Use of *Pyracantha coccinea* Roem. as a possible biomonitor for the selected heavy metals

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ABSTRACT: In this study, copper, iron, manganese and nickel levels of branches and leaves of *Pyracantha coccinea* Roem. (firethorn) were measured for determining the heavy metal pollution in Mugla Province. Plant samples were collected from 34 different localities in four different areas of Mugla Province, during 2006 vegetation period. Unwashed and washed leaf samples and unwashed branch samples were subjected to analysis and copper, iron, manganese and nickel concentrations of samples were measured by inductively coupled plasma optical emission spectrometry. The obtained data were analyzed with "statistical package for the social sciences" statistics program. As a result of measurements, the highest average and lowest values and their collected stations were as follows; the highest average $(5.89 \pm 0.04$ µg/g dw) and the lowest $(5.20 \pm 0.03$ µg/g dw) values of copper were measured near highways. The average highest iron value $(9.53 \pm 1.68$ µg/g dw) was in industrial area while the lowest was near highways $(1.73 \pm 0.54$ µg/g dw). The highest value of magnesium accumulation $(1.00 \pm 0.16$ µg/gdw) was measured near highways. The lowest value was determined in urban area $(0.40 \pm 0.14$ µg/g dw). The average highest level of nickel was in industrial area while the lowest was determined in urban area. The values were 14.34 ± 1.59 µg/g and 4.05 ± 0.51 µg/g dw. As a result, it was proven that *P. coccinea* could be used as a biomonitor species for some of these heavy metals especially for copper and nickel.

Keywords: *Biomonitor; Firethorn; Heavy metal; Mugla*

INTRODUCTION

A great quantity of heavy metals and other chemicals, especially produced by industries, agriculture, mining, combustion of fossil fuels and traffic are often released to the atmosphere, soil and water. They aggressively manifested on terrestrial and aquatic flora and fauna (Celik *et al*., 2005; Okafor and Opuene 2007; Mahvi, 2008; Yasar *et al*., 2010). Heavy metals easily accumulate in large quantities in plant tissues with no visible phytotoxicity, but exceed human and animal tolerance (Kovalchuk *et al*., 2001; Prasad and Freitas, 2003). For years, both tolerable and lethal dose of toxic compounds for different plants have been established and in some cases, the modification of chemical composition of the plants under the action of pollutants has been observed (Kabata-Pendias and Pendias, 2001; Nameni *et al.,* 2008).

The use of biological materials to measure environmental effects of atmospheric deposition was first reported by Nylander (1886). Since then, his report

on the epiphytic lichens of Paris and its surroundings has been performed in many areas (Wolterbeek, 2002). In the past few decades, an increasing use of higher plant leaves as biomonitors for heavy metal pollution has been seen in the environment and especially in urban areas (Aksoy *et al*., 1999; Yilmaz *et al.*, 2006; Yasar *et al.*, 2010). Today, not only the vegetal sources (lichens, mosses, tree barks, tree rings, pine needles, grasses and leaves) but also many other living organisms (mollusks, fishes, birds and *cyanobacteria*) are being used as biomonitors to detect deposition, accumulation and distribution of metal pollution (Sakurai *et al.*, 2000; Figueiredo *et al.*, 2007; Akguc *et al.*, 2008; Aksoy, 2008; Yasar and Ozyigit, 2009). Although there are many types of biomonitors, lower plants, especially mosses, algae and lichens are the most frequently used organisms for monitoring metal pollution in urban environments since they have a higher metal accumulation capacity (Aksoy and Ozturk, 1997; Augusto *et al*., 2007; Akguc *et al.*, 2008; Yasar

and Ozyigit, 2009). Therefore, selection of a biomonitor plant is an important criterion to be successful in a scientific study on heavy metal pollution in any environment (Akguc *et al.*, 2008; Malakootian *et al.,* 2009). However, a good biomonitor will indicate the presence of the pollutant and provide additional information about the quantity and intensity of the exposure (Wolterbeek, 2002). In addition, it should differentiate between both air- and soil-borne heavy metal contaminations, easy to sample and has no identification problems. It is expected to represent large numbers in all over the study area and to have a wide geographical range (Wittig, 1993). At present, no plant species that respond to a wide range of heavy metals as biomonitors are known (Mertens *et al.*, 2005). Although white poplars are good biomonitors for As, Cd and Zn pollution in the soil, the same monitoring strategy would fail to reveal soil pollution, for Pb and Cu (Mertens *et al.*, 2004). As a result, some plants can be more advantageous as biomonitors than other plants. For instance, perennial plants have a potential advantage over annual or short-living perennials, which could enable monitoring of short-term or yearly changes in pollution (Aksoy *et al.*, 1999). In that case, the importance of the selection of a good biomonitor plant is coming into question once more.

In this study, *Pyracantha coccinea* Roem. (firethorn) was used as a possible biomonitor plant in Mugla Province (during vegetation period, in 2006). In this manner, the pollution levels of Cu, Fe, Mn and Ni metals have been observed particularly in urban areas such as along roadsides, because of the following characteristics: It grows easily in both urban and rural areas of various

geographical ranges. Its ecological distribution can be found throughout Europe. It can be easily sampled, identified and cultivated and it is inexpensive to sample (Wittig, 1993; Akguc *et al.*, 2008).

MATERIALS AND METHODS
Study area

Mugla City is located in the southwestern 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 with 1.100 km (683 miles). The city is divided into 12 administrative districts (Fig. 1) (Mugla Municipality, 2010). Mugla is a rapidly growing city and its present population is estimated to be around 766.156 in whole city and 94.207 in city center (Tuikapp, 2010).

The economy of the city relies mainly on tourism (on the coast), agriculture (honey, pine-forest honey in particular and citrus fruits), forestry and marble quarries. 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 Yatagan 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; Akguc *et al.*, 2008).

Botanical characteristics of Pyracantha coccinea Roem.

Shrub up to 3 m high; young twigs grey-pubescent; spines leafless. Leaves lanceolate, elliptic or obovate elliptic, 2-4 (-5) × 1-1.5 (-2) cm, crenate-serrate, pubescent beneath, especially when young, or entirely glabrous;
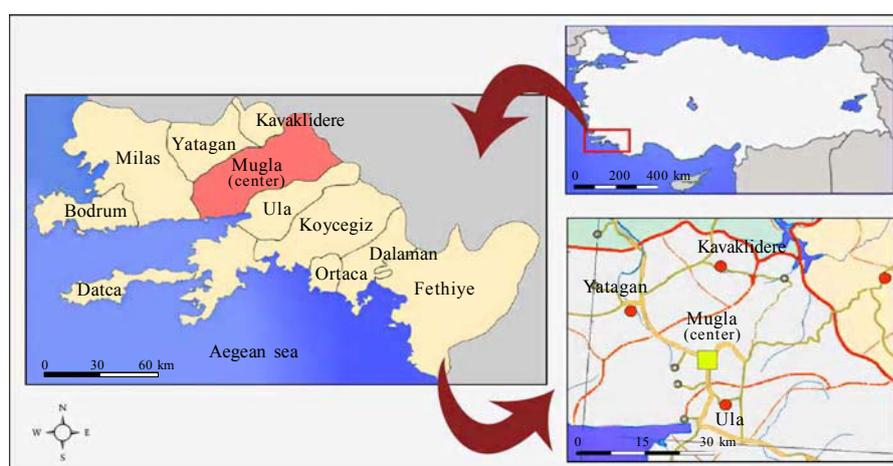

Fig. 1: Administrative districts of Mugla City in Turkey

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 (Davis, 1972).

P. coccinea is distributed especially North, Central and South Anatolia in Turkey and South Europe, Crimea, Caucasia, Northwest Iran in the world; Wild in Turkey, especially near the Black Sea Coast. It has a very wide range of horticultural use, for its ornamental berries (Pyracantha). Several cultivars and at 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). In cultivation, it does not prefer a particular type of soil and requires little or no supplemental fertilization. Full sun preferred but will grow in partial to fairly heavy shade. It tolerates atmospheric pollution and reasonable exposure. This species, especially the cultivar "Lelandii", is notably resistant to honey fungus (Huxley, 1992). It is intolerant to root disturbance except when young, a good bee plant and birds are particularly attracted to the fruit of this plant (Bean, 1981; Huxley, 1992).

Sample collection and preparation

The plant samples were collected from different sites of Mugla Province in September 2006. Totally, 34 plant samples were cut and then they were labeled and kept in standard paper envelopes. The localities were categorized as follows: urban (twelve stations), industrial (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; some of them were 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 oven-dried at 80 ºC 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 Erlenmeyers and then six mL mixture of 65 % nitric acid (HNO_3) and 70 % perchloric acid ($HClO_4$) in 1:4 ratios was added in it. Later, they were incubated in hot water in 52 ºC for at least 2 h (Kacar and Inal, 2008). After 2 h, the Erlenmeyers including dissolved samples in the acid mixture were put on hot-plates and the organic materials and acids were evaporated from the solutions. 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 Cu, Fe, Mn and Ni in the leaf and branch samples were then measured by inductively coupled plasma atomic emission spectroscopy (ICP-OES). The standard error values of the means were calculated for comparison of site categories. The wavelengths used for determination were 324.754 nm for Cu, 259.940 nm for Fe, 257.610 nm for Mn and 221.647 nm for Ni. The standard error values of the means were calculated to compare the site categories. A paired *t*-test was performed to determine the significance of washing of the leaves, comparing heavy metal contents of washed and unwashed plant samples for each type of site and *F*-test (ANOVA) was performed to compare different localities.

RESULTS AND DISCUSSION
Copper

The mean copper concentrations in leaves (washed and unwashed) and branches (unwashed) of *P. coccinea* are shown in Fig. 2a. As a result of measurements, the average highest value of Cu accumulation (5.89 ± 0.04 µg/g dw) was in the branch samples collected near highways, whilst the lowest was measured in the washed leaves (5.20 ± 0.03 µg/g dw) collected near highways as well. It was observed that the highest values were in branch samples, the second highest values were in leaf samples and the lowest values were in washed branch samples in all stations. However, the values of Cu concentrations in all samples and stations were very close to each other and it was seen that washing the leaves reduced Cu concentrations in small amounts; nevertheless, the reduction was significant (Table 1). In that case, it can be stated that the source of Cu accumulation in leaves is not mostly originated from airborne factors in Mugla Province like a similar study, realized in Spain by Madejon *et al.* (2006) with *Olea europaea* featured. In nature, copper occurs in rocks, water, air and it is an essential element for plant growth and development (Aksoy *et al.*, 2005). It is also an important component for many enzymes, which catalyzes oxidation and reduction reactions. The main sources of Cu are home tools production, metal manipulating, timber industry and ashes (Aksoy *et al.*, 2005). It is accepted that the normal limits of Cu concentrations in plants are in the range of 4-15

μg/g dw (Allaway, 1968; Bowen, 1979) and between 20-100 μg/g dw are accepted as toxic values (Kabata-Pendias and Pendias, 2001). According to these values, the Cu concentrations in this study were within normal limits in Mugla Province. In a similar research, Aksoy and Ozturk (1997) used *Nerium oleander* as a biomonitor in Antalya and found minimum and maximum limits as 3.2-6.18 μg/g dw in unwashed and 3-4.45 μg/g dw in washed leaves. In some other works realized in Turkey, Cu amounts were higher than in Kayseri with *Robinia pseudoacacia* (Aksoy *et al.* 2000) and in Sivas with *Pinus sylvestris* and *Robinia pseudoacacia* (Elik and Akcay, 2000). The Cu measurements in Antalya (*Nerium oleander*) which is a well-known touristic city like Mugla, have a broad agreement with the results obtained in this study. Kayseri and Sivas are industrialized cities in Turkey and they are affected by strong heavy metal pollutant factors. For instance, Kayseri has a very big metal processing factory called Çinkur (Manganese-Lead Metal Industry). The factory produces Cd, Pb and Zn based metallic products, which causes pollution due to lack of filter systems in its chimneys (Aksoy and Sahin, 1999). The highest values were obtained from the samples, which were collected near that factory. Sivas also has very big cement factories and other small industries. These factors could be the cause of the higher Cu concentrations in these cities. In different parts of Mugla City, Tuna *et al.* (2005) studied *Pinus* sp. and *Olea europaea* and their lower measurements (*Pinus* sp. 4.4 μg/g dw and *Olea europaea* 6.4 μg/g dw) could be an evidence that there is no Cu pollution in Mugla City.

Iron

The average iron concentrations in both types of (unwashed and washed) leaves and branches (unwashed) are shown in Fig. 2b. In this study, the findings of Fe values are as follows; the maximum amount was in the unwashed leaf samples in industrial area (9.53±1.68 μg/g dw) and the minimum Fe amount was in

Table 1: Total percentage of Cu, Fe, Mn and Ni removal from the leaf samples of *Pyracantha coccinea* Roem. through washing procedure in four different stations

Station type	Cu (%) removal	Fe (%) removal	Mn (%) removal	Ni (%) removal
Industrial	8.78	40.57	19.23	59.53
Urban	5.27	47.42	33.08	16.55
Highway	3.57	18.01	52.85	26.32
Control	2.97	30.80	20.72	50.74
F-test	***			**

Significance of comparison means by ANOVA (*F*-test) are indicated (**$p<0.01$, ***$p<0.001$ significant)

the branch samples near highways (1.73 ± 0.54). For all stations, the highest values were in unwashed leaf, the second highest values were in washed leaf and the lowest were in branch samples. Fe is mostly used in steel industry as a raw material, in paint industry with its oxidized form as pigment, as a compound in carbon and some other metals, in constructions and buildings (Taylor *et al.* 1988). Literature indicates that, the normal limits of Fe are in the range of 2-250 μg/g for plants (Kabata-Pendias and Pendias, 2001). Levy *et al.* (1999) mentioned that Fe concentrations over 500 μg/g are toxic. As it is seen above, values of the current study are accepted as normal. It was also observed that, washing the leaves reduced the Fe concentrations dramatically (Table 1).

In the present study, measured Fe levels were very low. In other studies realized both in Turkey and other countries, the results were within normal limits or higher, related to environmental conditions and/or high Fe accumulation capacity of the plants used as biomonitors (Uhlig and Junttila, 2001; Madejon *et al.*, 2006; Yasar *et al.*, 2010). Celik *et al.* (2005) found Fe concentrations between 100.2-3087 μg/g dw in Denizli using unwashed leaves of *Robinia pseudoacacia*. In another study, *Pinus sylvestris* (3800 μg/g dw) and *Robinia pseudoacacia* (6300 μg/g dw) were used in Sivas and the results were very high (Elik and Akcay, 2000). These higher Fe concentrations may be a result of long-time industrial activities in Denizli and Sivas. Czarnowska and Milewska (2000) measured leaf Fe amounts between 121-1056 μg/g dw using *Taraxacum officinale* in Warsaw Polonia related with the distance to the motorways. In Mugla, the Fe values of unwashed leaves were 214.5 μg/g dw in *Pinus* sp. and 241.6 μg/g dw in *Olea europaea* can be taken as an evidence that *P. coccinea* could not be a good biomonitor for Fe metal.

Manganese

Manganese amounts in washed and unwashed leaves and unwashed branches of *P. coccinea* in four different types of localities are shown in Fig. 2c. The results were as follows: the highest Mn (1.00 ± 0.16 μg/gdw) was measured in unwashed leaf samples collected near highways, whilst the lowest was measured in washed leaf samples (0.40 ± 0.14 μg/gdw) collected in urban area. The second highest values were found in unwashed leaves and the lowest values were in branch samples excluding highway stations (Fig. 2c). In the literature, the normal limits of Mn concentrations in

plants are in the range of 15-100 μg/g dw and 300-500 μg/g dw are toxic (Kabata-Pendias and Pendias, 2001). According to these values, in this study, the Mn concentrations are within normal limits and washing leaves reduced Mn levels (Table 1). In a study, *Pinus sylvestris* and *Robinia pseudoacacia* were used and the results were 50 μg/gdw and 93 μg/g dw, respectively (Elik and Akcay 2000). In many other studies realized in other countries, Mn values were variable. In *Empetrum hermaphroditum* and *Vaccinium myrtillus* Mn accumulation in unwashed leaves were between 533-3181 μg/g dw and 44-659 μg/g dw, respectively in Sor-Varanger, Norway (Uhlig and Junttila, 2001) and with *Quercus ilex* (318-2000 μgg dw unwashed leaves) and *Olea europaea* (46.2–418 μg/g dw unwashed and 148 μg/g dw washed leaves) in Spain (Madejon *et al.*, 2006). These results are high and mostly over toxic values and this situation could be a result of higher Mn accumulation capacity of used plants and/or the environmental Mn pollution. The sources of Mn are mainly fossil oil's particles and smog, chemical manures, drain wastes in cities. In addition, iron and steel, battery, glass, ceramics, paint, lacquer and ink industries are the other important sources of Mn, which dispersed in the atmosphere (Bowen, 1979). Tuna *et al.* (2005) in Mugla City, studied with *Pinus* sp. and *Olea europaea* and their results were 52.2 and 42 μg/g dw in unwashed leaves. When the results are compared with results of Tuna *et al.* (2005) in Mugla, it can be suggested that *P. coccinea* could not be a good biomonitor for Mn like Fe.

Nickel

Nickel concentrations of three different samples (unwashed-washed leaves and branches) of *P. coccinea* are shown in Fig. 2d. As a result of measurements, the average highest levels of Ni were in industrial area (unwashed leaves) while the lowest was determined in urban area (branches). The values were 14.34 ± 1.59 μg/g and 4.05 ± 0.51 μg/g dw. Moreover, it was observed that the Ni concentrations reached the highest values in unwashed leaf samples in all stations. The second highest values were in washed leaf samples and the lowest values were measured in branch samples in all four different stations as well. The accepted Ni values in plant tissues are in the range of 0.5-5 μg/g dw (Kabata-Pendias and Pendias, 2001). According to the results of this study, current values are higher than normal limits. In the current study, washing leaves reduced the Ni values significantly (Fig. 2d). It can be said that divided particles of Ni and Ni-compounds are mainly carried by

air. In nature, Ni is an abundant element and is naturally found in soil, water and food and is emitted from volcanoes. It occurs in the environment mainly in combination with arsenic, antimony and sulfur. Metallic nickel is found alloyed with iron in many meteors and the earth core is believed to contain substantial quantities. Commercially important nickel ores are garnierite, pyrrhotite and millerite. The sources of Ni are mainly vehicles running on petroleum and diesel fuel. They can easily contribute to nickel emissions to the atmosphere. Furthermore, consumer products that contain nickel and compounds are coins, jewellery, nickel-cadmium batteries, some paints and ceramics, magnetic tapes, computer components, goods containing stainless steel (sinks, cooking utensils, cutlery) (Nriagu, 1979; Kacar *et al.*, 2002). Some scientists in Turkey carried out similar studies with some other plant taxa and different results were obtained. Elik and Akcay (2000) carried out a study in Sivas by using *Pinus sylvestris* and *Robinia pseudoacacia*'s unwashed leaves and they found out the Ni values were 50 μg/g dw and 93 μg/g dw respectively. In different localities of Istanbul, Ni amounts were found between 2.19 μg/g dw and 4.47 μg/g dw by using *Cercis siliquastrum* subsp. *siliquastrum* L. (Yasar *et al.*, 2010). In Mugla, Tuna *et al.* (2005) measured Ni in *Pinus* sp. (3.2 μg/g dw) in *Olea europaea* (3.9 μg/g dw). Higher results in Mugla may be due to locality differences in the same city or rapid Ni increase in the city during two years (from 2005 to 2007) or *P. coccinea* could be a better biomonitor than *Pinus* sp. and *Olea europaea*. There are some similar studies carried out in other countries by using different plant taxa as possible biomonitors. Uhlig and Junttila (2001) measured Ni accumulation in unwashed leaves of *Empetrum hermaphroditum* (2-14 μg/g dw) and *Vaccinium myrtillus* (4-21 μg/g dw) in Norway. Madejon *et al.* (2006) used *Populus alba* in New Zealand (1.33 μg/gdw unwashed leaves) and Calzoni *et al.* (2007) measured Ni values between 8-14 μg/g dw in unwashed leaves of *Rosa rugosa* in Italy. As seen in the results of these studies, only the Ni values of *Populus alba* in New Zealand are between the normal limits. Other values showed broad agreement with the current study and higher than normal limits. It can be said that like in our country, Ni values have been increasing in many countries. In addition, all the values were belonged to unwashed leaf samples and in the current study, washing leaves reduced the Ni values significantly (Table 1). In this study, it was observed that the concentrations of those four elements (Cu, Fe Mn and Ni) increased with
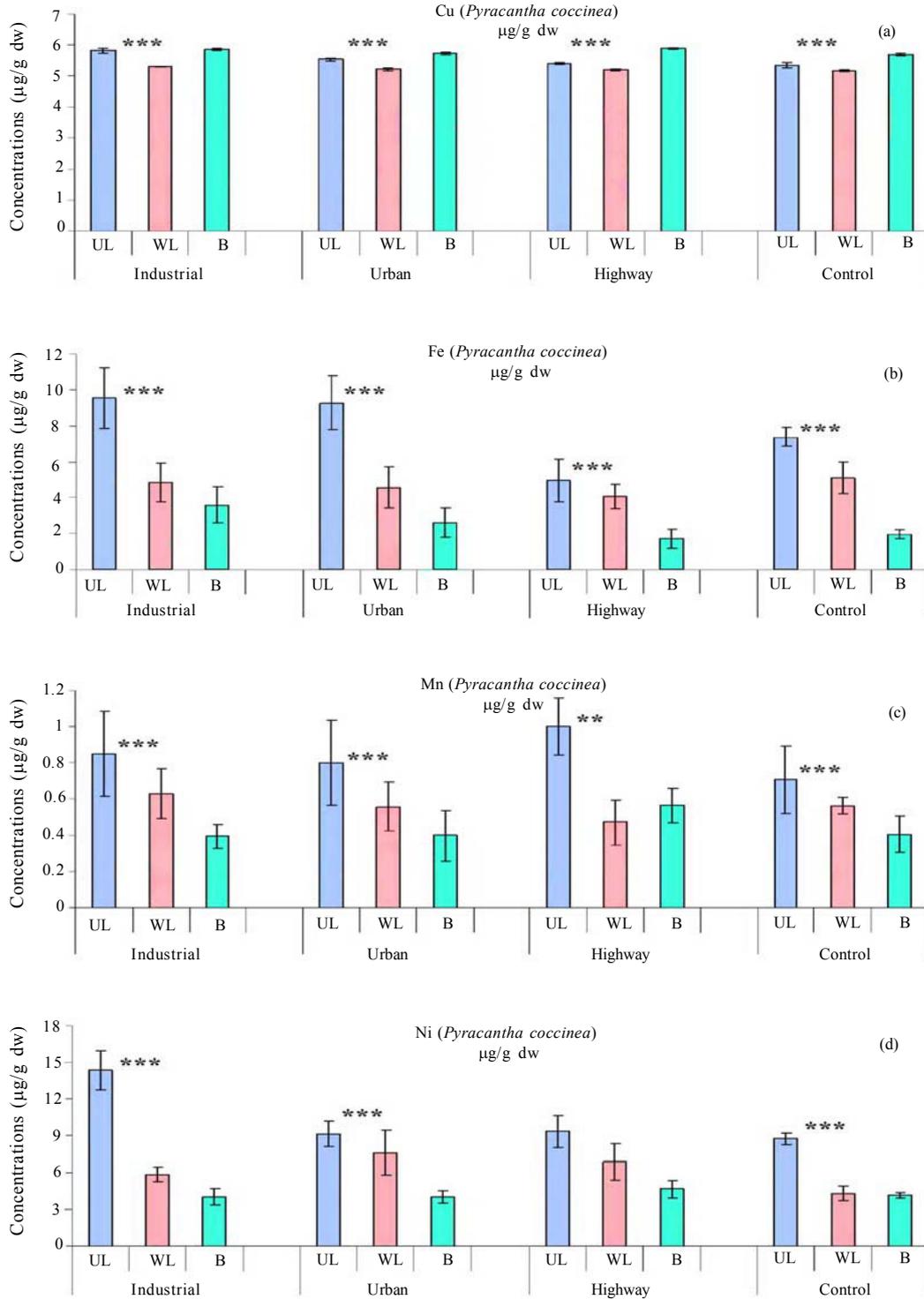
Fig. 2: Mean Cu (a), Fe (b) Mn (c) and Ni (d) concentrations (µg/g dw) in branches, washed and unwashed leaves as µg/g dw together with S.E. bars. UL: Unwashed leaves, WL: Washed leaves and B: Branch, significances of differences between washed and unwashed plants, from *t*-test, are indicated above the columns (**$P < 0.01$, ***< 0.001 significant)

increased urbanization and industry. The distinguishing ability between airborne and soil-borne contamination was assessed by washing the leaves with deionized distilled water. Although washing procedures reduced Fe, Mn and Ni levels of leaves on an average of 40 %, the reduction of Cu levels by washing was less in leaves (8-10 %). In some previous studies with other plants, the Cu reduction was also higher than current results (Aksoy and Ozturk, 1997; Aksoy *et al*., 2000; Celik *et al*., 2005). The results of Figs. 2 b, c and d indicate that there was substantial aerial deposition on the leaves for three of those elements (Fe Mn and Ni), which was removed by the washing procedure. Furthermore, the highest Fe, Mn and Ni levels were found in unwashed leaves, the second highest levels were found in washed leaves and the lowest values were found in branches of *P. coccinea*. Nevertheless, the highest Cu values were found in branches and then unwashed and washed leaves, respectively and all the values were closer to each other. In this study, although the Fe values were higher in industrial and urban areas compared to highway and rural, totally, all the values were very low. This could be a result of the Mugla Province is being mostly a touristic city, and in general not affected by Fe pollution or *P. coccinea* is not a good biomonitor for Fe element. In addition, the results of a similar study, which was carried out in industrial areas of Mugla City with *Pinus* sp. and *Olea europaea*, were higher than ours (214.5 μg/g dw and 241.6 μg/g) (Tuna *et al*., 2005). This situation could also support that *P. coccinea* is not a good biomonitor for Fe. In addition, Mn values of this study were under normal limits and when same comparison is made with the study of Tuna *et al*. (2005), the same conclusion is deduced as Fe. Ni values of this study were higher than normal limits and especially higher in industrial area and washing procedure reduced Ni values in leaves. This result shows that Mugla is under Ni pollution. In conclusion, this study supports the view that *P. coccinea* could be used as a biomonitor for Cu and Ni metals, especially with its branches and leaves. However, for Fe and Mn metals, it could not be used as a biomonitor or other supportive studies could be carried out in other cities especially in industrialized cities with *P. coccinea*.

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