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Original Research

Fraxinus excelsior as a Biomonitor of Heavy Metal Pollution

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Abstract

Fraxinus excelsior L. (Common Ash) is a deciduous tree that grows in wild and urban Turkey. *Fraxinus excelsior* has been tested as a possible biomonitor of heavy metals in the city of Kayseri, Turkey. Forty-eight sites (urban road side, road side, urban park, industrial, suburban and rural sites) in and around the city of Kayseri were investigated. In this study, the concentrations of Pb, Cd, Cu, Zn, Ni and Cr in unwashed and washed leaves and soils were determined. There were significant relationships between samples of the heavy metal concentrations from surface soil and washed leaves. The concentrations of heavy metals in the leaves of *F.excelsior* increased along with the urbanization of the sites. *Fraxinus excelsior* was found to be a useful biomonitor of the determined heavy metals.

Keywords: heavy metal, pollution, biomonitor, *Fraxinus excelsior*, urban environment.

Introduction

One of the first attempts for the assessment of environmental pollution coming from exhaust gases of automobiles in traffic by using plants is based on the analyses of different trees, grasses, and vegetables that grow near highways and cities, which was used as a common method in the early 1960s. Later on, this problem became an object of extensive investigation of many researchers [1, 5]. Mainly tree leaves and bark, herbs, lichen and moss were used as bioindicator species [1].

Biological indicators have been used for many years to detect the deposition, accumulation and distribution of heavy metal pollution [6]. During the past few decades there has been an increase in the use of higher plant leaves as biomonitors of heavy metal pollution in the terrestrial environment [3, 5, 7, 8].

Uptake and accumulation of elements in plants may follow different paths, i. e. the foliar surface and the root system. The relative importance of these routes for pollutant flux toward the leaves isn't clear on tree leaves [9, 11].

The aim of this study is to determine the Pb, Cd, Cu, Zn, Ni and Cr concentrations in surface soil and in unwashed and washed leaves of *F. excelsior* (ash) which was selected and tested as a biomonitor of these heavy metals in Kayseri, located in Central Anatolia.

Materials and Methods

Fraxinus excelsior L.

Fraxinus excelsior is a deciduous tree which under most favorable conditions may reach heights of over 40 m with a girth of up to 6 m, although under extreme conditions it may remain a shrub. *Fraxinus excelsior* is common on soils of high base status which prefer calcareous soils such as limestone and chalk, where it may become abundant avoiding the soils that are markedly acidic [12]. *Fraxinus excelsior* requires the soil to be rich in humus and nutrients and can multiply in rich soil that accumulates in cracks of mountain limestone.

Fraxinus excelsior, a tall tree, is readily distinguished by its light-grey bark and by its large compound leaves. Both bark and the leaves have medicinal use. This plant

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is native to Europe, the Caucasus and Turkey [13]. However, it's grown in gardens and is also popular as an urban ornamental plant throughout Asia and Europe. *Fraxinus excelsior* is called a pioneer tree, which is tolerant of the intensity of ranges of most environmental factors except a shortage of light.

Study Area

Kayseri is located in the Iran-Turan phytogeographical region of Turkey. Samples were taken from Kayseri. Six different types of sites were sampled, namely urban roadside, road side urban park, industrial, suburban sites with the aim of reflecting a progressive decrease in urbanization and thus likely pollution levels. Urban roadside sites were selected mainly near the city center along the busy main roads. Roadside sites were chosen along the Kayseri- Kırşehir highways at 20-35 km. The traffic density of this road was estimated to be 285 vehicles per hour. The industrial sites were chosen from the industrial area that is located approximately 15 km from the city of Kayseri. The suburban sites were chosen from the edge of the city in the residential areas with relatively low human activity. Rural sites located approximately 25 km away from center were chosen. However, a low level of contamination should also be expected even in these sites considering the farm and visitors' traffic.

Sample Collection and Preparation

Samples of plants and soils were collected from different sites during July 2002. The numbers of sites from each category sampled were as follows: roadside = 10, urban roadside = 10, urban park = 10, Industrial area = 6, suburban area = 6 and rural area = 6 (control). At each site, soils were sampled from the top 10 cm by means of a stainless steel trowel to avoid contamination. The soil samples were air dried and then passed though a 2 mm sieve. Approximately 150 g (fresh wt) of similar sized well-developed leaves of *F. excelsior* were selected and harvested. Plant samples were then divided into two sub-samples. One sub-sample was thoroughly washed with running distilled water to remove dust particles and the other remained untreated. All plant samples were oven- dried at 80^0C for 24 h, milled in a micro-hammer cutter and fed through a 1.5 mm sieve. Plant samples were stored in clean self-sealing plastic bags in silica gel.

Analytical Techniques

The method was adopted from Paveley and Davies [14], for the analysis of heavy metal concentrations in soil involved aqua regia digestion. One-gram samples of dried and sieved soil materials were ashed in muffle furnace at 460^0C for 24 hours. The weighed ash was digested in 10 ml aqua regia (1 HNO_3: 3 HCl) in a digestion tube on heating block for a total of 9 hours, in the following sequence and durations of temperatures: 2 hrs each at 25^0C, 60^0C and 105^0C and finally 3 hrs at 125^0C. All the digested samples were centrifuged and then made up to volume with 1% HNO_3.

The method used for plant digestion is the same as that described earlier by Al-Shayeb et al. [3]. One-gram samples of dry milled plant materials were ashed in a muffle furnace at 460^0C for 24 hrs. The weighed ash was digested in analar concentrated HNO_3 and evaporated to near dryness on a hot plate. Digested samples were centrifuged and then made up to volume with 1% HNO_3.

The concentrations of heavy metals (Cd, Pb, Ni, Cu, Zn and Cr) were measured in soil and plant samples by Varian ICP-OES (Inductively Couple Plasma Optical Emission Spectroscopy). The standard error values of the means were calculated for the comparison of site categories. To determine the significance of washing the leaves a paired t- test was performed, comparing the washed and unwashed plants for each type of the sites. The relationships between variables were assessed using linear regression and correlation analyses.

Results

Lead and Cadmium

The mean levels of Cd and Pb found in unwashed and washed leaves of *F.excelsior* in different sites are presented in Figure 1-2. The urban roadside with the highest human activities, together with high vehicular density congestion, shows the highest Pb levels, 18.41 μgg^{-1} which is significantly higher than that of the rural sites, 8.02 μgg^{-1} (Fig.1 a). The results showed that the mean Pb levels in the roadside (14.71 μgg^{-1}) was much greater than the Pb content range in the urban park (12.39 μgg^{-1}), industry (11.50 μgg^{-1}), suburban (10.53 μgg^{-1}) and rural (8.02 μgg^{-1}) areas. The urban roadside had significantly ($p< 0.05$) higher contamination than the rural one. Similar observations were made by Aksoy and Öztürk [4] and Al-Shayeb et al. [3]. Fig.1b shows that the higher levels of Pb are found in the urban roadside in soils (57.62 μgg^{-1}). The Pb concentrations in other stations are in roadside (20.15 μgg^{-1}), urban park (17.11 μgg^{-1}), industrial (14.28 μgg^{-1}), suburban (12.10 μgg^{-1}) and rural (9.12 μgg^{-1}) areas.

Although uptake from the soil is one possible way to raise Pb concentration in leaves, Markert [1] suggested that uptake of Pb is probably passive and translocation from root to other parts of the plant is low, but aerial deposition and foliar uptake may contribute significantly to leaf concentration.

Washing the leaves significantly reduced the Cd concentration in *Fraxinus excelsior* from all the sites. The washing of samples removes a large part of the particles from the leaves when compared to unwashed samples. The highest concentrations of Cd in Kayseri were recorded in the plant samples and also in the soil from the urban roadside (3.43 μgg^{-1}) with heavy traffic and considerable human activ-

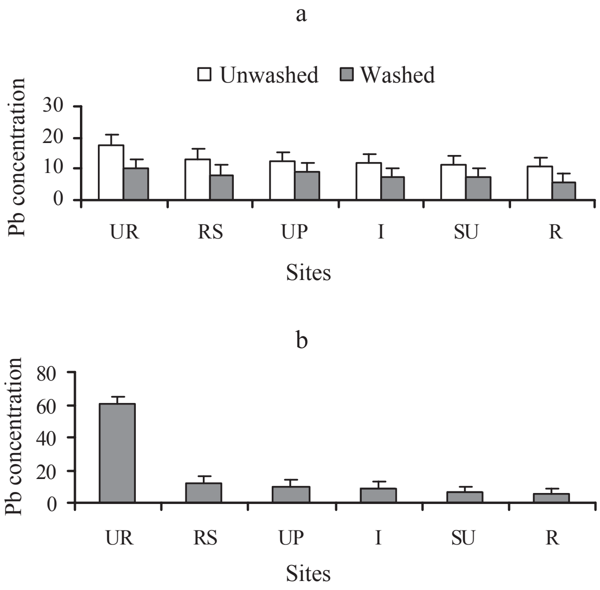

Fig.1. Mean Pb concentrations (µgg^{-1}, dry weight) (a) in unwashed and washed *F. excelsior* and (b) in soil, together with standard error bars.

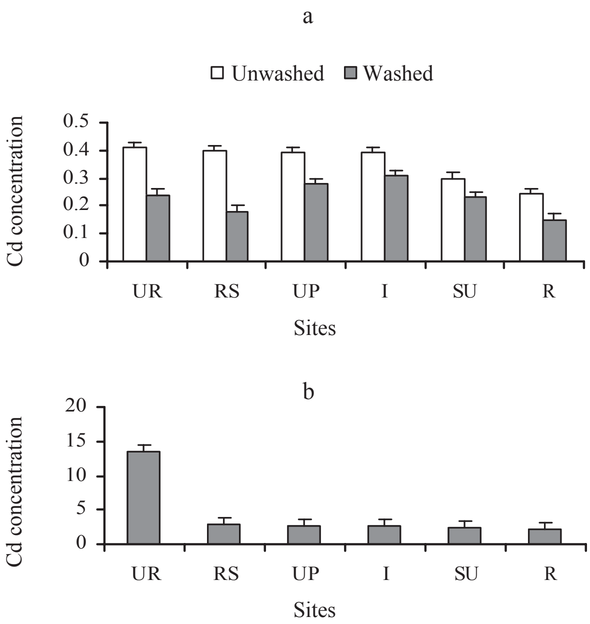

Fig.2. Mean Cd concentrations (µgg^{-1}, dry weight) (a) in unwashed and washed *F.excelsior* and (b) in soil, together with standard error bars.

ity and the lowest values were found in the rural sites (2.02 µgg^{-1}, Fig. 2 a). The highest mean of Cd concentrations, 0.428 µgg^{-1}, in leaves of the *F.excelsior* is significantly higher than that of all the other sites except the urban sites. The mean level of Cd contents in the roadside (0.397 µgg^{-1}) was much greater than the Cd content range in the urban park (0.362 µgg^{-1}), industrial (0.341 µgg^{-1}), suburban (0.288 µgg^{-1}) and rural (0.243 µgg^{-1}) areas. Significantly ($p < 0.01$) elevated cadmium concentrations were measured in urban roadside whereas the urban park and industrial area were also, but to a lesser degree, contaminated. The mean Cd concentrations in soils are in the urban roadside (3.43 µgg^{-1}), roadside (2.92 µgg^{-1}), urban park (2.67 µgg^{-1}), industrial (2.71 µgg^{-1}), suburban (2.34 µgg^{-1}) and rural (2.02 µgg^{-1}) areas.

Table 1 shows that the amounts of Pb, Cd, Zn, Cu, Ni and Cr removed by washing the leaves were higher than those of the rural site, indicating that atmospheric depositions of these metals differ in these sites. These differences are due to high and low densities of pollutants (traffic, industry, burning fossil fuels used in homes etc.) in study sites. In general, higher levels of heavy metals are removed by washing the leaves in the urban roadside and the roadside; the lower levels of heavy metals are removed by washing in the rural site. As seen in Table I, 41.35-18.52 % of Cd, 46.11-9.57 % of Pb, 38.16-9.92 % of Zn, 32.69-10.27 % of Cu, 46.45-27.94 % of Ni and 43.55-18.81 % of Cr were removed through the washing procedure from the leaves of *F.excelsior*.

Nickel and Copper

The mean concentrations of Ni and Cu found in unwashed and washed leaves in different types of sites for *F.excelsior*

Table 1. Total percentage of Pb, Cd, Ni, Cu, Zn and Cr was removal from the leaves of *Fraxinus excelsior* by washing procedure. Significance of comparison means by ANOVA (F- test) are indicated (Key: *$p< 0.05$ and **$p< 0.01$).

Sites	Urban roadside	Roadside	Urban park	Industry	Suburban	Rural	F-test
Cd	41.35 ± 1.29	41.31 ± 1.23	37.56 ± 1.12	37.53 ± 0.40	28.12 ± 1.33	18.52 ± 1.54	*
Pb	46.11 ± 1.23	41.46 ± 2.25	35.35 ± 1.81	36.34 ± 0.05	30.86 ± 2.56	9.57 ± 1.37	*
Zn	38.66 ± 0.99	33.81 ± 1.16	28.53 ± 2.02	25.16 ± 0.20	12.11 ± 0.35	9.92 ± 1.12	**
Cu	32.69 ± 1.62	31.20 ± 1.74	36.23 ± 2.42	30.18 ± 1.6	15.84 ± 1.56	10.27 ± 1.37	*
Ni	46.45 ± 1.28	45.01 ± 1.32	40.62 ± 2.13	50.41 ± 1.55	40.42 ± 1.95	27.94 ± 1.43	*
Cr	43.55 ± 0.99	29.68 ± 1.49	38.84 ± 1.51	42.85 ± 0.99	27.58 ± 0.19	18.81 ± 0.76	*

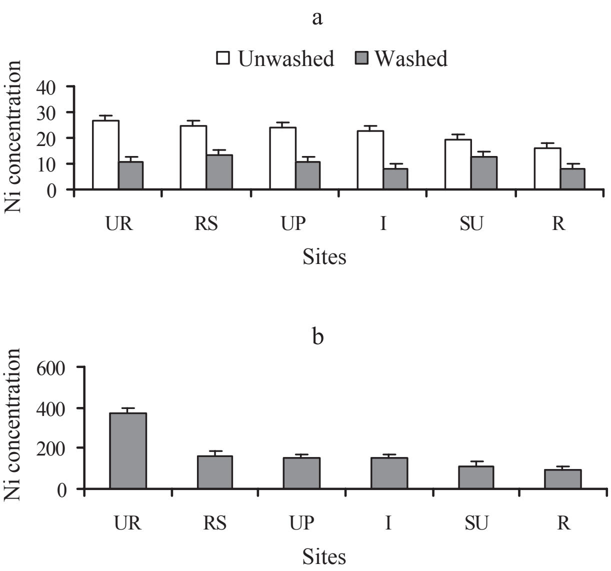

Fig.3. Mean Ni concentrations (μgg^{-1}, dry weight) (a) in unwashed and washed *F.excelsior* and (b) in soil, together with standard error bars.

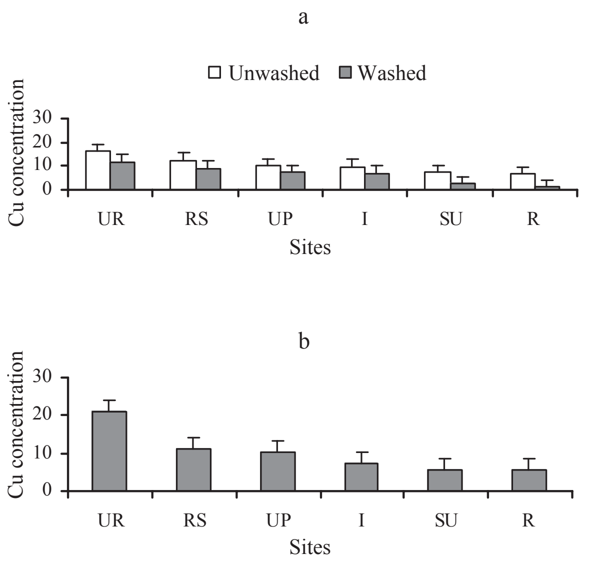

Fig.4. Mean Cu concentrations (μgg^{-1}, dry weight) (a) in unwashed and washed *F.excelsior* and (b) in soil, together with standard error bars.

are presented in Figures 3a and 4a. In addition, the mean concentration of metals in soils supporting *F.excelsior* in these areas is shown in Figs. 3b and 4b. The results indicated that leaves of *F.excelsior* could accumulate significant Ni and Cu concentrations. The concentrations of Ni and Cu were found to follow a similar general trend to that for Cd with the highest values generally found in the urban roadside (27.04 μgg^{-1}, 16.21 μgg^{-1}, respectively). The highest mean Ni concentration was 27.04 μgg^{-1} and the highest mean Cu concentration was 16.21 μgg^{-1} in *F.excelsior*. The Ni and Cu concentrations in the leaves of *F.excelsior* in the other stations are in roadsides (24.75 μgg^{-1}, 13.11 μgg^{-1}), urban park (20.01 μgg^{-1}, 11.26 μgg^{-1}), industry (21.86 μgg^{-1}, 10.03 μgg^{-1}), suburban areas (16.82 μgg^{-1}, 7.26 μgg^{-1}) and rural area (13.42 μgg^{-1}, 6.62 μgg^{-1}). Among the stations studied, the urban roadside, the urban park, the roadside and the industrial site had significantly ($p < 0.05$) elevated Ni concentrations. In addition, the highest mean Cu and Ni concentrations were 21.06 μgg^{-1} and 390.29 μgg^{-1} in soil, respectively.

Zinc and Chromium

The mean levels of Zn and Cr found in the unwashed and washed leaves of *F.excelsior* in the different sites are presented in Figs. 5-6.

The mean zinc and chromium concentrations in the leaves of *F.excelsior*, as well as in the respective soil samples, are shown in Figure 5b and 6b. The highest concentrations of Zn and Cr in the plants sampled in Kayseri were recorded from the urban roadside (29.41 μgg^{-1}) and the industrial site (1.70 μgg^{-1}) with the influence of heavy traffic and considerable human activity. The lowest values were found in the rural sites (Zn = 13.22 μgg^{-1}, Cr = 1.01 μgg^{-1}). The mean level of Zn contents in the roadside (23,60 μgg^{-1}) was much greater than the content range in the urban park (20.96 μgg^{-1}), industrial (18.72 μgg^{-1}), suburban (15.68 μgg^{-1}) and rural (13.22 μgg^{-1}) sites. Significantly ($p < 0.01$) elevated zinc concentrations were measured in the roadside whereas the urban park and industrial sites were also, but to lesser degree, contaminated. The results showed that the mean level of Cr contents in the urban roadside (1.63 μgg^{-1}) was much greater than the content range in the other stations.

The highest concentrations of Zn and Cr were found in the leaves of *F.excelsior* (29.41 μgg^{-1} and 1.70 μgg^{-1}). Washing the leaves reduced the Zn and Cr concentrations significantly in all the sites. Although washing the leaves reduced Zn concentrations in all the sites, the percentage of the element that was removed was considerably lower than Cu. In addition, the highest concentrations of Zn (92.64 μgg^{-1}) and Cr (124.42 μgg^{-1}) were found in soils in the urban roadside.

A least-squares linear regression analysis was obtained for each of the metals Pb, Cd, Cu, Ni and Cr, between the concentrations of the element in soils and in the washed leaves of *F.excelsior* (Table 2). The results show that the correlation coefficients (r) for Cu and Zn were significant at $p<0.001$, while for Cd correlations were significant at $p<0.01$ and for Ni, Pb and Cr it was not significant in any case.

Discussion

The results indicate an increase in their concentrations with increasing urbanization. The higher Pb content

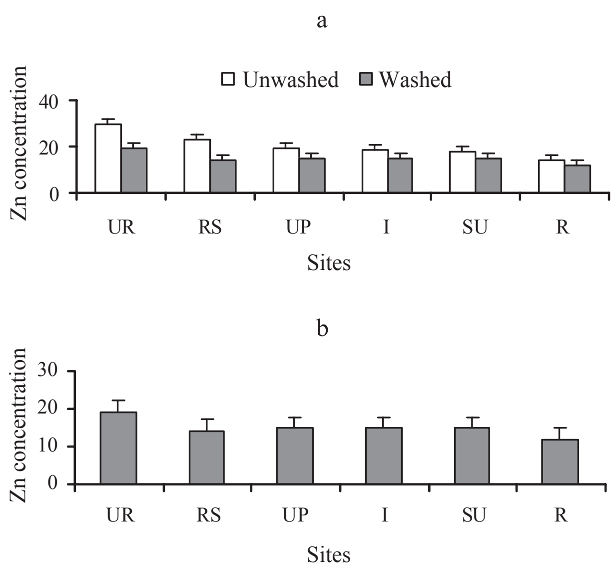

Fig.5. Mean Zn concentrations (μgg^{-1}, dry weight) (a) in unwashed and washed *F.excelsior* and (b) in soil, together with standard error bars.

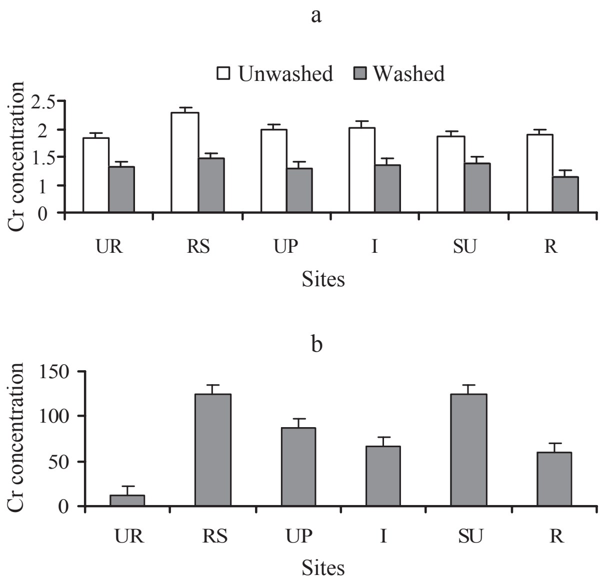

Fig.6. Mean Cr concentrations (μgg^{-1}, dry weight) (a) in unwashed and washed *F.excelsior* and (b) in soil, together with standard error bars.

in urban roadside soil and plant samples is due to traffic density, which is considered as one of the major sources of Pb contamination as shown by many investigations in different countries [5, 15, 16].

Lead is generally added to the environment by aerial deposition alongside the roads in proportion with the density of traffic and the distance from the roadside. Sawidis et al. [2] studied air pollution with heavy metals in the city of Thessaloniki (Greece) using trees as biological indicators and reported that high levels of heavy metals came from vehicular emissions. The rate of damage caused in the environment is greatly affected by meteorological factors, mainly wind direction.

Traffic emissions on roads are the main cause of heavy metal accumulation on the surrounding environment including vegetation, which might have an ecological effect. Elevated levels of heavy metals in urban and industrialized areas are reported in many parts of the world. Momani et al. [17] and Scerbo et al. [18] have found that some heavy metals such as Pb decrease in the leaves of plants with increasing distance from roads.

The high heavy metal content in the urban sites, urban roadsides and the soils and plant samples from these sites is mostly due to the density of traffic. One of the major sources of heavy metal contamination, especially Pb, is because people use leaded petrol. Depending on the pollutant level at the collection sites, for example, the amount of Pb removed from the leaves of *F. excelsior* by washing was the highest in urban roadside (46.11 %) and it was the lowest in the rural sites (9.57 %). According to Al- Shayeb et al. [3], 26 to 68 % of Pb was removed from the leaves of *Phoenix dactylifera* by the washing procedure. The mean Cd

Table 2. Relationships between heavy metal concentration in soil and washed leaves of *Fraxinus excelsior* (r: correlation coefficient; *p< 0.01, **p< 0.001).

Plant	Elements	r	Sample number
F. excelsior	Cd	0.645*	48
	Cr	0.427	48
	Cu	0.71**	48
	Pb	0.365	48
	Ni	0.272	48
	Zn	0.702**	48

concentrations in urban roadsides are slightly higher than the rural (control) site, probably indicating the accumulation of dust raised by motor vehicles and other human activities.

Washing the leaves reduced the heavy metal concentrations significantly in all the types of sites. Ni showed a larger effect than Cr in this respect. A plant foliar concentration of 0.05-3 μgg^{-1} Pb has been quoted by Dmuchowski and Bytnerowicz [16] as a critical indicator of whether the environment is polluted with Pb. The results indicate that the roadsides, urban roadsides, parks and industrial sites are seriously polluted with Pb. Mesanza and Casado [19] used the leaves of *Pinus radiata* to study highway heavy metal contamination in northern Spain and found a significant correlation between traffic density and forest damage. According to Allen [20], plants from unpolluted natural environments contain 0.01- 0.3 μgg^{-1} Cd. *Fraxinus excelsior* exceed the upper value in the urban sites and in urban parks (Fig.1).

Zn is an essential element for plant growth and plays an important role in the biosynthesis of enzymes. Normal Zn concentration in plants is 15-100 μgg^{-1} [20]. Zinc concentration in the unwashed plant samples was linearly correlated with the traffic.

Copper is essential for growth, but plant concentrations above 20 μgg^{-1} are considered toxic to plants [21]. Nriagu [22] reported that the main sources of pollutant copper in the atmosphere were Cu production and handling, fossil fuel combustion and iron steel production. The results of this study for all the sites did not reveal high levels of pollution by Cu since concentrations in *F.excelsior* plant did not exceed the upper limit.

Normal Ni concentration in plants is 0.5-5 μgg^{-1}. Fig. 1 showed that concentrations in *F.excelsior* plant exceed the upper limit. Chromium is not essential for plant, but plant concentrations above 0.5 are considered toxic to plants. Fig. 6a shows that all the sites chosen are highly polluted by Cr since concentrations in *F.excelsior* plant exceed the upper limit.

Another notable observation is that some of the stations investigated had high concentrations of certain metals and low concentrations of other metals. In other words, stations containing the highest concentrations of metals measured were not observed. For example, the industrial site had the highest Cr value (1.70 ± 0.06), but the lowest Zn value (18.72 ± 0.23) among all the stations. Similar correlations were observed by Sawidis et al. [11], even when the above data are compared with heavy metal concentrations from the control stations.

According to Wittig [23], some basic criteria for the selection of a species as a biomonitor are that it should be represented in large numbers all over the monitoring area, have a wide geographical range, be possible to differentiate between airborne and soil-borne heavy metals, be easy to sample and there should be no identification problems. *Fraxinus excelsior* embodies all these criteria and our study fully supports the view that it can be a useful biomonitor as a significant linear regression was obtained for each of the metals Pb, Cd, Cu, Zn, Ni and Cr between the concentrations of the element in surface soil and in the washed leaves of the plant and because it is possible to measure the differences between aerial depositions and root uptakes. This is supported by the findings of Seaward and Mashhour [24], Al-Shayeb et al. [3] and Aksoy and Öztürk [4, 5].

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