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PYRACANTHA COCCINEA ROEM. (ROSACEAE) AS A BIOMONITOR FOR CD, PB AND ZN IN MUGLA PROVINCE (TURKEY)

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Abstract

This study was firstly aimed to find out the measures of Cd, Pb and Zn as the heavy metal pollution in Mugla Province, and secondly if *Pyracantha coccinea* Roem. (firethorn) can be used as a biomonitor and phytoremediation. *P. coccinea* samples were collected from 34 localities in four different areas of Mugla Province, during vegetation period in 2006. The Cd, Pb, and Zn concentrations of unwashed and washed leaf samples and unwashed branch samples were measured by ICP-OES. As a result of measurements, the average highest value of Cd accumulation has been reached in unwashed leaf samples, which were collected near highways. The value was 0.36±0.60 µg g^{-1} dw. The lowest value was detected as 0.16±0.04 µg g^{-1} dw, in washed leaf samples which were found in industrial area. The average highest value of Pb (14.93±1.23 µg g^{-1} dw) was determined in the branch samples collected in industrial area whilst the lowest (5.60±1.47 µg g^{-1} dw) were in washed leaf samples collected in industrial area too. The highest Zn (15.57±1.66 µg g^{-1} dw) was measured in the branch samples collected in city center, whilst the lowest was measured in the branch samples (8.99±1.32 µg g^{-1} dw) collected in city center too. It was proven that *P. coccinea* can be used as a biomonitor species for these heavy metals. It was also observed that this species could be used for phytoremediation procedure.

Introduction

Environmental pollution with toxic metals has increased significantly in the last decades as a result of industrial revolution, and causing serious ecologic problems. Soil pollution with heavy metals, such as cadmium, lead and zinc and their mobility in the environment threatens to introduce these metals into life cycle, a danger for all living organisms, and an important problem, which must be solved in the future (Badora, 2002; Yılmaz *et al*., 2006).

The term biomonitor [Greek bios (βίος) = life and Latin monitor = anything displays viewable objects] is defined as an organism that provides quantitative information on the quality of the environment around it. Therefore, a good biomonitor will indicate the presence of the pollutant and also attempt to provide additional information about the amount and intensity of the exposure (Wolterbeek, 2002). With proper selection of organisms, the general advantage of the biomonitoring approach is related primarily to the permanent and common occurrence of the organism in the field, even in remote areas, the ease of sampling, and the absence of any necessary expensive technical equipment (Wittig, 1993; Wolterbeek, 2002).

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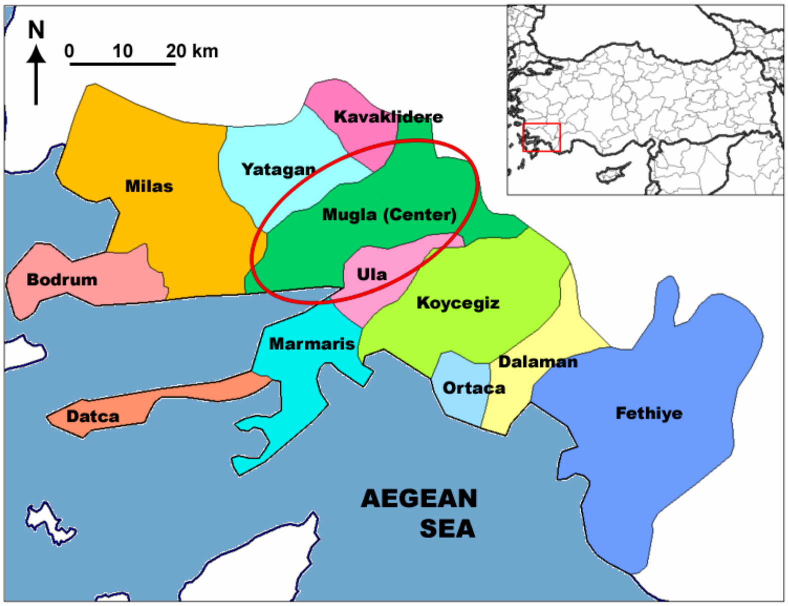

Figure 1: Mugla Province, its administrative districts and location in Turkey.

The use of biological materials for monitoring heavy metal air pollution was introduced 30 years ago. Since then, numerous biomonitors have been used to monitor heavy metal pollutions (Augusto *et al.,* 2007). These include mollusks, fishes, birds, cyanobacteria, lichens, mosses, and many parts of plants (tree barks, tree rings, pine needles, grasses and leaves) (Lovett *et al.*, 1997; Aksoy & Öztürk, 1997; Sakurai *et al.*, 2000; Augusto *et al*., 2007; Aksoy, 2008; Atiq-Ur-Rehman and Iqbal, 2008) Some plant species of these groups have more ability of accumulating high levels of metals and other toxic elements, without showing any visible injury, such as leaf necroses and discolorations. These are then denominated accumulator or biomonitor plants (De Temmerman *et al.*, 2004).

Mugla is a city, which locates in the south-west part of Turkey (37° 12' N, 28° 21' E) near the Aegean Sea. The coastline of the Mugla City is the longest among the Provinces of Turkey and longer than many countries' coastlines with 1,100 km (683 miles). The city is divided into 12 administrative districts (Figure 1) (http://www.mugla-bld.gov.tr). It is also a rapidly growing city and its present population is estimated to be around 766 156 in whole city and 94 207 in Mugla City Center. The economy of Mugla relies mainly on tourism (on the coast), and agriculture, forestry and marble quarries in the inland. Agricultural activities in Mugla are rich and varied; the province is one of Turkey's largest producers of honey, pine-forest honey in particular, and citrus fruits are grown in Ortaca, Fethiye, Dalaman and Dalyan districts of Mugla. The province is the second center of marble industry in Turkey after Afyon in terms of quantity, variety and quality. Other mines including coal in Yatağan and chrome in Fethiye are very important for Turkey's economy. The other industries in the province include SEKA Paper Mill in Dalaman and Yatagan Power Stations (Acar, 1998).

The aim of present study is to look into the pollution levels of Cd, Pb and Zn, by using *Pyracantha coccinea* Roem. (Firethorn) as bioindicator in Mugla Province. For numerous reasons *P. coccinea* is selected as a biomonitor of heavy metal pollution. It grows easily in both urban and rural areas of various geographical ranges. Its ecological distribution can be found throughout the Europe, and it can be easily sampled, identified and cultivated and inexpensive (Wittig, 1993, Juhásová et al, 2004).

Materials and Methods

Shrub up to 3 m high; young twigs grey-pubescent; spines leafless. Leaves lanceolate, elliptic or obovate elliptic, 2-4 (-5) x 1-1.5 (-2) cm, crenate-serrate, pubescent beneath, especially when young, or entirely glabrous; petioles 5-10 mm. Corymbus many-flowered. Flowers up to 8 mm diameter; pedicels 5 mm. Fruit globose, 5-7 mm diameter, red or occasionally yellow orange. Flowers between April-June. Limestone slopes, sand dunes, in open woodland and scrub, 30-1800 m.

P. coccinea is distributed especially North, Central and South Anatolia in Turkey and South Europe, Crimea, Caucasia, North-West Iran in the world; Wild in Turkey, especially near the Black Sea Coast; also cultivated for its ornamental berries (Pyracantha). Several cultivars and least three wild varieties have been described (incl. *P. coccinea* Roem var. *kuntayi* Kasapligil in Bauhinia 1, 2:124, 1958). Most of these refer to minor variants, differing in the color of the fruits or in leaf pubescence, not considered further here (Davis, 1972).

The plant samples were collected from different sites of Mugla Province in September 2006 (Figure 1). In total, 34 plant samples were cut, and then they were labeled and kept in the envelopes. The localities were categorized as follows: Urban (twelve stations), Industrial Areas (eight stations), Highway (eight stations), and Control (six Rural stations). The samples were especially taken from the sides, which are faced to the asphalts. Leaf samples were divided into two sub-samples; one of them was thoroughly washed with running deionized distilled water to remove dust particles in a standardized procedure, and rest of the leaves and the branch samples were untreated. All plant samples were owen-dried at 80 ^{o}C for 24 h, milled in micro-hammer cutter and fed through a 1.5-mm sieve. Plant samples were then stored in clean self-sealing plastic bags in silica gel.

Dried and milled plant samples were measured as 200 g and put into a 100 ml Erlenmeyer and then six ml of mixture, which had 1:4 ratios included 65 % nitric acid (HNO_3) and 70 % perchloric acid ($HClO_4$) added in it and then they were incubated in hot water in 52 ^{o}C at least two hours. After two hours, the Erlenmeyers included solved samples in the acid mixture were put on the hot-plate and the organic materials and the acids were evaporated from the solution. The rest of the solutions were completed to 25 ml by deionized distilled water and the new solutions were stored in glass bottles.

Concentrations of Cd, Pb and Zn in the leaf and branch samples were then measured by ICP-OES. The standard error values of the means were calculated for comparison of site categories.

Results and Discussion

Cadmium: The mean cadmium concentrations in leaves (washed and unwashed) and branches (unwashed) of *P. coccinea* are shown in figure 2-A. As a result of measurements, the average highest value of Cd accumulation has been reached in unwashed leaf samples which were collected near highway. The value was 0.36±0.60 µg g^{-1} dw. The lowest value was detected (Excluding Control-Rural area) as 0.16±0.04 µg g^{-1} dw in washed leaf samples which were collected in industrial area. Moreover, it was observed that the Cd concentrations reached to the highest values in unwashed leaf samples in all stations. In addition, control (rural) area's Cd concentrations are as follows; 0.23±0.05 µg g^{-1} dw in unwashed leaves, 0.15±0.04 µg g^{-1} dw in washed leaves and 0.22±0.04 µg g^{-1} dw in unwashed branches. For all stations, the second highest values were in branches whilst the lowest in washed leaves. It is accepted that the normal limits of Cd concentrations in plants are between 0.2-0.8 µg g^{-1} dw and between 5-30 µg g^{-1} dw is accepted as toxic values (Bowen, 1979; Kabata-Pendias & Pendias, 1986). According to these values, the Cd concentrations in this study were within normal limits. Moreover, Cd values were closer to each other in both unwashed leaf and branch samples and it was seen that washing the leaves significantly reduced the Cd concentrations (Figure 2-A).

Some researchers carried out similar studies in Turkey, obtained different results by means of different plant taxa. Aksoy & Öztürk (1997) carried out a study in Antalya (a city in Turkey) by using *Nerium oleander* and they found out that minimum and maximum limits were respectively 0.21-0.72 µg g^{-1} dw in unwashed leaves, and 0.2-0.49 µg g^{-1} dw in washed leaves. Aksoy *et al.* (2000) found out Cd amount between 0.47-3.39 µg g^{-1} dw in unwashed leaves and between 0.44-1.22 µg g^{-1} dw in washed leaves in their study realized in Kayseri (a city in Turkey) by using *Robinia pseudoacacia* as a biomonitor. Bayçu *et al.* (2006) carried out a similar study by using *Acer* sp. as a biomonitor in Istanbul (0.32 µg g^{-1} dw). A similar study was carried out in Kayseri and *Elaeagnus angustifolia* was used (Aksoy & Şahin, 1999). The results of that study were between 0.50-3.45 µg g^{-1} dw for the unwashed leaves.

The above Cd results obtained in our study have a broad agreement with the results obtained in Antalya (*Nerium oleander*) and in Istanbul (*Acer* sp.). However, the Cd values in Kayseri (*Robinia pseudoacacia* and *Elaeagnus angustifolia*) were higher than the results in our study. The reason for these high emissions could be due to a big factory called Çinkur (Zinc-Lead Metal Industry) in Kayseri. This factory produces metallic Cd, Pb and Zn which caused pollution due to lack of filter systems in its chimneys (Aksoy & Şahin, 1999).

The results obtained in Mugla by Tuna *et al.* (2005) for Cd were lower than the results in our study (*Pinus* sp-0.23 µg g^{-1} dw and *Olea europaea*-0.28 µg g^{-1} dw). Having the fact that *P. coccinea* is a better biomonitor than *Pinus* and *Olea europaea* for Cd can explain the differences of the above results. However, different parts of the same area can be the cause for the diversion of the results in Mugla.

In our study, the highest value of Cd was found near highway. As it is known, abrasion of the tires of the vehicles and wastes of the oils of the diesel engines cause the Cd pollution in highways (Lagerwerff & Specht, 1970). In addition, the manures including phosphate are also one of the most important sources of Cd (Adriano, 1986).

There are also some similar studies carried out in other countries. Palmieri *et al.* (2005) studied by using *Pittosporum tobira* (0.49 µg g^{-1} dw-unwashed leaves) and Calzoni *et al.* (2007) by using *Rosa rugosa* (0.05-0.1 µg g^{-1} dw-unwashed leaves) in Italy. Madejon *et al.* (2006) studied by *Quercus ilex* (0.13-0.25 µg g^{-1} dw -unwashed leaves) and *Olea europaea* (0.02-0.11 µg g^{-1} dw -unwashed leaves) in Spain. The Cd results obtained in these studies have a broad agreement with the results obtained with our study. Madejon *et al.* (2004) studied by *Populus alba* in New Zealand (3.82 µg g^{-1} dw -unwashed leaves). However, these values are ten times more than our results and this situation could be related with the Cd uptaking capacity of *Populus alba*.

Lead: The mean Pb concentrations in both types of (unwashed and washed) leaves and branches (unwashed) are shown in figure 2-B. In this study, the findings of Pb values are as follows; the maximum amount was in the branch samples in the industrial area in Mugla (14.92±1.22 µg g^{-1} dw) and the minimum Pb amount was in the washed leaf samples in the industrial area (5.60±1.47). In addition, for all stations, the second highest values were in unwashed leaf samples and lowest were in washed leaf samples. Literature indicates that, the normal limits of Pb are 0.1-10 µg g^{-1} dw and the amounts between 30-300 µg g^{-1} dw are toxic levels for plants (Kabata-Pendias & Pendias, 1986). As it is seen above, our values are accepted normal. It was also observed that, washing the leaves reduced the Pb concentrations like it happens in Cd, but the reduction was less than Cd's reduction (Figure 2-A-B).

Some scientists in Turkey carried out similar studies by means of some other plant taxa and different results were obtained. Aksoy & Öztürk (1997) carried out a study in Antalya by using *Nerium oleander* and they found out minimum and maximum limits as 2.65-28 µg g^{-1} dw in unwashed leaves and 2.4-16.5 µg g^{-1} dw in washed leaves. Aksoy *et al.* (2000) found out Pb amount between 15.98-176.88 µg g^{-1} dw in unwashed leaves and between 14.89-62.42 µg g^{-1} dw in washed leaves in their study that realized in Kayseri by using *Robinia pseudoacacia* as a biomonitor. Çelik *et al.* (2005) were realized a biomonitoring study in Denizli by using *Robinia pseudoacacia* as well. Their results were quite high (between 132.2-139 µg g^{-1} dw). Bayçu *et al.* (2006) carried out a similar study by using *Acer* sp. as a biomonitor in Istanbul (12.41 µg g^{-1} dw). A similar study was carried out in Kayseri and *Elaeagnus angustifolia* was used (Aksoy & Şahin, 1999). The results of that study were between 16.81-180.21 µg g^{-1} dw for the unwashed leaves. Elik & Akçay (2000) used *Pinus sylvestris* and *Robinia pseudoacacia* as biomonitors for Pb in Sivas (a city in Turkey). The mean results were consequently 32 and 39 µg g^{-1} dw.

The Pb results obtained in this study have a broad agreement with the results obtained in Antalya (*Nerium oleander*) and Istanbul (*Acer* sp.) like Cd values with these cities and taxa. However, the Pb results in Kayseri (*Robinia pseudoacacia* and *Elaeagnus angustifolia*), in Denizli (*Robinia pseudoacacia*) and Sivas (*Pinus sylvestris and Robinia pseudoacacia*) were higher than our study.

Kayseri, Denizli and Sivas are affected by strong heavy metal pollutant factors. For instance, Kayseri has a high traffic density and Pb processing factories. However, Denizli is a very big industrial city and Sivas has a very big cement factory. These factors could be the cause of the higher Pb concentrations in these cities. Another reason could be decreasing of leaded petrol in vehicles in the last decade, and the other studies were carried out earlier times than our study. Today, the usage of leaded petrol in vehicles is prohibited in Turkey.

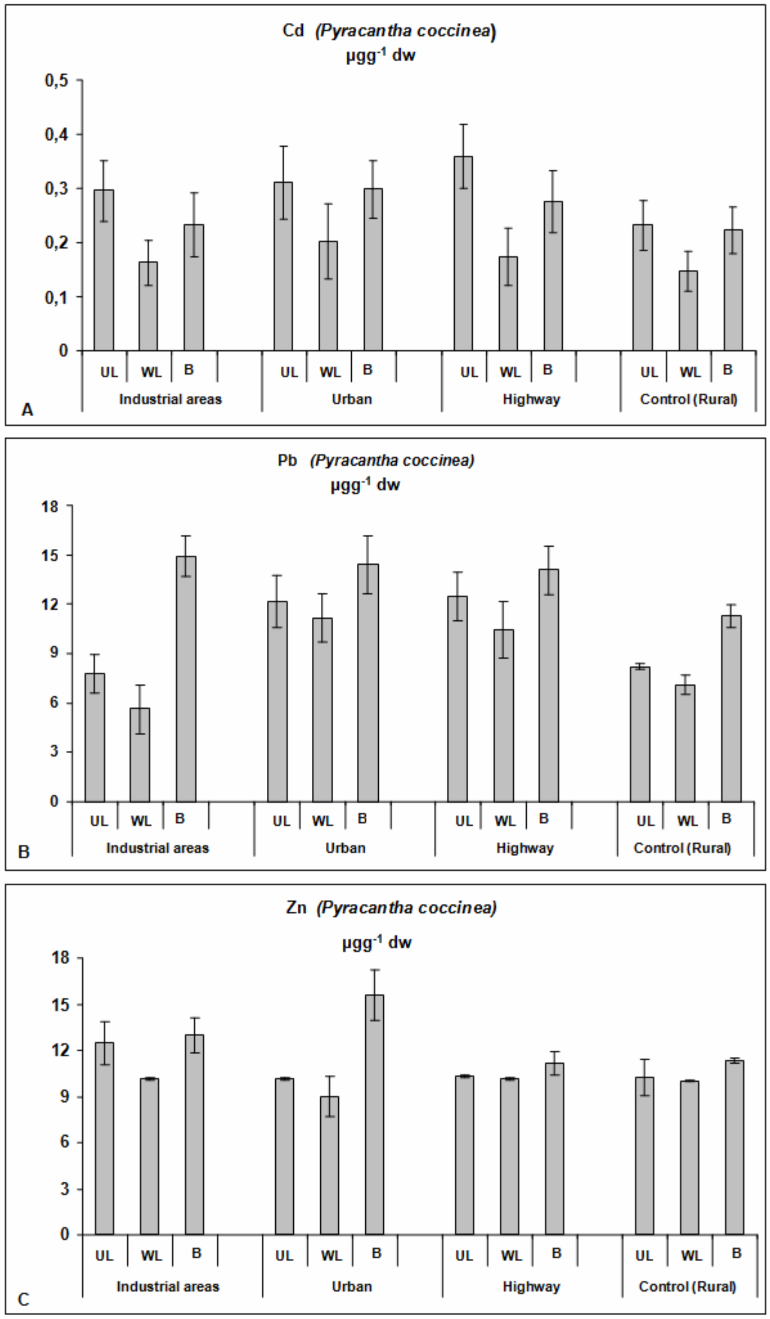

Figure 2: Mean Cd (A), Pb (B) and Zn (C) concentrations in branches, washed and unwashed leaves as µg g^{-1} dw together with S.E. bars. B: Branch, UL: Unwashed leaves, WL: Washed Leaves.

Tuna *et al.* (2005) used *Pinus* sp. and *Olea europaea* as biomonitor for Pb in Mugla. The values were 6.4 and 8.3 respectively. The differences of the results can be explained as the absorption capacity of *Pyracantha coccinea* for Pb is higher than *Pinus* and *Olea europaea.* In addition, locality differences in Mugla Province, decrease of the usage of leaded petrol in vehicles could be other reasons for this reduction.

In addition, Madejon *et al.* (2006) studied by *Quercus ilex* (4.7-20.5 µg g^{-1} dw -unwashed leaves) and *Olea europaea* (1.63-24.2 µg g^{-1} dw -unwashed leaves) in Spain. Our values are within these limits. Palmieri *et al.* (2005) studied by using *Pittosporum tobira* (4.1 µg g^{-1} dw-unwashed leaves) and Calzoni *et al.* (2007) by using *Rosa rugosa* (1.0-9.0 µg g^{-1} dw-unwashed leaves) in Italy. Madejon *et al.* (2004) studied by using *Populus alba* (5.0 µg g^{-1} dw-unwashed leaves) in New Zealand. Our Pb values are higher than later three studies.

The Pb values in New Zealand (*Populus alba*) and in Italy (*Pittosporum tobira* and *Rosa rugosa*) were lower than the Pb values in this study. The earlier usage of the unleaded oils in New Zealand and Italy can be the cause of these lower values.

Zinc: Zinc amounts in washed and unwashed leaves and branches (unwashed) of *P. coccinea* in four different types of localities are shown in figure 2-C. The results were as follows: the highest Zn (15.57±1.66 µg g^{-1} dw) was measured in the branch samples collected in city center, whilst the lowest was measured in the branch samples (8.99±1.32 µg g^{-1} dw) collected in city center too. The second highest values were found in unwashed and washed leaves. There was not much difference between the levels of highway and control-rural areas (Figure 2-C).

In the literature, it is accepted that the normal limits of Zn concentrations in plants are between 8-400 µg g^{-1} dw (Allaway 1968). Between 100-400 µg g^{-1} dw is accepted as toxic values in plants (Kabata-Pendias & Pendias, 1986). According to these values, in this study, the Zn concentrations are within normal or lower limits.

There are also some similar studies by using of different plant taxa as biomonitors in Turkey. In a study, Aksoy & Öztürk (1997) used *Nerium oleander* as possible biomonitor for Zn and they found out minimum and maximum limits were 8-21 µg g^{-1} dw in unwashed leaves and 7-14.8 µg g^{-1} dw in washed leaves in Antalya. In another study, Aksoy *et al.* (2000) found out that the Zn amount were between 21-242 µg g^{-1} dw in unwashed leaves and between 19-98 µg g^{-1} dw in washed leaves in Kayseri with *Robinia pseudoacacia*. Bayçu *et al.* (2006) also carried out a similar study by using *Acer* sp. as a biomonitor in Istanbul (30.06 µg g^{-1} dw). Çelik *et al.* (2005) were used *Robinia pseudoacacia* as a possible biomonitor in Denizli. Their results were between 15.11-206.2 µg g^{-1} dw. A similar study was carried out in Kayseri by Aksoy & Şahin (1999) and *Elaeagnus angustifolia* was used as a biomonitor. The results of that study were between 22.08-231.26 µg g^{-1} dw with unwashed leaves and 20.14-102.1 with washed leaves.

In our study, the Zn results obtained have a broad agreement with the results obtained in Antalya (*Nerium oleander*) and in Istanbul (*Acer* sp.) like Cd and Pb values with these cities and taxa. However, the Zn results of unwashed leaves in Kayseri (*Robinia pseudoacacia* and *Elaeagnus angustifolia*), and Denizli (*Robinia pseudoacacia*) were too much higher than our study, even their minimum levels were higher than our Zn levels.

In our study, the highest value of Zn was found in urban areas. As it is known, in urban areas, there are traffic density and abrasion of the tires of the vehicles that contain

ZnO and wastes of the oils of the diesel engines cause the Zn pollution in roadside (Vallee & Falchuk 1993). Kayseri and Denizli are industrial cities and affected by strong heavy metal pollutant factors. For instance, Kayseri has traffic density and Zn processing factories. These factors could be the most important factors for the higher Zn concentrations in these cities.

Tuna *et al.* (2005) used *Pinus* sp. and *Olea europaea* as biomonitor for Zn in Mugla. Their values were 25.8 for *Pinus* sp. and 21.9 for Olea *europaea.* However, their results were higher than our results. This could be due to the usage of different plants with different absorption levels and different localities.

There are also some similar studies carried out in other countries. Palmieri *et al.* (2005) by using *Pittosporum tobira* in Sicily, Italy. Their results were 129 µg g^{-1} dw in unwashed leaves and 128 µg g^{-1} dw in washed leaves. Although these results were higher than our Zn results, the closeness of Zn amounts between the unwashed and the washed leaves' in our study showed similarity with the study of Palmieri *et al.* (2005). In addition, Madejon *et al.* (2006) studied by *Quercus ilex* (44-113 µg g^{-1} dw -unwashed leaves) and *Olea europaea* (22.5-82.22 µg g^{-1} dw unwashed and 42 µg g^{-1} dw washed leaves) in Spain. In our study, the average concentrations of Zn in washed and unwashed leaves are closer to each other. Madejon *et al.* (2004) studied by using *Populus alba* (542.1 µg g^{-1} dw-unwashed leaves) in New Zealand. Their average Zn concentrations were 40 times higher than our average Zn concentrations.

The Zn values in New Zealand (*Populus alba*), in Italy (*Pittosporum tobira*) and in Spain (*Quercus ilex* and *Olea europaea*) are higher than our Zn values. It can be said that the higher Zn emission was due to the developed industries and industrial pollutants in those countries. In addition, those four plant species could be better biomonitors for Zn than *P. cocinea* with their wider leaves.

The concentrations of those three elements (Cd, Pb and Zn) show, as expected, an increase in their concentrations with increasing urbanization and industry. The distinguishing ability between airborne and soil-borne contamination was assessed by washing the leaves with deionized distilled water. The results of figures 2-A-B-C indicate that there was substantial aerial deposition on the leaves for all three elements, which was removed by the washing procedure. However, Cd showed the highest reduction and then Pb and Zn respectively. In addition, for all three elements, the concentrations of washed leaves are the lowest, while the highest are for Cd in unwashed leaves and for Pb and Zn in unwashed branches. High heavy metal levels in branches could be the result of being long time organs and having thorny structures on *P. coccinea*.

The results obtained in our study also reflect the differences in sources and mode of heavy metal uptake by plants. Pb has been added to the environment by aerial deposition alongside roads in proportion to the volume of traffic and close distance from the road (Tam *et al.*, 1987; Aksoy *et al.,* 1999). As it is known, Pb is less mobile than Cd and Zn and its uptake from the soil can raise foliar Pb concentrations. The uptake of Pb through the root system was demonstrated in a greenhouse conditions, in the field; Pb uptake was demonstrated through the leaves (Motto *et al.*, 1970; Aksoy *et al.,* 1999). The high proportion of Pb that was removed from the leaves by washing would suggest that *P. coccinea* would be very responsive to Pb uptake through the air. In contrast, Cd and Zn are elements, which actively taken up by the roots, easily transported within plants, and distributed to all plant organs (Kabata-Pendias & Pendias, 1986).

In conclusion, this study supports the view that *Pyracantha coccinea* Roem. could be used as a biomonitor for Cd, Pb and Zn heavy metals, especially with its branches and leaves.

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