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Research Note

Robinia pseudo-acacia L. as a Posssible Biomonitor of Heavy Metal Pollution in Kayseri

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Abstract : The leaves of *Robinia pseudo-acacia* L. *(Fabaceae)* were tested as a possible biomonitor of heavy metal pollution in Kayseri. Concentrations of Pb, Cd, Cu and Zn were determined in unwashed and washed leaves and soils collected from a wide range of sites with different degrees of metal pollution. Differences between the unwashed and washed samples varied according to the metal pollutant levels. Significant correlations were obtained between the heavy metal concentrations in surface soil and washed leaf samples. *R. pseudo-acacia* was found to be a useful biomonitor of the heavy metals investigated.

Key Words: *Robinia pseudo-acacia*, biomonitor, heavy metal pollution, Kayseri.

Robinia pseudo-acacia L. 'nın Kayseri'de Ağır Metal Kirlenmesinde Biyomonitör Olarak Kullanılması

Özet : Bu çalışmada *Robinia pseudo-acacia* L. *(Fabaceae)* bitkisinin yaprakları, Kayseri'de (Türkiye) ağır metal kirlenmesinin olası biyolojik monitörü olarak araştırıldı. Geniş ve farklı metal kirlenme düzeyinde olan alanlardan alınan yıkanmış ve yıkanmamış bitki örnekleri ve toprak örneklerindeki Pb, Cd, Cu ve Zn konsantrasyonu belirlendi. Yıkanmış ve yıkanmamış bitki örnekleri arasında metal kirlenme düzeyleri farklılıklar gösterdi. Yıkanmış bitki yapraklarında ve yüzey topraklarındaki ağır metal konsantrasyonları arasında istatiksel olarak önemli bir ilişki elde edildi. *R. pseudo-acacia*'nın araştırılan ağır metallerin kullanışlı bir biyolojik monitörü olduğu bulundu.

Anahtar Sözcükler: *Robinia pseudo-acacia, biomonitor*, ağır metal kirlenmesi, Kayseri.

Introduction

Botanical materials such as fungi, lichens, tree bark, tree rings and leaves of higher plants, have been used to detect the deposition, accumulation and distribution of metal pollution. Lower plants, especially mosses and lichens, in view of their higher capacity for metal accumulation, are probably the organisms most frequently used for monitoring metal pollution in urban environments (1-3). The past few decades have seen an increase in the use of higher plant leaves as biomonitors of heavy metal pollution in the terrestrial environment (4-6). Al-Shayeb et al., (2), for example, used date palm leaflets to monitor the distribution of airborne Pb, Zn, Cu, Ni, Cr and Li in the city of Riyadh in Saudi Arabia. A comparison of washed and unwashed samples showed that leaflet analyses gave a reasonably reliable measure of the total aerial fallout of heavy metal in the study area. Such studies have focused on evergreen plants rather than decidious trees. The latter, however, have the potential advantage that they could enable monitoring of short-term or yearly changes in pollution.

Robinia pseudo-acacia L. *(Fabaceae)* can survive under a wide temperature range and grows in almost any type of soil. In the city of Kayseri, Turkey, it is a typical urban deciduous tree up to 25 m. This plant is native in North America. It is commonly planted for ornament and for stabilizing dry soil and is extensively naturalized around Europe, Asia and Africa (7). It usually occurs along roadsides in industrial, urban parks and agricultural areas, even under high levels of urban pollution in Turkey.

Kayseri is located in Central Anatolia in Turkey. It is a rapidly growing city and its present population is estimated to be around 978, 927, compared with 403, 861 in 1950. The city enjoys dry hot summers and cold winters. Heavy metals are emitted into the Kayseri environment from different sources, i.e., transportation, industrial activities, fossil fuels, agriculture and other human activities (8,9).

The aim of this study was to determine Pb, Cd, Cu and Zn concentrations in surface soil and in unwashed and washed leaves of *R. pseudo-acacia,* which was tested as a possible biomonitor of heavy metal pollution in Kayseri.

Materials and Methods

Site selection: Plant and soil samples were taken from different sampling sites. Preferred urban sites for sampling were the most crowded parts of the city centre. Roadside sites were chosen along the Kayseri-Kırsehir highway at the 25th km. The traffic density of this road was estimated to be 285 vehicles per hour. Samples from the industrialised area were taken from different places between 0-10 m around a single but very large zinc producing industrial establishment (Çinkur Plant), which is nearly 22 km west of the city centre. Suburban sites were chosen from the edge of the city which is a shanty area. For uncontaminated controls, samples were collected from Ali Dagi, about 25 km south-east of the city of Kayseri.

Sample collection and preparation: Samples of plants and soils were collected from different sites during August 1997, the number of sites from each category sampled being as follows: industrial=8, roadside=12, urban=14, suburban=10 and rural area (as control)=8. 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 through a 2 mm sieve. About 200 g (fresh weight) of well developed leaves of *R. pseudo-acacia* were selected and collected. Plant samples were then divided into two sub-samples. One sub-sample was thoroughly washed with running distilled water to remove dust particles, the other remained untreated. All plant samples were oven-dried at 80 °C for 24 hours, milled in a micro-hammer cutter and fed through a 1.5 mm sieve.

Analytical techniques: The method adopted from Berrow and Ure (10) and Paveley and Davies (11) for analysis of heavy metal concentrations in soil samples involved aqua regia digestion. One gram samples of dried and sieved soil materials were ashed in a muffle furnace at 460 °C for 24 hours. The weighed ash was digested in 10 ml aqua regia (1 part concentrated HNO_3 to 3 parts HCl) in a digestion tube on a heating block for a total of 9 hours in the following sequence and duration of temperatures: two hours each at 25 °C, 60 °C and 105 °C, and finally three hours at 125 °C. All digested samples were centrifuged, then made up to volume with 1 % HNO_3.

The method used for plant digestion is the same as described earlier by Al-Shayeb et al.(2). One gram samples of dried and ground plant material were ashed in a muffle furnace at 460 °C for 24 hours. The weighed ash was digested in concentrated HNO_3 and evaporated to near dryness on a hot-plate. Digested samples were centrifuged, then made up to volume with 1 % HNO_3.

Concentrations of the heavy metals, Pb, Cd, Cu and Zn were measured in soil and plant samples by an atomic absorption spectrophotometer (Perkin Elmer model 1100). In order to ascertain the accuracy of the method employed and calibrate for any slight contamination, a reference material was used with every batch (SRM 1547 peach leaves).

The standard error values of the means were calculated for a comparison of site categories. To determine the significance of washing of the leaves, a paired t-test was performed, comparing heavy metal contents of washed and unwashed plants, for each type of site. Significance of comparison of means by ANOVA (F-test) are indicated. Relationships between variables were assessed using linear regression and correlation analyses.

Results and Discussion

The mean concentrations of heavy metals (Pb, Cd, Cu and Zn) found in unwashed and washed leaves of *R. pseudo-acacia* in different sites are presented in Tables 1 and 2. The mean heavy metal concentrations in industrial sites and urban roadsides are slightly higher than in urban and suburban sites, and significantly higher than in rural sites in washed and unwashed leaves.

Table 1. Mean Pb and Cd concentrations (μgg^{-1} dry weight) in leaves of *R. pseudo-acacia* collected from different sites of Kayseri, together with standard error of the mean. Significance of means by ANOVA (F-test) and comparison of washed and unwashed leaves by paired t-test are indicated. (Key:*** p<0.001, **p<0.01 and * p<0.05 significance).

	Pb			Cd		
Site	Unwashed	Washed	T-test	Unwashed	Washed	T-test
Industry	176.88 ± 12.2	62.42 ± 3.45	***	3.39 ± 0.14	1.22 ± 0.10	***
Roadside	74.46 ± 9.1	33.65 ± 3.30	***	1.34 ± 0.08	0.65 ± 0.08	***
Urban	48.96 ± 7.8	27.02 ± 3.01	***	1.12 ± 0.11	0.61 ± 0.08	***
Suburban	26.67 ± 6.2	21.04 ± 2.42	**	0.77 ± 0.07	0.58 ± 0.05	**
Rural	15.98 ± 1.9	14.89 ± 2.28	*	0.47 ± 0.05	0.44 ± 0.03	*
F- test	***	***		***	***	

Table 2. Mean Zn and Cu concentrations (μgg^{-1} dry weight) in leaves of *R. pseudo-acacia* collected from different sites of Kayseri, together with standard error of the mean. Significance of means by ANOVA (F-test) and comparison of washed and unwashed leaves by paired t-test are indicated. (Key:*** p<0.001, **p<0.01 and * p<0.05 significance).

	Zn			Cu		
Site	Unwashed	Washed	T-test	Unwashed	Washed	T-test
Industry	242 ± 11.10	98 ± 3.81	***	29.12 ± 2.54	14.04 ± 1.47	***
Roadside	80 ± 9.00	40 ± 2.76	***	22.98 ± 2.32	12.21 ± 1.32	***
Urban	67 ± 8.06	36 ± 2.75	***	18.56 ± 2.22	10.48 ± 1.14	***
Suburban	35 ± 5.08	26 ± 2.36	**	12.96 ± 1.30	8.96 ± 1.01	**
Rural	21 ± 1.66	19 ± 1.23	*	8 ± 1.11	7.32 ± 0.86	*
F- test	***	***		***	***	

The mean concentrations of heavy metals in the soils supporting *R. pseudo-acacia* in the same areas are presented in Table 3. The mean Pb, Cd, Zn and Cu concentrations in industrial sites are slightly higher than in the roadside, urban and suburban sites, and significantly higher than in rural sites in soil. Similar observations were made by Aksoy and Sahin (12) while studying *Elaeagnus angustifolia* as a biomonitor of Pb, Cd and Zn in Kayseri. They found higher levels of heavy metals in the industrial, roadside and urban areas.

The highest pollution levels were found in the samples taken from the industrial sites. It may be concluded that the Çinkur plant is a large source of pollution for its surroundings. This plant produced significant levels of metallic Zn, Cd, Cu and Pb pollution, due to a lack of a filter system in its chimneys. Certainly, a filter system for the flues of the plant might reduce these levels and is thus necessary to bring down this load of heavy metal pollution considerably. The residuals of processed mines accumulated around the factory is the second source of

Table 3. Mean Pb, Cd, Zn and Cu concentrations (μgg^{-1} dry weight) in soils collected from different sites of Kayseri, together with standard error of the mean. Significance of comparison of means by ANOVA (F-test) is indicated. (Key:*** p<0.001 significance)

Site	Pb	Cd	Zn	Cu
Industry	468± 18.60	9.88 ± 0.43	1189 ± 19.31	79 ± 5.12
Roadside	141 ± 13.03	2.05 ± 0.32	166 ± 15.02	36 ± 4.04
Urban	120 ± 10.98	1.68 ± 0.24	137 ± 11.04	30 ± 3.68
Suburban	70± 7.44	1.20 ± 0.22	106 ± 8.85	16 ± 4.42
Rural	39 ± 3.15	0.64 ± 0.13	63 ± 4.14	11 ± 2.16
F-test	***	***	***	***

pollution. Spreading the residuals by wind erosion to the environment causes pollution in the soil and plants. Pollution could be prevented by storing those residual mines (12). Kartal et al.(9) studied soil pollution levels of six elements around the Çinkur plant. They reported that Pb, Cd and Zn pollution was very high, originating from the zinc ore used in the factory. The heavy metal levels decrease with an increase in the distance from the factory.

The high heavy metal content in roadside and urban soils and plant samples is mostly due to the density of the traffic, which is considered one of major sources of heavy metal contamination, because unleaded gasoline is expensive and drivers prefer leaded gasoline. In Turkey, this results in high Pb pollution alongside the roads. This is also a major source of heavy metal pollution in the city of Kayseri. Kartal et al., (8) studied Pb, Ni, Cd and Zn pollution of traffic in Kayseri. They found a good correlation between the numbers of cars and the heavy metal contents.

Washing the leaves significantly reduced the Pb concentrations in *R. pseudo-acacia* from all sites (as indicated by t-test results). A comparison of the amount of metal extracted from unwashed leaves with that from washed leaves (Table 4), shows that removal of the metals from the leaves by washing was significantly different; for example, 7-65 % of the Pb was removed by the washing procedure, depending on the pollutant level at the sampling sites. The ability to distinguish airborne and soil borne contamination was assessed by washing the leaves. The results given in Table 4 indicate that there was substantial aerial deposition on the leaves of all four elements, which were removed by washing.

Table 4. Total percentage of Pb, Cd, Zn and Cu removal from the leaves of *R. pseudo-acacia* through washing procedure. Significance of comparison means by ANOVA (F-test) are indicated (Key: *** $p<0.001$ significant).

Sites	Pb	Cd	Zn	Cu
Industrial site	65	64	61	52
Roadside	55	52	50	47
Urban	45	46	47	45
Suburban	21	25	26	30
Rural	7	6	9	8
F-test	***	***	***	***

A least squares linear regression was obtained for each of the metals, Pb, Cd, Zn and Cu, between concentrations of the element in surface soils and in the washed leaves of *R. pseudo-acacia*. Table 5 shows the values of the correlation coefficient (r) for each heavy metal. A perusal of the table shows that Zn, Cd and Cu are all highly significant at $p<0.001$. Lead is significant at $p<0.01$. However, lead is less mobile than the other metals determined by us, thus, lower values are obtained. It can therefore be inferred that with an increase in the amount of heavy metals in soil due to percolation, the uptake of heavy metals by *R. pseudo-acacia* also increases. Aksoy and Şahin (12) investigated Elaeagnus angustifolia as a biomonitor of heavy metal pollutions in Kayseri. They reported that correlations between various elements in washed leaves and soils were highly variable (e.g., Pb=0.401, Cd=0.665 and Zn=0.901).

Table 5. Relationships between heavy metal concentration in surface soil and the washed leaves of *Robinia pseudo-acacia* (Key: r, correlation coefficient; **, $p<0.01$ ***, $p<0.001$ significant).

Plant	Element	Sample number	r
	Pb	52	0.415**
R. pseudo-acacia	Cd	52	0.678***
	Zn	52	0.812***
	Cu	52	0.769***

Lead is generally added to the environment by aerial deposition alongside the roads in proportion with the density of traffic and distance from the roadside. Sawidis et al. (13) 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. Tam et al.(14) used surface soil in a survey of roadside heavy metal contamination in Hong Kong and found a significant correlation between traffic density and Pb, Cu and Zn concentrations. Lead is less mobile than Cd and Zn, although uptake from the soil can raise foliar Pb concentrations, and an uptake of Pb through the root system has been demonstrated under greenhouse conditions as well. In the field most uptake has been demonstrated to be through the leaves (15).

Table 6 presents a comparison of the heavy metal concentrations in the literature considered toxic or contaminated (16) with the values from this study. According to the Ross criteria, the concentration of heavy metals in *R. pseudo-acacia* did not exceed the upper limit. However, all sites showed values whose upper limit was higher than the minimum levels of contamination in Ross. However, in soils the upper values of Pb, Cd and Zn were higher than the upper levels of contamination in Ross. These high concentrations may be due to aerial deposition of heavy metals over a long time period.

According to Markert (1) and Wittig (17), the 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, should be able to differentiate between airborne and soil borne heavy metals, be easy to sample, inexpensive to sample and there should be no identification problems. *R. pseudo-acacia* embodies all these criteria and our study fully supports the view that it can be a useful biomonitor all through Turkey, the U.S.A., Asia, Europe and Africa, because a highly significant linear regression was obtained for each of the metals Pb, Cd, Zn and Cu between concentrations of the element in surface soil and in the washed leaves of plants.

Table 6. Comparison of heavy metal concentrations ($\mu g g^{-1}$ dry wt) considered toxic or contaminated, taken from the literature (adapted from Ross, 1994), with values from this study

Element	Concentrations in soil considered toxic	Concentration in contaminated plants	Present results	
			Soil	Plants
Pb	100-400	30-300	3.15-468	15.98-177
Cd	3-8	0.03-3.8	0.14-9.88	0.05-3.39
Zn	70-400	100-400	4.14-1189	21-242
Cu	60-125	20-100	2.16-79	8-29.12

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