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Flame Atomic Absorption Spectrometric Determination of Pb, Cd, and Cu in *Pinus nigra* L. and *Eriobotrya japonica* Leaves Used as Biomonitors in Environmental Pollution

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Abstract The assessment of trace metal pollution in Gaziantep city-Turkey has been studied using plant leaves of *Pinus nigra* L. and *Eriobotrya japonica* as biomonitor. The concentrations up to 3,056 mg Pb kg^{-1} in the needles of *Pinus nigra* L., and 367 ng Cd g^{-1} in the leaves of *Eriobotrya japonica* were determined. The observed Cu concentrations were in range of 1.6–7.1 mg kg^{-1}. The Pb, Cd, and Cu levels in soils were determined to be in the range of 17–602, 0.142–0.656, and 12–38 mg kg^{-1}, respectively. It was concluded that *Pinus nigra* L. can be considered as both biomonitor of atmospheric Pb pollution and hyperaccumulator plant.

Keywords *Pinus nigra* L. · *Eriobotrya japonica* · Atomic absorption · Metals

Lead and cadmium are among the most determined elements in environmental and biological matrices due to their high toxicity for humans and animals. Interest in Cu determination in environmental and biological samples has been originated from both its necessity as a component of more than 30 enzymes, and its adverse effects on human health at high concentrations (Yaman 2006). It was reviewed that the accumulation of Pb, Cd and Cu in human body can cause middle and long-term health risks and adversely affects the physiological functions (Flora 2002; Nordberg 2004; IARC 1993). The populations of, at least, 100 countries have been still exposed to considerable lead levels owing to air pollution despite the banned of lead in gasoline (Flora 2002; Papanikolaou et al. 2005). Due to its malleability, low melting point, and ability to form compounds, Pb is used in hundreds of products such as pipes, solder, brass fixtures, crystal, paint, cable, ceramics, and batteries. Although maximum tolerable intake of cadmium recommended by the World Health Organization (WHO 1996) is 70 µg Cd/day, the advised intake is 2–25 µg Cd/day for children and 15–50 µg Cd/day for adults. However, the exposure to these metals even at small concentrations can cause significant effects over the long term since Pb and Cd accumulate in the body. Human's exposure to Cd includes employment in primary metal industries, production of certain batteries, some electroplating processes, and use of tobacco products. Furthermore, it was reported by the International Agency Research on Cancer (IARC) that Cd can cause lung cancer in humans and animals through inhalation (IARC 1993). Consequently, humans have been exposed to Pb, Cd and Cu through food and air which are polluted by environmental conditions and industrial emissions. In order to measure/monitor and to assess the extent of pollution, a high quality database is required. Biomonitoring is one of the indicators for environmental pollution, and it includes trace element analysis of plants and similar biological matrices. Lead and Cd absorption from food by ingestion are between 3% and 10% in humans, and their absorption increases depending on special conditions such as low dietary Ca, low Fe stores and Fe deficiency during pregnancy. The biomonitoring has, particularly, high importance in the case of air pollution because the absorption rates of lead and cadmium by inhalation are significantly higher (up to 50%–60%) than those by ingestion described above (Mulgrew and Williams 2000).

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However, there are fewer studies available on Pb and Cd determinations in air samples compared to other environmental matrices such as soil and water due to, probably, the difficulties in obtaining air samples and the excessively low concentrations of these metals in aerial matrix. In order to overcome the difficulties in obtaining of air sample and lower metal concentrations than the sensitivities of analysis methods, there have been increased attentions to the usage of plant parts such as leaves, shoots and barks as biomonitoring (Bargagli 1998; Singh et al. 2005; Markert 1993; Kaya and Yaman 2008a). Furthermore, the sources of emissions can be identified, and the overland transportation of individual elements can be verified by using biomonitor plants. Although intensive industrial activities and rapid urbanization in the past three decades cause dramatic increase in the emission of pollutants in Gaziantep, there are fewer studies to monitor atmosphere pollution in this region (Kaya and Yaman 2008a, b).

In this study, plant and soil samples were collected from industrial areas in Gaziantep-Turkey. Concentrations of Pb, Cd and Cu in the plant species grown in this city were determined to examine biomonitor and/or hyperaccumulator plants. For this purpose, amounts of those metals were analyzed in soils and leaves of plants interested by using flame atomic absorption spectrometry (FAAS).

Materials and Methods

The concentrations of Pb, Cd, and Cu were determined using a Model ATI UNICAM 929 flame atomic absorption spectrophotometer (Unicam Ltd., Cambridge, England). The optimum conditions in FAAS for lead, cadmium and copper were applied as: wavelength: 217.0, 228.8 and 324.8 nm; HCL current: 9.5, 4.0 and 3.0 mA; acetylene flow rate: 0.6, 0.6 and 0.5 L min^{-1}, respectively, and air flow rate: 4.0 min^{-1} and slit width: 0.5 nm for three metals. A domestic microwave oven (MW) (Kenwood) was used for digestion of the leaves. A slotted tube atom trap (STAT) made from quartz (Quarzschmelze Ilmenau, Gmbh, Germany) was used to enhance the sensitivity of FAAS. The applied conditions for the quartz tube were taken from elsewhere (Yaman 2005; Kaya and Yaman 2008b). To assess the reliability of measurements, some samples were analyzed by PerkinElmer SCIEX ELAN® 9000 inductively coupled plasma mass spectrometer (ICP-MS) (PerkinElmer SCIEX, Concord, ON, Canada). The operation conditions for this instrument were applied as recommended by the manufacturers. The metal stock solutions (1,000 mg L^{-1}) were prepared from their nitrate salts (Merck, Darmstadt, Germany). Nitric acid (65%, Merck) and hydrogen peroxide (35%, Merck) were used for sample digestion. All chemicals used were of analytical reagent grade. Doubly distilled water (Millipore direct Q 3) was used for all preparations. All glass apparatus (Pyrex) were kept permanently filled with 1 mol L^{-1} nitric acid when not in use.

The representative locations, in surrounding lead battery-production plant and cement factory around a city (Gaziantep) of 1,200,000 population in SE Turkey, and uncontaminated areas, were chosen for this study. The Sampling was conducted in the summer of 2006. The samples studied including *Pinus nigra* L., *Eriobotrya japonica* (Thunb.) Lindl., *Armeniaca* sp. and *Pinus sylvestris* L., were planted in years ago. The healthy looking leaves (about 100 g fresh plant) were taken from three trees for per site. The soil samples were also obtained in depth of 10.0 cm and distance of 2.0 m around the sampled trees. The plants were transferred to the laboratory in plastic bags, washed with tap water, and then rinsed with distilled water. After drying procedure at 70°C, the samples were ground with agate mortar and then homogenously mixed. 0.2–1.0 g of the samples was transferred into Pyrex flasks and digested by using dry and/or microwave (MW) ashing methods. Each of the samples was analyzed in triplicate and the average of three values was used as a mean value. The dry ashing (at 480°C for 4 h) and microwave methods described elsewhere (Kaya and Yaman 2008a) were applied for digesting plant and soil samples.

Results and Discussion

The recoveries of Pb, Cd and Cu from the plant leaves fortified with these elements were used to check the accuracy. The concentrations of Pb, Cd, and Cu added to the samples were in the range of 5–20, 0.05–0.1, and 1–2 mg kg^{-1}, respectively. It was found that at least 94% of Pb, Cd, and Cu added to the plant leaves were recovered. Some samples were also analyzed by ICP-MS to assess the accuracy. It was observed that there was no significant difference between the data obtained from FAAS and those obtained from ICP-MS using the *t*-test at a confidence level of 90% (Table 1). Furthermore, the accuracy of the method was checked by examining the Standard Reference Material-Tomato Leaves-1573a. The recoveries for Cd and Cu in Tomato Leaves-1573a were found to be at least 96% and 98%, respectively. Related to determination of the best digestion procedure, it was found that there is no significant difference between the data obtained from the both methods namely dry and microwave ashing (considering *t* test at confidence level of 90%) as shown in Table 2. Although the losses of Pb and Cd in dry ashing were reported for some matrices such as fruits and vegetables, no losses of Pb and Cd in this study by using dry ashing can be attributed to the differences in matrices (Yaman and Gucer 1995).

Table 1 FAAS determination of metals in plant leaves taken from different points around battery manufacturing plant (n = 3)

No.	Sampling point	Soil			*Pinus nigra* L. (ICP-MS)			*Eriobotrya japonica* (ICP-MS)		
		Pb (mg kg^{-1})	Cd (ng g^{-1})	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Cd (ng g^{-1})	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Cd (ng g^{-1})	Cu (mg kg^{-1})
1	Area of 50 m around battery	602 ± 65	444 ± 45	37 ± 5	3,056 ± 360	57 ± 7	6.2 ± 0.8	195 ± 26	283 ± 30	6.4 ± 1.1
2	200 m from battery south	415 ± 36	623 ± 58	38 ± 6	257 ± 46 (243 ± 41)	52 ± 10 (60 ± 8)	7.1 ± 0.7 (7.3 ± 0.4)	171 ± 18 (172 ± 21)	208 ± 23 (200 ± 25	5.2 ± 0.4 (5.9 ± 0.8)
3	200 m from battery SE	191 ± 23	344 ± 32	24 ± 4	166 ± 22	55 ± 6	5.6 ± 0.7	158 ± 17	257 ± 28	5.3 ± 0.5
4	200 m from battery north	261 ± 22	142 ± 6	31 ± 4	23 ± 5 (19 ± 3)	32 ± 6 (28 ± 4)	4.5 ± 0.8 (5.0 ± 0.5)	NA	NA	NA
5	200 m from battery west	227 ± 25	156 ± 14	27 ± 3	69 ± 10	68 ± 8	5.4 ± 0.9	NA	NA	NA
6	300 m from battery west	193 ± 20	172 ± 16	24 ± 4	37 ± 5	61 ± 10	8.0 ± 1	NA	NA	NA
7	300 m from battery NW	92 ± 9	284 ± 25	20 ± 4	52 ± 9	79 ± 11	6.1 ± 1	NA	NA	NA
8	800 m from battery North	27 ± 3	656 ± 69	21 ± 2	13 ± 2	126 ± 17	2.9 ± 0.5	NA	NA	NA
9	1,500 m from battery NW	29 ± 3	236 ± 20	23 ± 2	5.3 ± 1	68 ± 13	2.8 ± 0.5	NA	NA	NA
10	400 m from cement plant	42 ± 5	323 ± 29	20 ± 3	10 ± 1	88 ± 10	3.5 ± 1	10 ± 2	176 ± 26	2.8 ± 0.5
11	Uncontaminated (control) area away from plant	17 ± 2	268 ± 26	12 ± 1	0.7 ± 0.1 (0.7 ± 0.1)	36 ± 5 (40 ± 5)	1.6 ± 0.2 (1.8 ± 0.2)	1.7 ± 0.2	123 ± 15	1.8 ± 0.3

The values in parenthesis were obtained by ICP-MS

Concentrations in leaves of *Armeniaca* sp. plant taken from point of 1 were found to be 97 mg Pb kg^{-1}, 20 ng Cd g^{-1}, and 5 mg Cu kg^{-1}

NA not available

Table 2 FAAS determination of metal levels in the studied plants taken from relatively uncontaminated sites (around textile industry) by using dry ashing method

No.	Pb mg kg^{-1}	Cd ng g^{-1}	Cu mg kg^{-1}
1 *Pinus nigra* L.	0.7 ± 0.1	76 ± 15	2.3 ± 0.3
2 *Pinus nigra* L.	0.8 ± 0.2	41 ± 9	3.3 ± 0.6
3 *Pinus nigra* L.	1.2 ± 0.2	47 ± 8	4.1 ± 0.1
4 *Pinus nigra* L.	1.4 ± 0.2	99 ± 6	2.6 ± 0.5
5 *Pinus nigra* L. (MW)	1.8 ± 0.3 (2.0 ± 0.2)	36 ± 6 (41 ± 4)	1.8 ± 0.2 (2.1 ± 0.2)
6 *Pinus nigra* L. (MW)	2.0 ± 0.3 (2.2 ± 0.2)	70 ± 11 (66 ± 8)	1.6 ± 0.1 (1.7 ± 0.2)
7 *Pinus nigra* L.	2.2 ± 0.2	62 ± 8	3.5 ± 0.4
8 *Eriobotrya japonica*	2.3 ± 0.2	367 ± 44	4 ± 0.6
Pinus sylvestris L. taken 800 m far from battery plant	6.6 ± 0.6	45 ± 3	3.2 ± 0.5

n = 3

The values in parenthesis were obtained by using microwave oven

Levels of Pb, Cd and Cu in the reagent blanks in the total analytical steps were, respectively, found to be 8.0, 0.5 and 3.5 ng mL^{-1} with standard deviations (s) of 1.0, 0.1 and 0.7 ng mL^{-1}. Therefore, the limit of detection, defined as three times of the *s* values of blanks, was calculated as 3.0, 0.3 and 2.1 ng mL^{-1}. The effects of contamination were eliminated by subtracting the obtained values from the blank. In order to overcome the enhancement or suppression due to the presence of major components such as calcium and magnesium of the plant matrix, calibration solutions were performed within the sample matrix itself. On the other hand, standard additions method for Pb, Cd and Cu was also applied to the plant samples to figure out possible matrix interferences. The obtained equations from the standard additions and calibration graphs by using STAT-FAAS are as follows:

$$Y = 0.268X + 0.5$$
$$R^2 = 0.9998 \text{ for Pb calibration (between } 30 - 200\,\text{ng mL}^{-1})$$

$$Y = 0.262X + 11$$
$$R^2 = 0.9996 \text{ for Pb standard addition,}$$

$$Y = 1.74X + 0.5$$
$$R^2 = 0.9999 \text{ for Cd calibration (between } 4 - 40\,\text{ng mL}^{-1})$$

$$Y = 1.7X + 39.5$$
$$R^2 = 0.9993 \text{ for Cd standard addition,}$$

$$Y = 0.314X$$
$$R^2 = 0.9998 \text{ for Cu calibration (between } 30 - 200\,\text{ng mL}^{-1})$$

$$Y = 0.312X + 11.6$$
$$R^2 = 0.9995 \text{ for Cu standard addition.}$$

The slopes of the calibration curves were compared with the slopes obtained from the standard additions method. There was no difference in the slopes of both methods. These results indicate the absence of chemical interferences, because the slopes of the calibration curves and standard additions are identical for the studied elements. Therefore, the calibration graphs were used to determine Pb, Cd, and Cu in the samples.

Concentrations of Pb, Cd, and Cu in the leaves of plants are given in Tables 1 and 2. It can be seen that the Pb concentrations are in range of 0.7–3,056.0 μg/g (dw) for *Pinus nigra* L. and 1.7–195.0 μg/g (dw) for *Eriobotrya japonica* in the studied locations. This large variation may be depending on the uncontaminated and the highly polluted areas from the lead battery factory as well as plant species. In the literature (Singh et al. 2005), it was described that Pb may enter into the leaves through translocation of precipitated atmospheric Pb along with accumulation through urban air, particularly in the summer season. As a result, the authors generally determined the metal concentrations in plants to evaluate atmospheric pollution (Aboal et al. 2004; Rossini Oliva and Mingorance 2006; Sawidis et al. 2001). Aboal et al. (2004) examined oak leaves and pine needles as biomonitors of airborne trace element pollution in rural areas throughout Galicia, Spain. They found the following metal concentrations in *P. pinaster*: 0.016–0.121 (mean 0.059) mg Pb kg^{-1}, 0.033–0.187 (mean 0.085) mg Cd kg^{-1}, and 0.34–5.38 (mean 3.52) mg Cu kg^{-1}. Rossini Oliva and Mingorance (2006) observed that the Pb and Cu concentrations in *Pinus nigra* grown in contaminated site were between 0.05–23.4 mg Pb kg^{-1} and 8.8–172 mg Cu kg^{-1} while the Pb and Cu levels in *P. pinea* grown in background sites were found to be between 4.28–5.82 mg Pb kg^{-1} and 3.59–3.74 mg Cu kg^{-1}. Sawidis et al. (2001) studied metal distribution from lignite fuels using trees as biological monitors. They found that Cd and Cu concentrations in *Pinus nigra* were between 0.89–1.72 mg Cd kg^{-1} (0.46 for control) and 1.5–3.1 mg Cu kg^{-1} (0.8 for control). Cicek and Koparal (2004) determined trace heavy metals in soil and tree leaves surrounding the Tuncbilek Thermal Power Plant in Kutahya, Turkey, to assess environmental impact. They found the Pb, Cd, and Cu concentrations in the studied plants including *Salix* sp., *populus nigra* L., *Robinia pseudoacacia* L., and *Pinus nigra* in the range of 0.1–55.0 mg kg^{-1} for Pb, 0.1–7.2 mg kg^{-1} for Cd, and 2.1–59.0 mg kg^{-1} for Cu.

Table 1 shows that the Pb concentrations in the needles of *Pinus nigra* L. taken from around the battery factory are higher than 3,000 mg kg^{-1} (0.3%). These concentrations are extremely high when compared to the values described above for similar plants as well as the plants growing around the cement factory and uncontaminated fields sampled for this study. These data can be attributed to aerial accumulation of Pb by *Pinus nigra* L. As shown in Table 1, statistically significant relationships were observed in the Pb concentrations of the *Pinus nigra* L. needles depending on distance from the polluted area and/or wind direction. The extremely high Pb concentrations up to 3,056 mg kg^{-1} in the *Pinus nigra* L. plant (taken at 50 m from the battery manufacturing plant) decreased sharply to about 250 mg Pb kg^{-1} at a 200-m distance. The decrease in Pb concentration in plant leaves (Table 1) depends not only on the distance of the sampling point from the contaminated area but also seems to be related to the wind direction. In the studied area, the predominant wind direction is mainly in the North–South directions. As a result, significantly higher Pb concentrations were found in all samples (including soil and *Pinus nigra* L. plants) when sampled from a southern direction rather than the northern. Particularly, Pb absorption by the *Pinus nigra* L. plant varies significantly depending on wind direction. As a result, the accumulation of Pb in *Pinus nigra* L. leaves is not only related to soil pollution but also to air pollution. Mertens et al. (2005) concluded that foliage sampling and analysis is a useful tool for studying the effects of metals on ecosystems and for monitoring atmospheric pollution, but it has little value for the straightforward biomonitoring of soil pollution. The observed metal concentrations in the plant samples including *Pinus nigra* L., *Eriobotrya japonica*, *Armeniaca* sp., *Spruce* (*Picea*), *Cedrus libani* and *Pinus sylvestris* L. collected from different points are given in Tables 1 and 2. In respect to the first view of biomonitoring definition: “10-times higher metal concentrations in biomonitor plants in comparing with the other plants taken from the same sampling point”, *Eriobotrya japonica* can be used as biomonitor of atmospheric Cd pollution because Cd concentration in this plant was found higher 14 times than in the *Armeniaca* sp. leaves (20 ng g^{-1}) grown in the same location (Table 1). With respect to the second view of biomonitoring definition that “metal concentration in biomonitor plant grown in a polluted media is, at least, 10-times higher than in the same plant grown in an unpolluted media, the needles of *Pinus nigra* L. can be used as a biomonitor for Pb (Tables 1, 2). It can be seen that the Pb levels emitted from the battery plant can influence the plants far beyond 300 m because higher Pb levels than the phytotoxic limit (30 mg kg^{-1}) were found in the plants taken at longer distances (Table 1). The Pb concentration in the *Pinus nigra* L. taken from an uncontaminated area was 0.7 mg kg^{-1}, and the corresponding value for the cement factory (a relatively uncontaminated area) was 10 mg kg^{-1}. Lead concentrations in soil samples collected at a distance of 300 m from the battery plant were higher than the limit levels for agricultural purposes. The Cd and Cu concentrations in the studied soil and plant samples were found to be lower than the allowable limits since there are no industrial sources in the region that emit these two metals. However, the observed slightly higher Cu concentrations in some leaves taken from industrial areas including the battery factory and the cement plant (Tables 1, 2) can be attributed to the incineration of tires and personal metallic activities.

The Pb levels in *Pinus nigra* L. needles grown around a battery manufacturing plant were found over 3,000 times greater than in plants grown around uncontaminated areas. It can, therefore, be concluded that *Pinus nigra* L. needles can be used as a biomonitoring-tool of Pb for air pollution studies. Furthermore, *Eriobotrya japonica* leaves have a potential for use in biomonitoring because the observed Cd level in this plant grown around a battery plant are 14 times higher than in the *Armeniaca* sp. leaves grown in the same site. Finally, it was observed that there is no significant difference between the data obtained from FAAS and ICP-MS methods using *t* test at confidence level of 90% for the metals of interest (Table 1).

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