iran (Isfahan Governor's Office, 2013),

the city has a semi-arid climate with a small precipitation. According to the most recent weather reports, it receives an annual rainfall of 120 mm, which is half the average rainfall in Iran and one-sixth the world average (Isfahan Meteorological Organization, 2013). Figure (1) shows a map of the city and its location with the 22 spots in the city selected for sampling. In selecting the sites, efforts were made to strike a balance between the highly congested areas and those with a small traffic.

Sampling Method

At each sampling site, two glass dust traps (each 1 m² in size) were installed on the structural roof of a building. The buildings were selected so that the three species of *Platanus orientalis* L, *Ulmus boissieri*, and *Morus alba* (i.e., the most abundant trees in Isfahan) were located around each to a radius of about 500 meters. At each sampling interval and in each of the 22 selected areas, samples were collected over the seven months of the study period (May to November 2014) from both tree leaves and glass dust traps (both mounted at the same height).

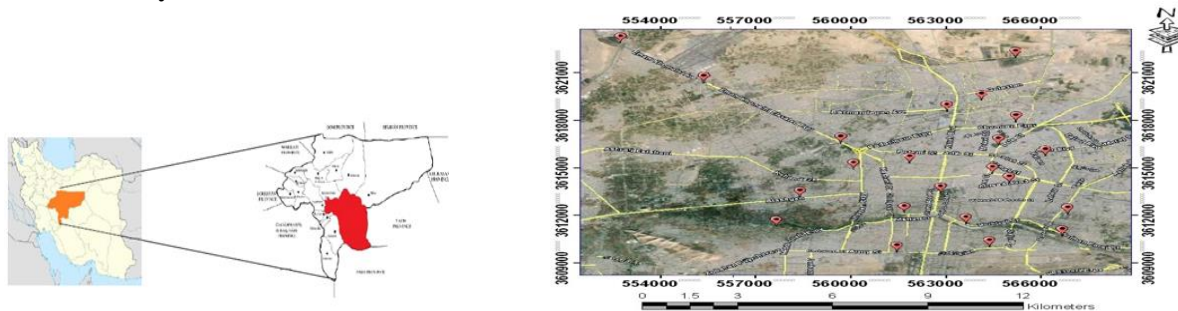
Dust samples from the glass dust traps

In this study, the glass dust traps recommended by Menendez et al. (2007) were used. Each trap is made of a glass sheet 1 m² in size with a plastic lid in which holes 2 × 2 mm are screwed to trap dust (Figure 2). At the end of each month, the dust on both traps would be collected with a metal blade at specified intervals and transferred in disposable bags to the laboratory.

Leaf samples

Samples from the tree leaves were simultaneously analyzed since it was hypothesized that the dust collected on the leaves of all the species dated back to the beginning of the study period in May because the selected species were all deciduous trees and their burgeoning would occur at the beginning of spring in May. This meant that the dust deposited each month would add on to that in the previous month. The leaf samples taken in each month from each tree species at each sampling site would be placed in special paper bags and transferred to the lab.

Figure 1 A) Map of Iran showing the location of Isfahan Province and the city of Isfahan; B) the sampling sites in the city



A

B

Laboratory studies

The leaf samples from each tree species were randomly divided into two groups of equal amounts. One group was completely washed with tap water and then with distilled water. The other group was left intact so that the different parameters of interest could be compared. The leaf samples in both groups were then placed in an oven for 24 hours to prepare them for grinding and digesting. The dried samples were then crushed in a mill. The dust samples were passed through a 2 mm sieve to separate sand, silt, and clay from the brushwood and other materials.

Determining heavy metal concentrations in the dust and leaf samples

Total concentrations of Zn, Mn, Co, Fe, Cu, Ni, and Pb present in the dust samples were measured according to the method described in Meza-Figueroa et al. (2007). Briefly, 0.5 gr of each dust sample was digested in normal nitric acid in a 250 ml Erlenmeyer flask for 48 hours before it was heated for half an hour at 80 °C. The solution was then passed through a Wattman paper No. 42 and finally placed in 26 ml of nitric acid 1%. The total concentrations of Zn, Mn, Cu, Ni, Co, and Fe were determined using the atomic absorption set of Perkin Elmer 3030 and Pb using the atomic absorption set of Analyst 200. The plant sampling digest method was used to determine the concentrations of Zn, Mn, Co, Fe, Cu, Ni, and Pb in the two types of washed and unwashed leaf samples. Briefly, 1 gr of the leaf sample was placed in a digester oven and heated to 550 °C for 4 hours. Once the organic content had been burnt, 10 ml of 2% normal hydrochloric acid was added and the digestion was completed by placing the samples on a heater. Finally, the ingredients were extracted on a Wattman paper No. 42 in a 50 ml balloon and the volume was made using distilled water (Chapman and Partt, 1961). Preparation of the tree leaf and dust samples was accomplished according to the quality assurance and laboratory control procedures of the Standard Reference Materials (SRM).

Figure 2. Glass traps used for dust sampling



Results & discussion

Heavy metals on unwashed (UL) and washed leaves (WL)

Tables 1 to 3 show heavy metal concentrations in the washed and unwashed leaf samples for each tree species over the seven months of sampling. Clearly, iron recorded the highest concentration in all the three tree species. This finding confirms those of a previous study by Norouzi et al. (2015) who used *Platanus orientalis L* leaves as their biomonitor to find that iron recorded the highest concentration among the heavy metals detected in the atmospheric dust in Isfahan.

Iron is one of the main elements in the earth's crust that is needed by plants for producing the required enzymes and proteins. When incorporated into the chloroplast, it plays an important role in plant photosynthesis and respiration processes. Another naturally occurring element with important roles in photosynthesis and chloroplast oxidation processes is Mn. Zn, essential for all living cells, takes part in producing hormones and proteins. While its excessive amounts in plants causes damages to plant production, Zn deficiency leads to leaf deformation. Although one of the less used elements, copper (Cu) is also essential for plant growth and metabolism. About 70% of the Cu in plant leaves is present in the chloroplast and plastocyanin, indicating the vital role it plays in photosynthesis. Copper (Cu) is also a main component of plant enzymes such that its deficiency leads to significant changes in the biochemical processes of the plant, while its abundance causes plant cancer which has the same symptoms as iron deficiency (Celik et al., 2005). Nickel (Ni), as part of urease, is also considered as an important element for plant growth. Its excessive amounts cause disorder and irregularity in plant protein formation (Gerendas et al., 1999). In addition to these metals, the two metals of Co and Pb have been considered in the present study as they have been shown to undertake small roles in plant life. Goerg and Vollenweider (2007) observed that oxidated iron, Mn, and Pb play important roles in fixing heavy metals including Pb and Co. They also reported that Pb is absorbed in small quantities and moves to the aerial parts.

In this study, small quantities of the two metals of Co and Pb were detected on the leaf samples taken from all the three tree species, especially in samples taken during the first months of the study period when the tree leaves still had small surface areas. At this stage, heavy metal concentrations were not detectable by the instruments; hence, the designation non-distinguishable (ND) in Tables 1 to 3 representing concentrations below detection limits.

Tables 1, 2, and 3 present the data obtained from the samples taken from *Platanus -orientalis L*, *Morus alba*, and *Ulmus boissieri* trees in a descending order of leaf surface area. Clearly, the leaves from

Platanus -orientalis L (Table 1) have the highest heavy metal concentrations in both washed and unwashed leaf samples while those from *Morus alba* (Table 2) stand second to *Platanus orientalis L*. Finally, leaf samples from *Ulmus boissieri* trees (Table 3) have the least heavy metal concentrations. This indicates the importance of leaf surface area in tracing dust-borne heavy metals. In other words, the larger the leaves, the more will be the dust collected and, hence, the higher will be the concentration of heavy metals in the leaves.

It is also clear from these same Tables that heavy metal concentration increases on unwashed leaf samples in all the three species during the growing season such that heavy metal concentrations increase from T₁ to T₆. This indicates the growing collection of dust particles on the tree leaves. The low concentration of heavy metals on unwashed leaf samples at T₁ is obviously their small surface area. Furthermore, heavy metal concentrations on unwashed leaf samples exhibit a sharp rise at T₇, obviously because of the heavy rainfall in that month which washed away the dust accumulated on the leaves. It has been reported that other natural forces such as strong winds may cause decreases in the dust collected on tree leaves (Tomašević et al., 2011). Norouzi et al. (2016) studied the biomagnetic monitoring of heavy metal contamination in the atmospheric dust in Isfahan. They found that the dust deposited on the leaf surface was the main source of magnetic signals from leaves which underwent variations due to rainfall or wind events.

According to the same Tables above, significant differences are observed in all the three tree species between the unwashed leaves and those washed with distilled water in terms of their heavy metal deposits, indicating the effect of washing and, simultaneously, confirming the effects of rain and wind on reducing the amounts of dust collected on leaves. This finding is in agreement with those reported by Norouzi et al. (2015) who used *Platanus orientalis L* leaves as bioindicators to study some heavy metals in the atmospheric dust in Isfahan. The authors found a significant difference in heavy metal concentrations between their washed and unwashed leaf samples. They also observed that this difference would rise in magnitude as the end of the growing season drew near, obviously due to the larger leaf surface area at the end of the growing season. Hovmand et al. (2009) studied Pb concentrations in the *Pinus Sp* and *Populus nigra* species in Norway. They compared washed leaves and unwashed ones to conclude that while atmospheric dust accounted for 98% of the Pb content of their leaf samples, plant uptake accounted

for only 2%. Celik et al (2005) compared the heavy metal concentrations on washed and unwashed leaves of *Robinia Pseudo acacia L* to find that not all the metals would be equally and totally removed by washing the leaf surfaces. The authors observed that washing removed only 15 to 48.5% of the iron content, for instance.

The T-test was conducted on the data reported in Tables (1) to (3) to shed more light on the significance of the differences between washed and unwashed leaves in each sampling period. Clearly, no significant differences are observed between the washed and unwashed leaf samples at the beginning of the sampling season due to the small surface area of the leaves. It is also obvious that Mg and Fe concentrations in the same period exhibited a statistically significant difference between the washed and unwashed leaves at the 95% confidence level. This is evidence of the fact that the high amounts of Fe and Mg in the atmospheric dust led to significant differences between the washed and unwashed leaf samples from the very beginning of the growing season whereas this was not the case with the other metals. In other words, the washed and unwashed leaves showed no significant differences in their concentrations of the three metals of Ni, Co, and Zn.

As for the question whether the heavy metals were absorbed by the roots or the leaves, it is obvious from the data in these three Tables that the temporal trend in Mn, Pb, and Co aggregation is an increasing one (from May to November), indicating the time-dependent accumulation of these three metals in leaves. This is while a reverse trend is observed in the case of Cu, Zn, and Fe in washed leaves such that, in all the three tree species investigated, they recorded their maximum leaf concentrations at the beginning of the growing season (T₁) and declining toward the end of the growing season when tree leaves start to fall off. It may, therefore, be claimed that in tree leaves the young parts of the trees accumulate more of these three metals. This is in agreement with the findings of Kim and Fergusson (1994) who reported higher concentrations of such heavy metals as Cu and Zn in young leaves. They also observed their declining concentrations throughout the growing season. Aznar et al. (2009) observed that such essential metals as Cu would generally move from the older to the younger parts of the plant during the growing season. Finally, it is worth mentioning that Ni is the only metal that did not exhibit any significant variations in its absorption by the different plant organs.

Table 1- Heavy metal concentrations on *Platanus orientalis L* leaf samples during the 7 sampling months ($\mu\text{g.Kg}^{-1}$ dry weight for Co and Pb and mg.Kg^{-1} dry weight for the other metals)

Sampling Time	Elements							
	UL	WL	UL	WL	UL	WL	UL	WL
	Ni		Cu		Zn		Co	
T1(May)	7.5 ^a (±6.8)	6.9 ^a (±6.1)	16.7 ^a (±5.4)	16.1 ^a (±5.9)	35.3 ^a (±9.9)	34.2 ^a (±8.5)	N.D	N.D
T2(Jun)	8.4 ^a (±2)	6.7 ^b (±2.1)	17.6 ^a (±3.3)	13.3 ^b (±3.5)	39.8 ^a (±10)	31.3 ^b (±8.3)	N.D	N.D
T3(Jul)	9.1 ^a (±2.6)	7 ^b (±2)	17.8 ^a (±7.1)	10.2 ^b (±6.9)	43.9 ^a (±23)	29.3 ^b (±9.3)	28.6(±26)	N.D
T4(Aug)	10.1 ^a (±3.5)	6.5 ^b (±2.6)	18 ^a (±6.3)	10.7 ^b (±6.4)	49.1 ^a (10.8)	29.2 ^b (±8.5)	35.7(±30)	N.D
T5(Sep)	10.3 ^a (±2.3)	6 ^b (±1.5)	20 ^a (±5.7)	11.7 ^b (±5.6)	57.9 ^a (±11.7)	28.3 ^b (±6.2)	66.1 ^a (±20)	25.7 ^b (±20)
T6(Oct)	11.2 ^a (±3.8)	6.2 ^b (±2.8)	21.8 ^a (±4.5)	12.1 ^b (±3.3)	61.2 ^a (±8.3)	28.9 ^b (±9.4)	86.1 ^a (±10)	36.2 ^b (±20)
T7(Nov)	8.9 ^a (±6.6)	5.9 ^b (±5.1)	16.5 ^a (±5.8)	10.5 ^b (±5.1)	41.8 ^a (±9.7)	27.7 ^b (±5.3)	70.4 ^a (±17)	42.3 ^b (±25)

Sampling Time	Elements					
	UL	WL	UL	WL	UL	WL
	Pb		Fe		Mn	
T1(May)	N.D	N.D	287.3 ^a (±91.6)	231.5 ^b (±86)	71.1 ^a (±6.4)	54.9 ^b (±48.1)
T2(Jun)	266 ^a (±260)	58 ^b (±50)	680 ^a (±194)	294.5 ^b (±179)	129.5 ^a (±37.7)	94.1 ^b (±45.4)
T3(Jul)	496 ^a (±400)	53.7 ^b (±22)	728 ^a (±275.5)	278 ^b (±93)	137.6 ^a (±33.2)	89.6 ^b (±30.5)
T4(Aug)	609 ^a (±330)	109.6 ^b (±30)	858 ^a (±261)	253.5 ^b (±104)	150.7 ^a (±31)	90.9 ^b (±29.1)
T5(Sep)	954 ^a (±710)	135.5 ^b (±140)	1128 ^a (±324)	241.5 ^b (±64)	167.1 ^a (±27)	92.4 ^b (±37)
T6(Oct)	1235 ^a (±337)	339 ^b (±190)	1291 ^a (±475)	239 ^b (±90)	179 ^a (±18.2)	101.3 ^b (±21.8)
T7(Nov)	822 ^a (±270)	440.7 ^b (±160)	790 ^a (±670)	232.5 ^b (±132)	119.8 ^a (±25)	99.4 ^b (±20.4)

Table 2- Heavy metal concentrations in *Morus alba* leaf samples during the 7 months of sampling ($\mu\text{g.Kg}^{-1}$ dry weight for Co and Pb and mg.Kg^{-1} dry weight for other metals)

Sampling Time	Elements							
	UL	WL	UL	WL	UL	WL	UL	WL
	Ni		Cu		Zn		Co	
T1(May)	7.1 ^a (±3.9)	6 ^b (±2.5)	10.7 ^a (±6.5)	10.2 ^a (±5.4)	32.4 ^a (±11.6)	31 ^a (±10.5)	N.D	N.D
T2(Jun)	8.3 ^a (±1.1)	6.7 ^b (±1.2)	11.2 ^a (±7)	10.7 ^a (±4.8)	34.2 ^a (±10)	30.5 ^b (±9.6)	N.D	N.D
T3(Jul)	9.3 ^a (±2.8)	6.8 ^b (±2.3)	12.1 ^a (±5.2)	10 ^b (±3.9)	37.8 ^a (±10.7)	30 ^b (±9.6)	N.D	N.D
T4(Aug)	10.5 ^a (±1.8)	6.6 ^b (±2.1)	13.7 ^a (±6.8)	9.9 ^b (±2.9)	40 ^a (±9.2)	30.5 ^b (±8.5)	23.3(±16)	N.D
T5(Sep)	10.6 ^a (±1.5)	6.1 ^b (±1.3)	15.1 ^a (±5.5)	9.6 ^b (±4.3)	46.1 ^a (±10)	30.7 ^b (±8)	66 ^a (±22.6)	25 ^b (±19.5)
T6(Oct)	11 ^a (±2)	6.5 ^b (±1.1)	15.8 ^a (±7)	9 ^b (±6.2)	52 ^a (±12.5)	30.4 ^b (±11.7)	69 ^a (±23)	34.3 ^b (±17.4)
T7(Nov)	9.9 ^a (±3.9)	6.2 ^b (±2.9)	19.1 ^a (±5.8)	9 ^b (±4.3)	36.8 ^a (±10.2)	29.9 ^b (±10.4)	54.3 ^a (±26.5)	29.4 ^b (±20)

Sampling Time	Elements					
	UL	WL	UL	WL	UL	WL
	Pb		Fe		Mn	
T1(May)	N.D	N.D	390 ^a (±101)	215.5 ^b (±81)	73.7 ^a (±19)	57.7 ^b (±15.8)
T2(Jun)	70.1 (±19.7)	N.D	634.5 ^a (±138)	241 ^b (±122)	81.2 ^a (±16.5)	60.1 ^b (±15)
T3(Jul)	98.9 (±100.7)	N.D	785 ^a (±290)	273 ^b (±154)	93 ^a (±24)	61.5 ^b (±16.5)
T4(Aug)	155 ^a (±116)	30 ^b (±20)	822.5 ^a (±215)	245.5 ^b (±97)	104 ^a (±17.2)	63.8 ^b (±16.5)
T5(Sep)	282.3 ^a (±74)	52.6 ^b (±25)	835.5 ^a (±310)	232 ^b (±141)	113.5 ^a (±21.8)	65 ^b (±16.4)
T6(Oct)	409 ^a (±205)	101 ^b (±94)	955.5 ^a (±280)	220.5 ^b (±83)	126.8 ^a (±34.5)	70 ^b (±27)
T7(Nov)	210 ^a (±192)	102 ^b (±64)	582 ^a (±242)	219.2 ^b (±75)	111.2 ^a (±20)	69.1 ^b (±17.2)

Table 3 - Heavy metal concentrations on *Ulmus boissieri* leaf samples during 7 months of sampling ($\mu\text{g.Kg}^{-1}$ dry weight for Co and Pb and mg.Kg^{-1} dry weight for other metals)

Sampling Time	Elements							
	UL	WL	UL	WL	UL	WL	UL	WL
	Ni		Cu		Zn		Co	
T1(May)	7.4 ^a (±5.9)	7 ^a (±4.8)	12.5 ^a (±7.6)	11.8 ^a (±5.9)	30.7 ^a (±16.9)	29.8 ^a (±13.8)	N.D	N.D
T2(Jun)	7.8 ^a (±6.2)	7 ^b (±5.5)	12.9 ^a (±8.5)	11 ^b (±7.2)	30.9 ^a (±19)	30.2 ^a (±17.4)	N.D	N.D
T3(Jul)	8.5 ^a (±6.1)	7.3 ^b (±3.8)	13.3 ^a (±7)	10.2 ^b (±6.6)	32 ^a (±10.2)	30.1 ^b (±10.2)	N.D	N.D
T4(Aug)	9.3 ^a (±7)	7.3 ^b (±4.9)	13.5 ^a (±8)	10 ^b (±4.9)	33.2 ^a (±14.3)	29.2 ^b (±13)	22.3 (±16)	N.D
T5(Sep)	9.6 ^a (±4.6)	7.6 ^b (±4.6)	14.1 ^a (±8.5)	10.1 ^b (±6)	36 ^a (±11.4)	29 ^b (±11.8)	77 ^a (±33)	24 ^b (±17.5)
T6(Oct)	10.1 ^a (±6.1)	7.3 ^b (±4)	14.7 ^a (±7.7)	10.2 ^b (±7.2)	36.8 ^a (±10.5)	28.9 ^b (±9)	92 ^a (±32)	22 ^b (±17)
T7(Nov)	8.5 ^a (±4.5)	6.9 ^b (±3.9)	12 ^a (±6.4)	9.7 ^b (±4.2)	30.3 ^a (±9.8)	28 ^b (±10)	40 ^a (±31)	28.5 ^b (±23)

Sampling Time	Elements					
	UL	WL	UL	WL	UL	WL
	Pb		Fe		Mn	
T1(May)	N.D	N.D	410.5 ^a (±180)	318 ^b (±125)	62 ^a (±28.5)	58 ^b (±24)
T2(Jun)	56 (±25)	N.D	530.5 ^a (±235)	297 ^b (±227)	65 ^a (±22.5)	57.1 ^b (±21)
T3(Jul)	72.1 (±26.5)	N.D	829 ^a (±373)	299 ^b (±239)	68.8 ^a (±17.8)	57.8 ^b (±17.5)
T4(Aug)	95.5 ^a (±24)	32.2 ^b (±17)	882 ^a (±229)	290 ^b (±128)	70.2 ^a (±19.1)	58 ^b (±18.2)
T5(Sep)	132 ^a (24.5)	85.5 ^b (±24)	912.5 ^a (±294)	289 ^b (±192)	74 ^a (±19.2)	58.2 ^b (±17.9)
T6(Oct)	203 ^a (±24)	125.5 ^b (±19)	1218 ^a (±306)	292.5 ^b (±231)	79.2 ^a (±27.5)	58 ^b (±21.8)
T7(Nov)	179 ^a (±35)	131 ^b (±29)	871.5 ^a (±319)	303 ^b (±214)	66.8 ^a (±21.8)	57.5 ^b (±22)

ND: not detected; Common letters between washed and unwashed leaves for each metal and each month indicate the non-significance of the differences between the means at the 95% confidence level in the T-test; UL: unwashed leaves; WL: washed leaves; SD: standard deviation (For Tables 1 to 3).

Heavy metals accumulated on tree leaves (UL-WL)

The difference between washed and unwashed leaves was defined as the heavy metal concentrations in the dust that accumulated on leaves through time. For the three bio monitors, the difference between concentrations in washed and unwashed leaves was calculated in each month in all the 22 sampling sites as shown in Figure 3. According to this Figure, the maximum and minimum concentrations belonged to Fe and Co, respectively.

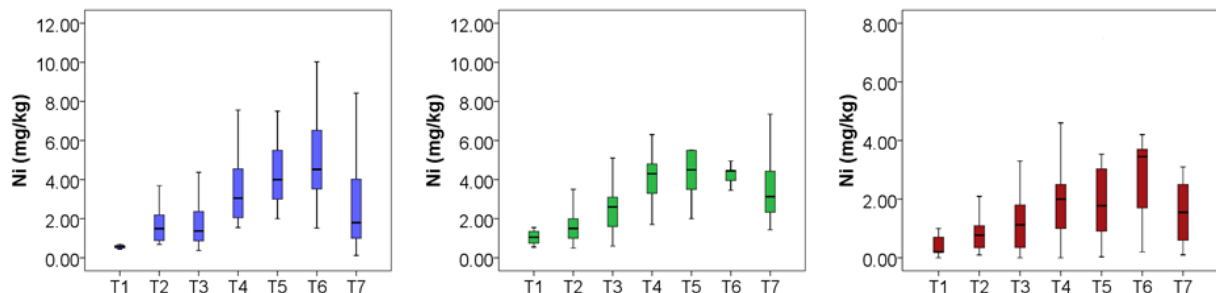
Accumulated dust on tree leaves and dust-borne heavy metal concentrations have been investigated in many studies (Simon et al., 2014). Among the three species investigated in the present study, *Platanus orientalis L* exhibited the greatest differences between the washed and unwashed leaves for all the heavy metals examined. Thus, this species may be regarded as the best among the studied biomonitors since washing eliminated much of the atmospheric dust accumulated on its leaves. Meanwhile, this is also evidence showing that the leaves of *Platanus orientalisL* are capable of trapping atmospheric dust due to their surface characteristics. Hwang et al. (2011) stated that the hairs on the back of the *Platanus orientalis L* leaves make them more effective in trapping aerosols than their smooth surface. This is in agreement with many previous studies which reported that the amount of dust accumulated on tree leaves depends greatly on their surface characteristics such as fuzzes, cuticles, stomata, and capillaries. Tree height and form have also been found effective in trapping

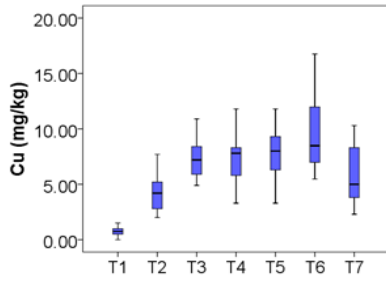
atmospheric dust (Simon et al., 2014; Sæbø et al., 2012; Ram et al., 2014; Bonanno, 2014). Simon et al (2014) found micro-fuzzes on leaves capable of trapping much of the atmospheric dust and found leaf surface and size as well as capillary density to be important parameters involved in dust accumulation. They also reported that the size of stomata and their high density could be possibly the main results of accumulating atmospheric pollutants on tree leaves. Bayco et al. (2006) suggested that not only tree species but also the growing period affect the trapping of atmospheric dust.

According to Figure 3, there is an increasing temporal trend in heavy metal concentrations due to atmospheric dust trapped on leaves during the 7 months of the sampling period from T₁ to T₆. This is the period during which tree leaves grow and their surface area increases; hence, the increasing heavy metal concentrations. On the other hand, the air-borne dust increases each month during the same period. The combined effect of larger surface area and growing air-borne dust leads to increasing heavy metal concentrations on tree leaves. At T₇, much of the accumulated dust was washed off due to a heavy rainfall in that month, leading to a declining heavy metal concentration from T₆ to T₇. Clearly, there is a negative and highly significant correlation between dust deposition and rainfall as the accumulated dust plummeted to its lowest value at T₇.

According to Figure 3, *Morus alba* and *Ulmus boissieri* also show the same process to some extent in terms of the difference in heavy metal concentrations between their washed and unwashed leaves. Moreover, the concentrations of Co and Pb accumulated on tree leaves are not detectable during the first months of the study period due to their low concentrations in the atmospheric dust during this period.

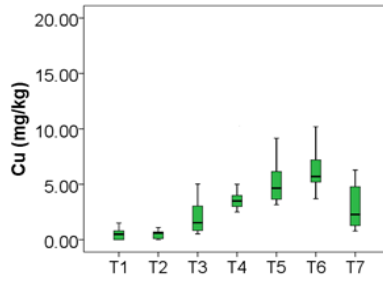
Figure 3- Effect of time on heavy metal deposition on the tree leaves studied (UL-WL)





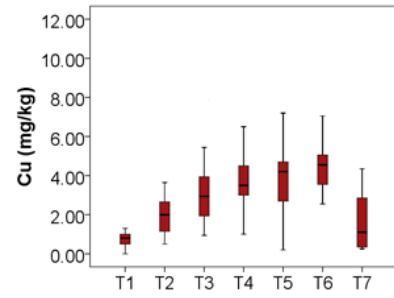
Sampling time

(*Morus alba*)



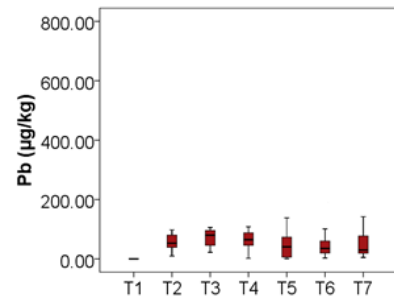
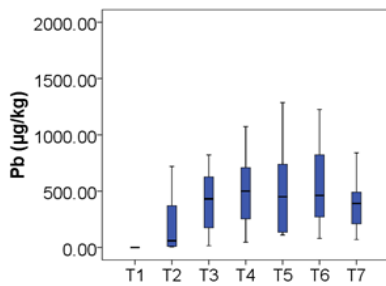
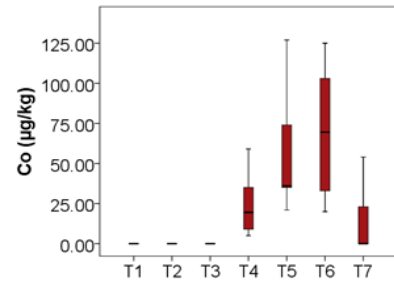
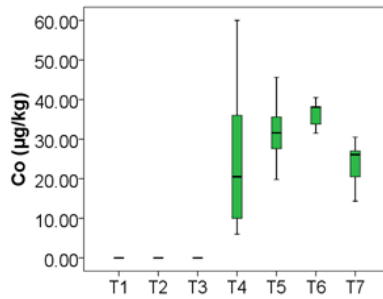
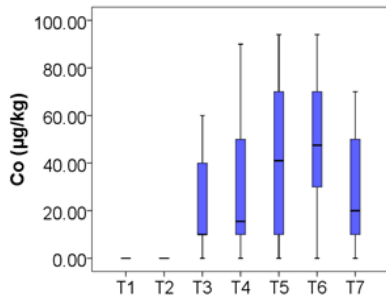
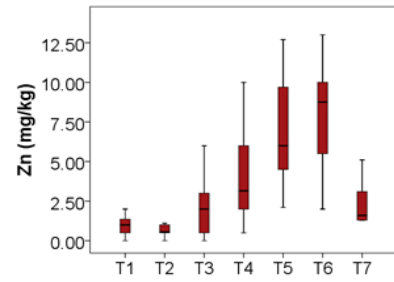
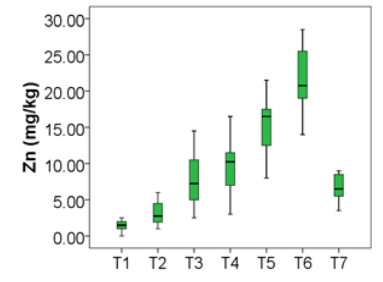
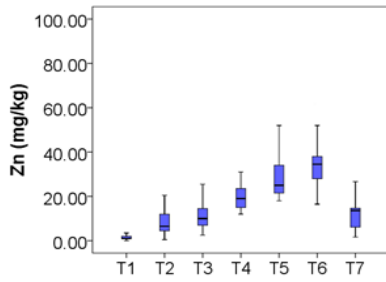
Sampling time

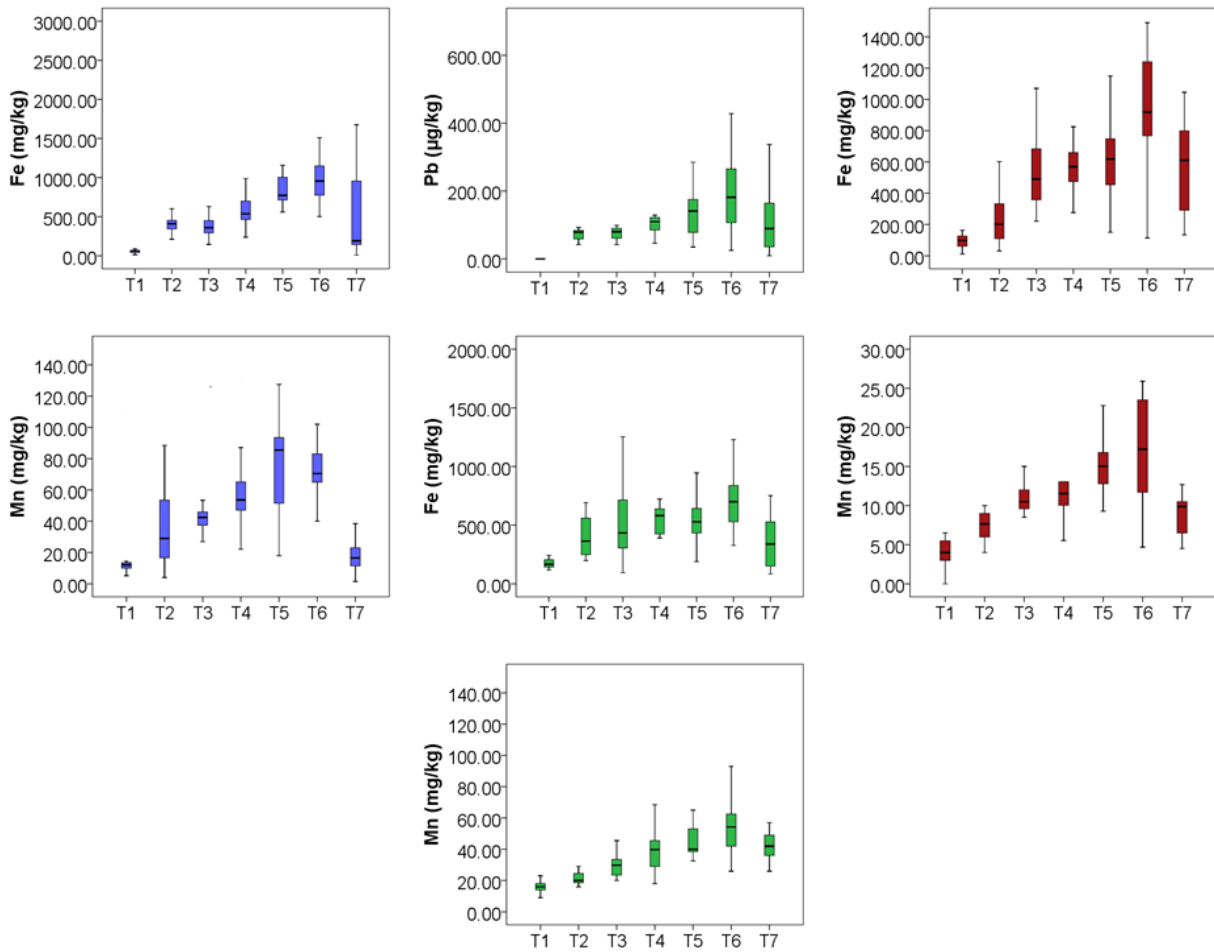
(*Platanus orientalis L*)



Sampling time

(*Ulmus boissieri*)





Heavy metal concentrations in the atmospheric dust and their relationship with those accumulated on tree leaves

Table 4 shows the heavy metal concentrations of the atmospheric dust collected during the sampling months in the glass traps in a descending order: Fe> Zn> Mn> Pb> Cu> Ni> Co. Clearly, the maximum concentrations for most of the heavy metals investigated belong to T6 and T7. Bing et al (2016) used several Alpine tree species for bimonitoring heavy metals to find that Pb and Zn concentrations in the trees were markedly affected by anthropogenic activities; hence, their high concentrations. They, however, observed that Cu and Ni which are mainly absorbed by plant roots were of rather low concentrations. Iodice et al. (2016) studied air pollution monitoring. They found that the major sources of air pollutants and the dust-borne heavy metals of Cr, Cu, Ni, Pb, and Zn could be traced to anthropogenic activities and that they especially affect plants during the cold season. The

reason for this they claimed was the temperature drop during the last two months of the sampling period that led to elevated use of fossil fuels and, thereby, an increase in heavy metal concentrations in the urban atmosphere. One additional cause in our case might be the beginning of the academic year in Iran when the urban areas face increased traffic loads and traffic jams which introduce more fossil fuel emissions bearing heavy metals into the atmosphere. Krietkus et al. (2011) conducted a research on the trends of atmospheric heavy metal deposition in Lithuania to find that more Pb and Zn were detected in the air during the cold season because of the large fuel consumption. Rodriquez et al (2014) studied the biomonitoring of atmospheric trace elements in agricultural areas and a former uranium mine. They found that the highest physiological damage to trees took place in the winter due to the adverse climate conditions and the high air pollution in winter.

Table 4- Heavy metal concentrations (mean \pm S.D) in the atmospheric dust collected in traps

Sampling Time	Elements (Mg/Kg ⁻¹)						
	Ni	Cu	Zn	Co	Pb	Fe	Mn
T ₁	78.8 (\pm 12)	108.5 (\pm 59.1)	962 (\pm 698.5)	28.8 (\pm 0.8)	207 (\pm 55.2)	13949.5 (\pm 2081.3)	356 (\pm 38.2)
T ₂	76.7 (\pm 11.9)	94.7 (\pm 59.4)	933.7 (\pm 649)	25.3 (\pm 0.9)	199 (\pm 54.8)	8025.7 (\pm 1866.9)	219 (\pm 43.3)
T ₃	75.7 (\pm 12.8)	83.5 (\pm 30.3)	860.9 (\pm 817.8)	21.4 (\pm 0.8)	194.3 (\pm 26.2)	9581.1 (\pm 4282.6)	330 (\pm 60.9)
T ₄	52.2 (\pm 16)	96.7 (\pm 59.7)	728.4 (\pm 650.6)	26.7 (\pm 1.3)	183.4 (\pm 25.6)	8158.9 (\pm 1583.8)	300.4 (\pm 42.1)
T ₅	46.8 (\pm 17.3)	102.6 (\pm 58)	790.9 (\pm 611)	27.2 (\pm 2)	183.8 (\pm 34.2)	8445.3 (\pm 2396.2)	303.7 (\pm 43.5)
T ₆	65.1 (\pm 16.8)	104.6 (\pm 57.1)	832.6 (\pm 603.4)	24.8 (\pm 1)	191 (\pm 57.1)	12478.1 (\pm 2106.1)	394 (\pm 40.9)
T ₇	93.7 (\pm 16.9)	140 (\pm 72.6)	1098.9 (\pm 893.7)	26.3 (\pm 1.5)	231.4 (\pm 59.7)	12020 (\pm 1210.4)	373.7 (\pm 36.5)

Studying the temporal trend in the accumulation of some heavy metals on *Quercus robur* and *Tilia cordata* trees in Belgrade, Serbia, Aničić et al. (2011) hypothesized that the best biomonitors for tracing heavy metals were the ones which would yield an output most similar to that of measurement instruments. They then compared the air-borne heavy metal concentrations measured on glass dust traps with those on tree leaves (both UL and WL) to determine the relationship between the two biomonitor variants for all atmospheric heavy metals.

In the present study, this was accomplished using the Pearson correlation. Thus, sampling in each month consisted of 22 measurements of heavy metal concentrations in the atmospheric dust captured on glass traps and the same number of measurements of heavy metals accumulated on both washed and unwashed leaves of the three tree species. The shared property reflected in the calculated correlation coefficients (Table 5) was the fact that the correlation coefficients increased for the majority of metals in all the three biomonitors during the growing season. Thus, T₆ for each of the biomonitors represented the maximum correlation coefficient among the concentrations of the heavy metals both captured by the traps and on the tree leaves. Moreover, the correlation coefficients of all the three bioindicators dropped sharply at T₇ due to the heavy rain that washed the accumulated dust on the tree leaves. Another common property of these three biomonitors, in relation to Pearson correlation coefficients, involves the low correlation coefficients for Pb and Co, indicating that the trees used in this research are not appropriate for tracing Pb and Co. It was observed that *Platanus orientalis L* absorbed more Pb from the atmosphere than the other two species as it showed no significant

correlation coefficients among the concentrations of the metals except for a few months of the study period. Sawidis et al (2011) maintained that while some tree species absorb most of the heavy metals in their areal parts, this is not necessarily the case for all heavy metals (Sawidis et al., 2011).

Table 5 presents the correlation coefficients among the concentrations of heavy metals from the atmospheric dust collected in glass traps as well as the differences between the heavy metal concentrations in washed and unwashed tree leaves. Clearly, high correlation coefficients are obtained for the metals Ni, Cu, Fe, and Mn during the growing period of *Platanus orientalis L*. This might be due to the increase in leaf surface area during the growing season such that the highest correlation was observed at T₆ when the leaves were growing well in size, enough dust was being deposited on the leaves, and no natural event such as rain or wind occurred. A point of interest about the ability of *Platanus orientalis L* leaves to trace heavy metals is that positive and meaningful correlations between washed and unwashed leaves were obtained for the heavy metals Ni, Cu, Zn, Fe, and Mn in the second month of the sampling period when the leaves had not yet fully grown and little dust had deposited on the leaves. Nakazato et al (2016) investigated tropical trees used as biomonitors of atmospheric toxic elements to find that *P. guajava* was a better option for biomonitoring toxic elements in Cubatao.

According to Table 5, the increasing trend in the correlation coefficients obtained between heavy metal concentrations in atmospheric dust collected on glass traps and those deposited on washed and unwashed leaves of *Morus alba* is similar to that established for *Platanus orientalis L*. Indeed, the correlation coefficients exhibit a great increase due

to the aggregation of dust during the growth of tree leaves. The only difference between *Morus alba* leaves and those of *Platanus orientalis L* with respect to the correlation coefficients lies in the lower correlation coefficients recorded for *Morus alba* for the absorption of all the metals except Ni. It may, thus, be concluded that *Platanus orientalis L* is preferable to *Morus alba* for tracing atmospheric heavy metals in the city of Isfahan.

According to Table 5, the correlation coefficients obtained for *Ulmus boissieri* for all the studied heavy metals are smaller than those obtained for *Platanus orientalis L* or *Morus alba*. This indicates that *Ulmus boissieri* does not function well as a bio-indicator of heavy metals under the conditions in Isfahan, especially where the two other species of *Platanus orientalis L* and *Morus alba* are available in this city. More specifically, the data in the same Table indicate that *Ulmus boissieri* is not a suitable bio-indicator for Co or Pb since no meaningful correlations were obtained for these metals in any of the seven months of the sampling period. The reason for this might be the small leaf surface area of this species compared to those of *Platanus orientalis L* and *Morus alba*; the large leaf surface area of the latter two species led to more dust-borne Co and Pb to be absorbed, which yielded meaningful correlation coefficients for these metals.

Generally speaking, based on the correlation coefficients reported in Table (6), the *Platanus orientalis L* leaves yielded higher correlation coefficients as compared with those of the two other species. Moreover, the concentrations of heavy metals in the leaves of this tree exhibit a greater similarity to those recorded for the dust traps than do those of *Morus alba* and *Ulmus boissieri*. It may, therefore, be concluded that the heavy metal concentrations in the washed and unwashed leaves of *Platanus orientalis L* are meaningfully correlated with those recorded for the dust traps so that the leaves are capable of being used as bio-indicators of heavy metals. This is also confirmed by Norouzi et al. (2015) who studied the ability of tree leaves to assess and trace the origin of air-borne heavy metals in Isfahan to conclude that the leaves could be successfully used as replacements for dust traps. Wannaz et al. (2012) studied the accumulation of Al and the physiological status of tree foliage in the vicinity of a large Aluminum smelter. They found

that tree leaves are the best indicators of atmospheric pollution, especially in industrial regions. In their investigation into the sources of heavy metals in the atmospheric dust of Isfahan, Norouzi and Khademi (2015) used the *Platanus orientalis L* leaf as a biomonitor. The authors showed that the leaf of this tree could be successfully used as a replacement for the costly devices employed for measuring atmospheric pollution.

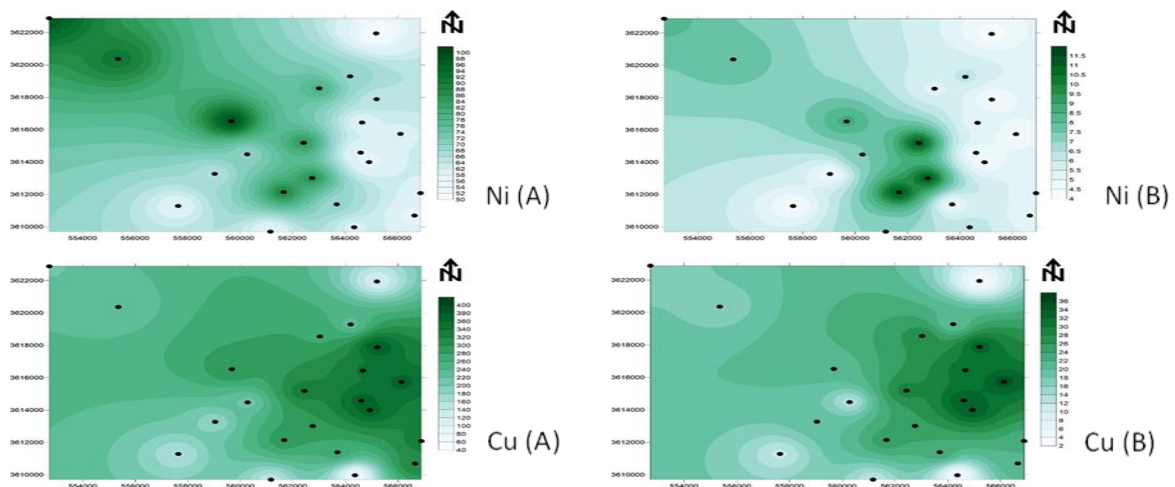
In this study, we developed an atlas of the distribution and concentrations of atmospheric heavy metals across all the 22 sites investigated using the data obtained on heavy metals deposited on traps and accumulated on the leaves of *Platanus orientalis L* (expressed as the difference between heavy metal concentrations in washed and unwashed leaves). In developing the same type of atlas, Wannaz et al. (2012) took advantage of special types of lichen to determine the sources of the pollutants in Cordoba, Argentina. For this purpose, they initially used the principal component analysis (PCA) method and assigned the accumulated heavy metals on lichens from different sources. They then exploited the interpolation method to determine the severity of pollution in each area of the city and, thus, developed a map of the urban environmental pollution distribution for the first three main components. Using a map thus developed, they were able to determine the causal factor for each of the heavy metals. Norouzi et al (2015) also compared the distributions of dust accumulated on *Platanus orientalis L* leaves and that collected in sampling traps. They finally discovered a strong correlation between the spatial distributions of heavy metals collected on the *Platanus orientalis L* leaves and on dust traps. Figure 4 shows the spatial distribution of heavy metals collected on both glass traps and (UL-WL) leaf surfaces of *Platanus orientalis L*. Clearly, there is a good match between the two and, thus, the tree leaves proved to be the best bio-indicators of heavy metals during the study period. Moreover, the similarity in the spatial distribution of heavy metals between the dust collected in the traps and on *Platanus orientalis L* leaves indicates the satisfactory difference between the washed and unwashed leaves, which obviously makes the species a good bio-indicator of atmospheric heavy metals in Isfahan

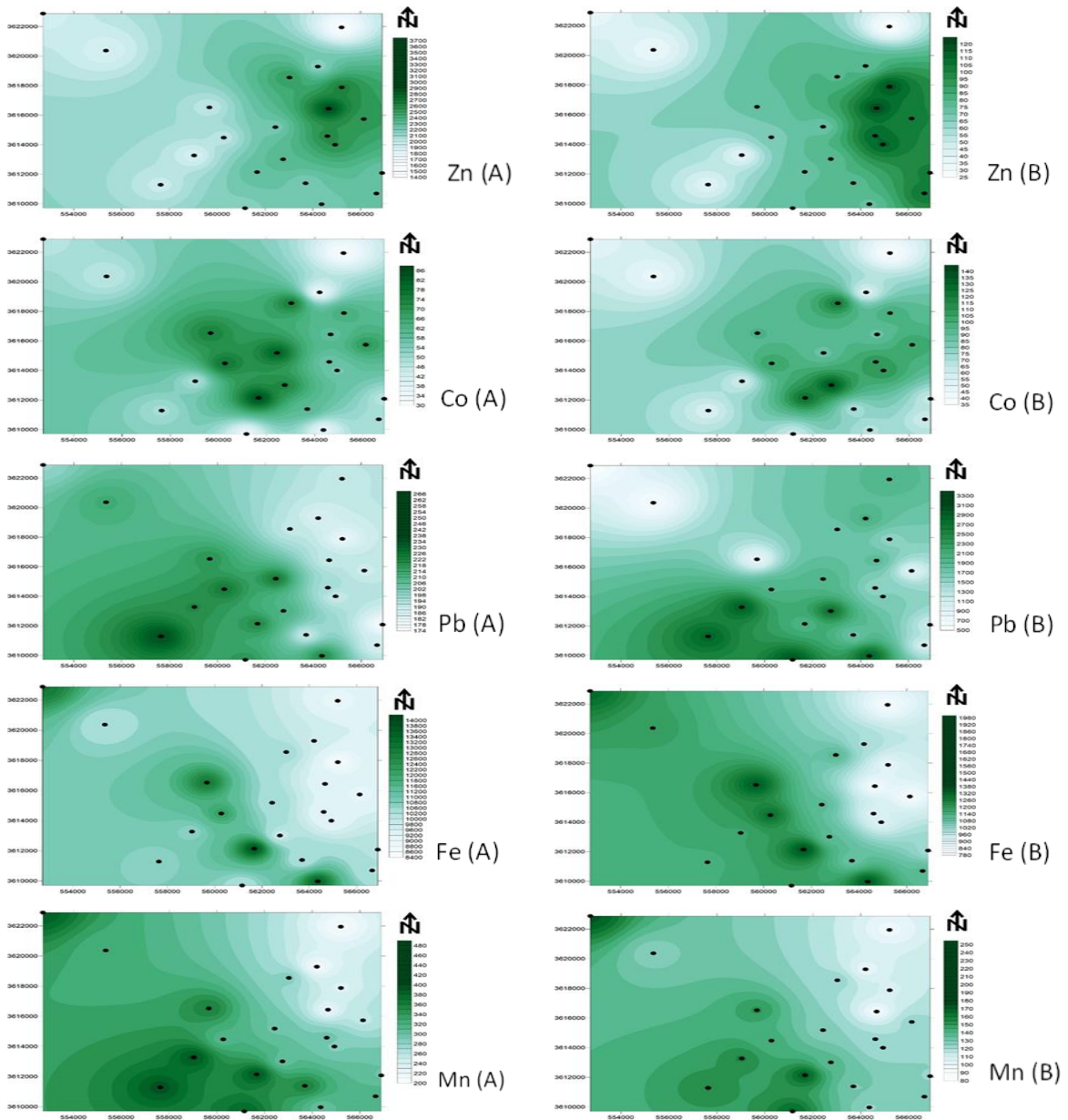
Table 5- Pearson correlations between heavy metals in the atmospheric dust and on trees leaves (UL-WL)

Sampling Time	Trees Leaf	Ni	Cu	Zn	Co	Pb	Fe	Mn
T1	<i>Platanus orientalis L</i>	0.3	0.42	0.4	0.17	0.35	0.41	0.4
	<i>Morus alba</i>	0.43*	0.4	0.38	0.23	0.3	0.25	0.42
	<i>Ulmus boissieri</i>	0.34	0.37	0.37	0.3	0.39	0.41	0.4
T2	<i>Platanus orientalis L</i>	0.43*	0.43*	0.51*	0.27	0.4	0.45*	0.49*
	<i>Morus alba</i>	0.5*	0.45*	0.38	0.3	0.3	0.28	0.46*
	<i>Ulmus boissieri</i>	0.37	0.37	0.42	0.36	0.37	0.44*	0.43*
T3	<i>Platanus orientalis L</i>	0.45*	0.47*	0.54**	0.38	0.43*	0.51*	0.53*
	<i>Morus alba</i>	0.56**	0.44*	0.41	0.44*	0.4	0.4	0.49*
	<i>Ulmus boissieri</i>	0.38	0.44*	0.41	0.32	0.4	0.48*	0.45*
T4	<i>Platanus orientalis L</i>	0.56**	0.44*	0.63**	0.4	0.42	0.62**	0.57**
	<i>Morus alba</i>	0.61**	0.5*	0.53*	0.43*	0.46*	0.44*	0.53*
	<i>Ulmus boissieri</i>	0.44*	0.45*	0.47*	0.4	0.4	0.52*	0.43*
T5	<i>Platanus orientalis L</i>	0.55**	0.61**	0.58**	0.56**	0.5*	0.67***	0.63**
	<i>Morus alba</i>	0.7***	0.49*	0.48*	0.5*	0.47*	0.44*	0.54**
	<i>Ulmus boissieri</i>	0.47*	0.45*	0.49*	0.41	0.39	0.64**	0.5*
T6	<i>Platanus orientalis L</i>	0.62**	0.71***	0.64**	0.55**	0.52*	0.7***	0.74***
	<i>Morus alba</i>	0.72***	0.64**	0.5*	0.48*	0.46*	0.49*	0.6**
	<i>Ulmus boissieri</i>	0.52*	0.5*	0.52*	0.4	0.38	0.68***	0.53*
T7	<i>Platanus orientalis L</i>	0.5*	0.56**	0.55**	0.42	0.46*	0.49*	0.61**
	<i>Morus alba</i>	0.44*	0.51*	0.4	0.42	0.42	0.34	0.43*
	<i>Ulmus boissieri</i>	0.29	0.39	0.41	0.38	0.4	0.55**	0.43*

(* , ** , *** respectively significant in 0.05, 0.01, 0.001)

Figure 4- Spatial distributions of heavy metals in atmospheric dust (A) and (UL-WL) (B). (Metal concentrations are in mg.kg⁻¹ in all the maps except for Pb (B) which is expressed in µg.kg⁻¹)





Conclusions

The study revealed that the leaves of *Platanus orientalis L* leaf are better bio-indicators of heavy metals in the urban atmosphere than are those of *Morus alba* and *Ulmus boissieri*. More particularly, it was shown that Zn, Fe, Mn, Ni, and Cu could be better traced by the leaves of this species than the two metals of Co and Pb. In order to understand whether heavy metals existing on plant leaves are the result of root uptake or atmospheric dust accumulated on tree leaves, the difference between metal concentrations in washed and unwashed leaves was determined for each sampling site and each tree species. The evidence obtained showed that the closer we drew to the end of the sampling period when leaf growth reached its culmination, the greater would become the difference between heavy

metal concentrations in washed and unwashed leaves. This was ascribed to the temporal trend in aggregation of dust deposition on tree leaves and the increase in tree leaf surface area. During the early months of the sampling period, the difference between the heavy metal concentrations in washed and unwashed leaves was found to be insignificant for some metals; however, the difference became significant toward the later months of the sampling period. Contrary to our expectation, the difference between washed and unwashed leaves disappeared in the last month of the sampling period (T₇), which was attributed to the rainfall event which washed the dust off the leaves. Another finding of the study was the higher heavy metal concentration in the dust on the leaves of *Platanus orientalis L* than on those of the other two species. Moreover, the correlation

found between the heavy metal concentrations in the dust collected on traps and that in the dust accumulated on the leaves of *Platanus orientalis* L – particularly during the final months of the sampling period – was greater than that established between the concentrations of the dust traps and those on *Morus alba* and *Ulmus boissieri* leaves. It was, hence, concluded that *Platanus orientalis* L was a better replacement for the costly measuring instruments.

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