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CHICORY *(CICHORIUM INTYBUS* L.): A POSSIBLE BIOMONITOR OF METAL POLLUTION

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Abstract

Leaves of *Cichorium intybus* L., (Chicory) were tested as a possible biomonitor of heavy metal pollution in Kayseri, Turkey. Forty-five sites (industrial, urban, roadside, suburban and rural) in and around Kayseri were investigated. The mean heavy metal concentrations in industrial site and roadside were non-significantly higher than the urban, suburban sites, significantly higher than rural sites in washed and unwashed leaves of *C. intybus*. 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. The mean Pb, Cd, Zn and Cu concentrations in industrial site were non-significantly higher than the roadside, urban and suburban sites, significantly higher than rural sites in soil. Significant correlations were obtained between the heavy metal concentrations in surface soil and washed leaf samples. *C. intybus* was found to be a useful biomonitor of the investigated heavy metals.

Introduction

During the last few decades, heavy metal contamination of biotic component of environment has attracted the attention of many investigators. The main reason of these researches based on the heavy metal concentration may have a potential hazard in our food chain after a long period of procrastination.

Using biological materials in the determination of environmental pollution as indicators is a cheap and reliable method. 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 (Markert, 1993; Al-Shayeb *et al*., 1995 & Aksoy *et al*., 1999). During the past few decades there has been an increase in the use of higher plant leaves as biomonitor of heavy metal pollution in the terrestrial environment (Aksoy & Ozturk, 1996, 1997; Aksoy & Demirezen, 2006). Aksoy *et al*., (1999), for example, used *Capsella bursa-pastoris* to monitor the distribution of airborne Pb, Zn, Cu and Cd in the city of Bradford in England. The comparison of washed and unwashed samples of leaves gave a reasonably high measure of the total aerial fallout of heavy metal in the studied area.

Cichorium intybus L., (Chicory) belongs to the family Asteraceae. It can survive under a wide temperature range and any type of soil. It is a world-wide species and commonly found in Kayseri, Turkey. This plant is a native of Europe, W. Asia and N. Africa (Davis, 1975). It usually occurs along roadside in industrial territories, urban parks, wasteland, grassland and agricultural areas, even under higher levels of urban pollution in Turkey.

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Kayseri is located in the Central Anatolia in Turkey. It is a rapidly growing city and its present population is 1, 060, 432, compared to 403, 861 in 1950. The city enjoys terrestrial climate with dry hot summers and cold winters. Heavy metals are emitted into the Kayseri environment from different sources; transportation, industrial activities, fossil fuels, agriculture and other human activities (Kartal *et al*., 1993; Aksoy *et al*., 2000).

Cichorium intybus was selected as a possible biomonitor of heavy metal pollution, particularly in urban areas, such as along roadsides, because of its widespread occurrence from urban to rural areas, its wide geographical range throughout the world, and easiness to sample, grow and cultivate it.

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

Materials and Methods

Site selection: Plant and soil samples were taken from different sampling sites. The sampling sites were selected based on previous studies (Aksoy *et al*., 1999; Aksoy & Sahin 1999) where the heavy metal pollution is in different levels. Preferred urban sites for sampling were the most crowded parts of the city centre. Roadside sites were chosen along the Kayseri-Kırsehir highway at 15^{th} km. The traffic density of this road was estimated to be 326 vehicles per hour. Samples from industrialised area were taken from different places between 0-10 m around single but very large zinc producing industrial establishment (Çinkur Plant) which is nearly 22 km away to the west of the city centre. Suburban sites were chosen from the edge of city which is under a shanty development. For uncontaminated controls, samples were collected from Ali Dagi, about 25 km south-east of Kayseri.

Sample collection and preparation: Samples of plants and soils were collected from different sites during June, 1998. The number of sites from each category have sampled as follows: industrial site=9, roadside=14, urban=16, suburban=10 and rural area (as control)=6. 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 *C. intybus* 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 ^{o}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 & Ure (1981) and Paveley & Davies (1988) 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 ^{o}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 the heating block for a total of 9 hours, in the following sequence and duration of temperatures: two hours each at 25^{o}C, 60^{o}C and 105^{o}C, and finally three hours at 125^{o}C. All digested samples were centrifuged, then made up to volume with 1% HNO_3.

The method used for plant digestion is same as described earlier by Al-Shayeb *et al*., (1995). One gram samples of dried and ground plant material were ashed in a muffle furnace at 460ºC 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 upto 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) is 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 *C. intybus* in different sites are presented in Tables 1 and 2. The mean heavy metal concentrations in industrial site and roadside are non-significantly higher than the urban, suburban sites, significantly higher than rural sites in washed and unwashed leaves.

The mean concentrations of heavy metals in the soils supporting *C. intybus* in the same areas are presented in Table 3. The mean Pb, Cd, Zn and Cu concentrations in industrial site are non-significantly higher than the roadside, urban and suburban sites, significantly higher than rural sites in soil. Similar kinds of this observation were made by Aksoy *et al*., (2000) while studying *Robinia pseudo-acacia* as a biomonitor of Pb, Cd, Cu and Zn in Kayseri. They found higher levels of heavy metals in the industry, roadside and urban area.

The highest pollution levels were found in the samples taken from the industrial sites. It may be concluded that, Çinkur Plant is a great pollution source for its surroundings. This plant produced significant levels of metallic Zn, Cd, Cu and Pb pollution, due to lack of a filter system in its chimneys. This plant has been closed but the residuals of processed mines accumulated around the factory are the main source of pollution. Spreading residuals by wind erosion to the environment cause pollution in the soil and plants. Pollution could be prevented by storing those residual mines (Aksoy & Sahin, 1999). Kartal *et al*., (1993) studied soil pollution level for six elements around Çinkur plant and reported that Pb, Cd and Zn pollution was very high which originates from the zinc-ore used in the factory. The heavy metal levels decrease gradually with increase distance from the factory.

The high Pb, Cd and Zn content in roadside and urban soils and plant samples is mostly due to the density of the traffic which is considered as one of the major sources of heavy metal contamination. As unleaded gasoline is expensive and drivers prefer leaded gasoline in Turkey, a high Pb pollution occurs along the roadsides. The latter one is also a major source of heavy metal pollution in Kayseri. Kartal *et al*., (1992) studied Pb, Ni, Cd and Zn pollution of traffic in Kayseri. They found a meaningful correlation between the numbers of cars and the heavy metal contents.

Table 1. Mean Pb and Cd concentrations (μgg^{-1} dry weight) in leaves of *C. intybus* collected from different sites of Kayseri, together with standard error of the mean.

Site	Pb			Cd		
	Unwashed	Washed	T-test	Unwashed	Washed	T-test
Industry	103.21 ± 10.4	61.82 ± 3.01	***	2.79 ± 0.13	1.35 ± 0.09	***
Roadside	70.22 ± 8.2	34.32 ± 2.88	***	1.38 ± 0.08	0.63 ± 0.08	***
Urban	46.54 ± 8.1	24.08 ± 2.36	***	1.20 ± 0.12	0.66 ± 0.06	***
Suburban	27.02 ± 5.8	21.06 ± 2.56	**	0.80 ± 0.09	0.60 ± 0.06	**
Rural	17.12 ± 2.1	15.64 ± 2.21	*	0.45 ± 0.07	0.42 ± 0.04	*
F- test	***	***		***	***	

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).

Table 2. Mean Zn and Cu concentrations (μgg^{-1} dry weight) in leaves of *C. intybus* collected from different sites of Kayseri, together with standard error of the mean.

Site	Zn			Cu		
	Unwashed	Washed	T-test	Unwashed	Washed	T-test
Industry	233 ± 9.10	149 ± 3.24	***	30.08 ± 2.54	20.40 ± 1.05	***
Roadside	82 ± 8.00	40 ± 2.46	***	24.45 ± 2.32	12.24 ± 1.17	***
Urban	70 ± 8.06	38± 3.01	***	19.20 ± 2.22	10.88 ± 1.08	***
Suburban	37 ± 5.68	29 ± 2.10	**	12.50 ± 1.30	9.12 ± 0.96	**
Rural	20 ± 2.02	18 ± 0.99	*	8.20 ± 1.11	7.79 ± 0.68	*
F-test	***	***		***	***	

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).

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.

Site	Pb	Cd	Zn	Cu
Industry	430± 15.0	8.24 ± 0.34	1068 ± 14.12	75 ± 4.20
Roadside	138 ± 11.25	2.15 ± 0.40	164 ± 13.10	34 ± 3.04
Urban	118 ± 9.38	1.65 ± 0.31	132 ± 10.60	29 ± 3.54
Suburban	69± 5.12	1.18 ± 0.20	103 ± 7.11	15 ± 2.11
Rural	40 ± 3.75	0.63 ± 0.10	60 ± 5.18	10 ± 1.68
F-test	***	***	***	***

Significance of comparison of means by ANOVA (F-test) is indicated. (Key:*** *P*<0.001 significance).

Washing the leaves significantly reduced the Pb concentrations in *C. intybus* from all sites (as indicated by t-test results). A comparison of the amount of metal extracted from unwashed and washed leaves (Table 4), shows that removal of the metals from the leaves by washing is significantly different; for example 5-54 % of the Pb is 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 substantial aerial deposition on the leaves for all four elements are removed by washing procedure.

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 *C. intybus*. 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 $p<0.001$, except Pb which is also significant at $p<0.01$. However, latter is less mobile than other metals determined by us, as such, lower values are obtained. It can be inferred that with an increase in the amount of heavy metals in soil due to percolation, the uptake of heavy metals by *C. intybus* also increases. Aksoy *et al*., (2000) investigated *Robinia pseudo-acacia* 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=0.415, Cd=0.678, Zn=0.812 and Cu=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*., (1995) studied air pollution with heavy metals in Thessaloniki city (Greece) using trees as biological indicators and reported that high levels of heavy metals came from vehicular emissions. Tam *et al*., (1987) conducted surface soil 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; an uptake of Pb through the root system has been demonstrated under greenhouse conditions as well. In the field most uptakes have been demonstrated to be through the leaves (Kope, 1981).

Table 6 presents a comparison of the toxic heavy metal concentrations already reported in literature (Ross, 1994) with the values recorded at this study. According to Ross criteria, the concentration of heavy metals in *C. intybus* did not exceed the upper limit. However, all sites show values whose upper limit is higher than the minimum levels of contamination reported by Ross (1994). Several trace metals are essential micronutrients for plant and animal growth. Tyler (1981) suggests that general requirements of Cu, Zn, Fe and Mn for plant growth are around 1-100 μgg^{-1} dry biomas. The upper values of Pb, Cd and Zn are higher than the upper levels of contamination in soils according to Ross (1994). These high concentrations may be due to aerial deposition of heavy metals over a long time period.

Some basic criteria for the selection of a species as a biomonitor are: it should be represented in large numbers all over the monitoring area; it should have a wide geographical range; it should be able to differentiate between airborne and soil borne heavy metals; it should be easy to sample and there are no identification problems and inexpensive to sample. *Cichorium intybus* embodies all these criteria and our study fully supports the view that it can be a useful biomonitor throughout Turkey, W. Asia, Europe and Africa, because a highly significant linear regression is 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 plant.

It would suggest that *C. intybus* can be used to monitor polluted sites, and regular monitoring of metal levels on, as well as in, leaves could be used as an inexpensive and simple biomonitor of atmospheric metal pollution.

The leaves of *C. intybus* are used in salad and tea in Turkey. For the above reason, it is strongly recommended that plants growing alongside roadways, industry and urban sites should be washed before eating or otherwise not be eaten.

Table 4. Total percentage of Pb, Cd, Zn and Cu removed from the leaves of *C. intybus* through washing procedure.

Sites	Pb	Cd	Zn	Cu
Industrial site	40	41	36	32
Roadside	56	54	51	50
Urban	48	45	46	43
Suburban	22	24	22	27
Rural	8	7	10	5
F-test	***	***	***	***

Significance of comparison means by ANOVA (F-test) are indicated (Key: *** P<0.001 significant).

Table 5. Relationships between heavy metal concentration in surface soil and the washed leaves of *C. intybus*.

Plant	Element	Sample number	*r*
	Pb	55	0.435**
C. intybus	Cd	55	0.690***
	Zn	55	0.848***
	Cu	55	0.803***

(Key: r, correlation coefficient; **, P<0.01 ***, P<0.001 significant).

Table 6. Comparison of heavy metal concentrations (μg 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	37-435	14-107
Cd	3-8	0.03-3.8	0.58-23	0.36-2.95
Zn	70-400	100-400	58-1078	17-242
Cu	60-125	20-100	9.7-77	7.48-32

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