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USE OF *AESCULUS HIPPOCASTANUM* L. AS A BIOMONITOR OF HEAVY METAL POLLUTION

RUYA YILMAZ[1], SERDAL SAKCALI[2], CELAL YARCI[2], AHMET AKSOY[3] AND MUNIR OZTURK[4*]

[1]*Trakya University, Department of Landscape Architecture, Faculty of Agriculture, Tekirdağı, Turkey*
[2]*Marmara University, Institute of Sciences, Goztepe, Istanbul, Turkey*
[3]*Erciyes University, Biology Department, Kayseri, Turkey*
[4]*Ege University, Botany Department, Bornova, Izmir, Turkey*

Abstract

Aesculus hippocastanum was studied as a possible biomonitor of the heavy metal pollution in the region of Thrace situated in the European part of Turkey. The urban roadside, city centre, and suburban sites were investigated. The Pb, Cd, Zn and Cu concentrations varied between 0.020-0.051; 0.001-0.002; 0.391-0.594; 0.256-0.387 µg g^{-1} dry weight in the washed leaves, between 0.023-0.119; 0.002-0.068; 0.374-0.532; 0.322-0.466 µg g^{-1} dry weight in the unwashed leaves, between 0.063-0.628; 0.005- 0.006; 0.406-0.660; 0.345-1.026 µg g^{-1} dry weight in the bark respectively. In the soils supporting these plants the values of Pb, Cd, Zn and Cu varied between 0.812-6.745; 0.002-0.006; 2.196-4.598; 0.517-1.117 µgg^{-1} dry weight respectively. When we compare the concentrations of the heavy metals in the leaves, bark and soils, we can see that the values in the urban roadside are higher than other sites. According to these results concentration of heavy metals in *A. hippocastanum* did not exceed the upper limit. A highly linear regression was obtained for Pb, Zn and Cu between concentrations of the element in surface soil and in the washed leaves of plant but the correlation in the case of Cd was insignificant.
Key words: *Aesculus hippocastanum* , heavy metals, biomonitor, Thrace, Turkey.

Introduction

The population explosion in Turkey during the last few decades has lead to an uncontrolled and unplanned urbanization and industrialization. It is creating severe environmental problems in the urban areas. The pollutants in the urban atmosphere are discharged from many sources the major contributors are traffic and industrial establishments. A number of heavy metals such as Pb, Cd, Cu, Zn and Cr originating from the said pollution may accumulate in toxic concentrations in the soils of urban areas (Adermorti, 1986; Ho & Tal, 1988; Ara *et al*., 1996; Arindam, 1999), pass on to the edible portions of our crops and through food chain cause different clinical problems. As such, an increased concern all over the world has been observed lately over metal pollution. A lot of work has been done all over the world related to the heavy metal biomonitoring features of plants; Seaward & Mashhour (1991), Ozturk & Turkan(1993), Sawidis *et al*. (1995), Aksoy & Ozturk (1996;1997), Aksoy & Shahin (1999), Aksoy *et al*. (1999; 2000a; 2000b), Aksoy & Demirezen (2006) and Baycu, (2003). For this purpose several species of lichens, mosses and higher plants have been used since 1950's (Djingova & Kuleff, 1993).

* Corresponding Author: Munir Ozturk; munirozturk@gmail.com

According to Wittig (1993), the basic criteria for the selection of a species as a biomonitor, 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 and there should be no identification problems.

Aesculus hippocastanum has been widely planted all over the Thrace region as a ruderal ornamental tree due to its attractive flowers and shade bearing characteristics. It was selected for biomonitoring studies because it fulfills all the basic criteria given by Wittig (1993). The aim of this study was to investigate the metal pollution levels in the Thrace Region of Turkey including the cities of Edirne, Tekirdag, Chorlu and Luleburgaz (Fig.1). Both industrial activities as well as heavy traffic load are the main cause of pollution in this area in particular heavy metal pollution. It is an important industrial sector with more than 155 industrial establishments. A heavy vehicular traffic is observed due to the central position of this region on the link road between Turkey and Europe.

Materials and Methods

The study area is located in the Southeast Europe (Fig. 1). The total annual precipitation varies between 575- 609 mm/m^2. The minimum temperatures in winter are; -22.2 ^{0}C in Edirne; -13.5 ^{0}C in Tekirdagi; -16.9 ^{0}C in Chorlu and -24.2 ^{0}C in Luleburgaz; whereas the maximum temperatures in summer are; 41.5 ^{0}C in Edirne; 37.8 ^{0}C in Tekirdagi; 39.2 ^{0}C in Chorlu and 42.8 ^{0}C in Luleburgaz.

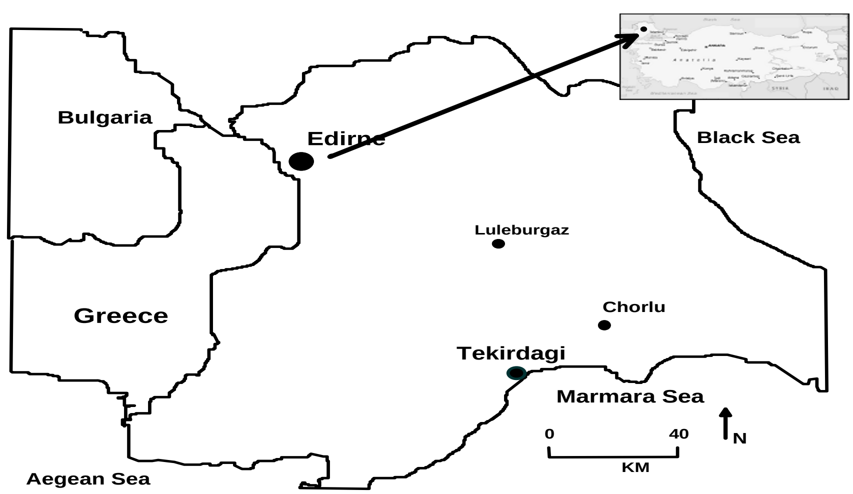


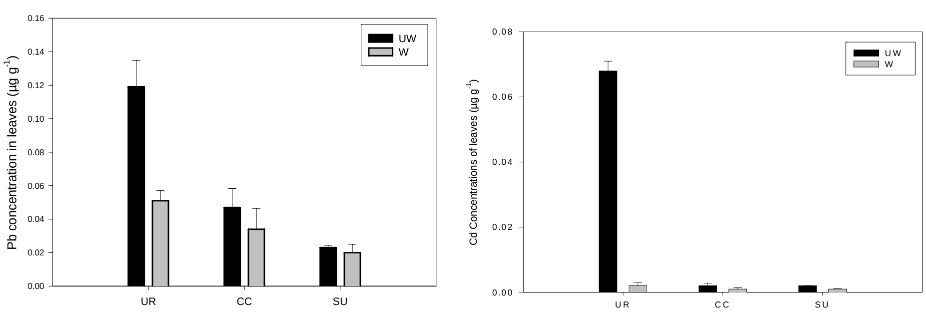
Fig. 1. Map showing the study area.

Sample collection and treatments: The leaves and bark samples of *Aesculus hippocastanum* L. were collected from twenty sites; 13 from Edirne, 4 from Tekirdagi, 2 from Chorlu and 1 from Luleburgaz; at a height of 15 m from the ground level and soil samples were taken from the top 10 cm. at the base of trees used for leaf collection with the help of stainless steel trowel to avoid contamination. These were grouped into 3; urban roadsides (15 samples), from suburban area (3 samples) and city centre (2 samples). The soil samples were air dried and passed through a 2-mm. sieve. About 150 g. of mature leaves of *A. hippocastanum* were harvested and divided into two sub-samples; one sub-sample was thoroughly washed with distilled water to remove dust particles, the other sub sample was used as unwashed. The plant samples were oven-dried at 80^0C till constant weight and milled in a micro-hammer cutter. These were stored under silica gel in a glass dessicator. The cutter was washed after every grinding, first with absolute alcohol then with bi-distilled water to avoid any contamination.

Extraction: Samples (1 gr in weight) of soils and milled plant were ashed in a muffle furnace at 460 ^{0}C for 24 h, and weighed ash digested in concentrated HNO_3, evaporated to near dryness on a hot-plate and made up to volume with 1 % HNO_3. The weighed ash of soils was digested in 10 ml Aqua Regia (1 part concentrated HNO_3 to 3 parts HCl, v/v) in a digestion tube on a heating block for a total of 9 h (2 h at 25 ^{0}C, 2 h at 60^0C, 2 h at 105 ^{0}C and 3 h at 125 ^{0}C). All digested samples were centrifuged then made up to volume with 10 % HNO_3. Lead, cadmium, zinc and copper concentrations were measured by atomic absorption spectrophotometer (Perkin Elmer Model 1100), following the method given in detail by Aksoy & Ozturk (1997). A reference material was used with every batch (SRM 1547 peach leaves) to ascertain the accuracy of the method used. All data were subjected to *t*-test analysis using the SAS TTEST procedure (Anon.1987).

Results and Discussion

The mean concentrations of heavy metals (Pb, Cd, Zn and Cu) in the unwashed and washed leaves, bark and soils of *A. hippocastanum* from different sites are presented in figures 2(a, b), 3(a, b), 4(a, b, c, d) and 5(a, b, c, d). A comparison of concentrations of heavy metals in the leaves, barks and soils reveals that the values in the urban roadside are higher than other sites due to higher human activity and higher vehicular traffic. A similar finding is reported by Tam *et al*. (1987) from Hongkong who found a significant correlation between traffic density and Pb, Cu and Zn concentrations. The heavy metal concentration in the bark and soils are higher than in the leaves as in our findings.



(a) **(b)**

Fig. 2. Mean Pb (a) and Cd (b) concentrations (µg g^{-1} dry wt.) in unwashed (UW-black columns) and washed (W-grey columns) leaves of *Aeasculus hippocastanum* (UR: urban roadside; CC: City Centre; SU: suburban).

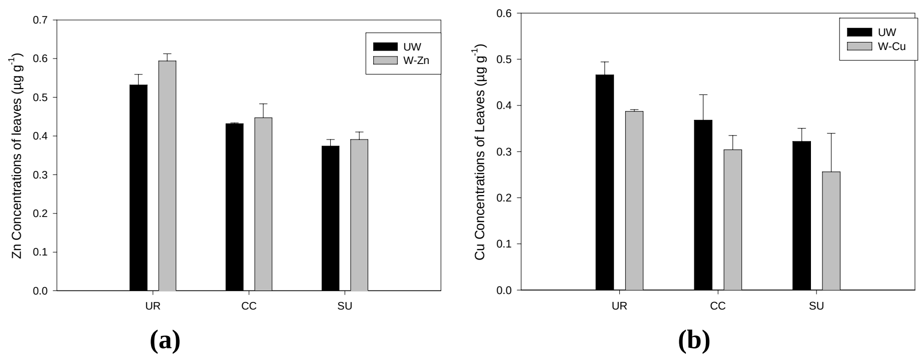


Fig. 3. Mean Zn (a) and Cu (b) concentrations (µg g^{-1} dry wt.) in unwashed and washed leaves of *Aeasculus hippocastanum*.

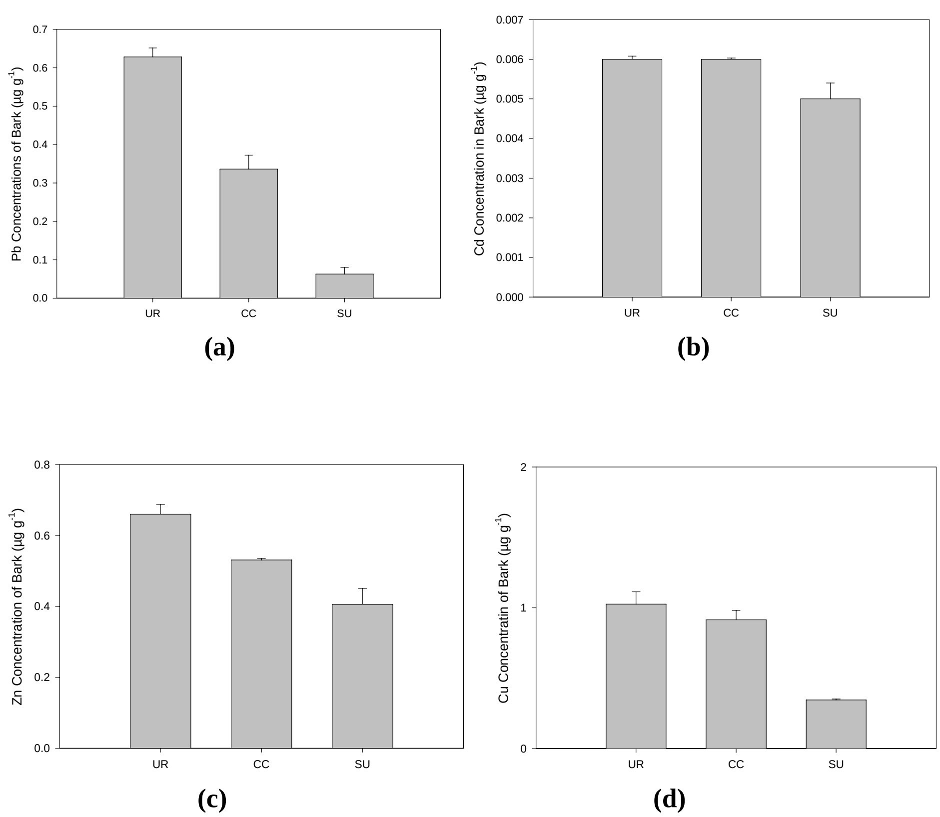
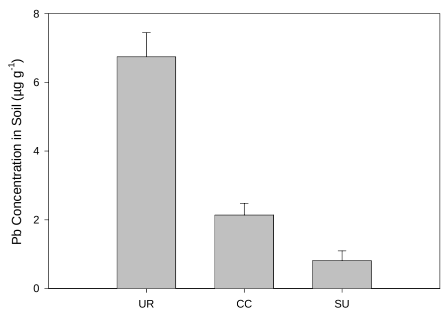
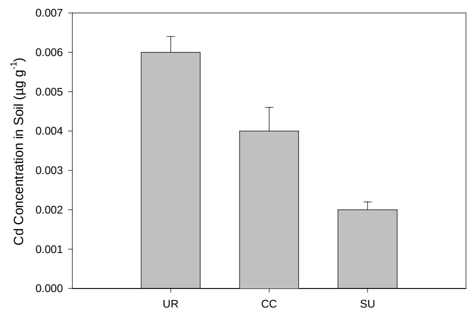


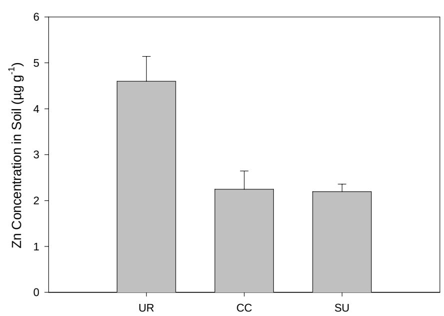
Fig. 4. Mean Pb(a), Cd(b), Zn(c) and Cu(d) concentrations (µg g^{-1} dry wt.) in the bark of *A. hippocastanum*.



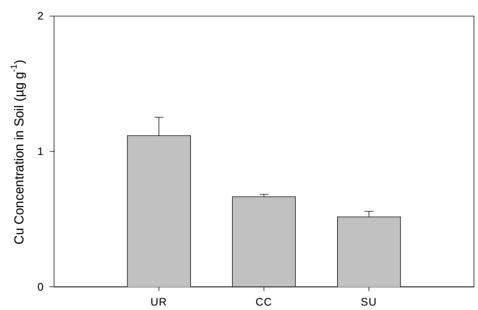
(a)



(b)



(c)



(d)

Fig. 5. Mean Pb(a), Cd(b), Zn(c) and Cu(d) concentrations (µg g^{-1} dry wt.) in the soils.

The highest lead concentration was found in the urban roadside soils (6.745 µg g^{-1}) as compared to the suburban soils (0.812 µg g^{-1}). Similar findings have been reported by Aksoy & Ozturk (1996) in *Phoenix dactylifera* (189.47-85.52 µg g^{-1}), but the values obtained in this study are very low than these, however in normal uncontaminated soils, the total content of Pb is given as 2-200 µg g^{-1} (Freedman & Hutchinson, 1981), present values fall within this range. The occurrence of Pb in higher amounts in the urban roadside soil is due to traffic volume, which is regarded as one of the main sources of contamination as reported by other workers too, but the concentration decreases more rapidly between 0 and 5 m from the road than over greater distances (Tam *et al*., 1987; Ozturk & Turkan, 1993). Low levels of lead were found in young leaves of *Tussilago farfara* and high levels in old leaves of *Beta vulgaris* which were collected from highway near Siena (Italy). In the *A. hippocastanum* leaves the highest values of lead in the urban roadside samples (0.119 µg g^{-1}), but these values too are much lower than those reported for *P. dactylifera* (24.37 µg g^{-1}) (Aksoy & Ozturk, 1996). In fact the values obtained in *A. hippocastanum* are very near to the values reported for the samples taken from the rural areas (2.18 µg g^{-1}) of Antalya (Aksoy & Ozturk, 1996). In the case of *Nerium oleander* the values of lead in the soil range between 34-193 µg $g^{-}1$ and in the leaves between 2.65-28 µg g^{-1}. Lead concentration of the bark too as expected was high in the urban roadside samples (0.628 µg g^{-1}), but quite below the values reported for the bark of *Cupressus semervirens* (302.60 µg g^{-1}) (El-Hasan *et al*., 2002). According to Markert (1993) aerial deposition and foliar uptake contributes significantly to the Pb concentration in plants.

Many workers have studied the toxic effect of Cd on plants. In general toxicity has been reported to cause a reduction in the plant growth as well as chlorosis. The Cd values in our samples in general are very low, but highest values were recorded in the urban roadside unwashed samples (0.068 µg g^{-1}). The values for unpolluted natural environments should lie between 0.01-0.03 µg g^{-1} (Allen, 1989). Our values in general are much below than these. The values in our soil samples too were very low (0.002 to 0.006 µg g^{-1}). In the uncontaminated soils, these values vary between 0.01-0.7 µg g^{-1} (Freedman & Hutchinson, 1981). The reason for the low values could be due to a low atmospheric deposition in this region because of long lasting rainy season. Aksoy & Ozturk (1997) have reported 1.01 to 3.38 µg g^{-1} of Cd in the soils supporting *Nerium oleander* and 0.02-0.72 µg g^{-1} in the leaves. These values too are higher than ours.

Zinc has been described as an essential plant nutrient element. A positive and significant correlation has been reported by some workers in the amount of total Zn in *Betula pendula* and *B. pubescens* leaves. It was two to three orders of magnitude higher than of Cu, Pb and Cd. In our study the values of Zn varied between 0.391-0.594 µg g^{-1} in unwashed leaves, 0.374-0.532 µg g^{-1} in washed leaves, 0.406-0.660 µg g^{-1} in the bark and 2.196-4.598 µg g^{-1} in the soil. The normal levels of Zn in plants are reported to lie between 10 to 100 µg g^{-1} (Allen, 1989) and 17 to 25 µg g^{-1} in the surface soils. In the natural habitats of *Thlaspi caerulescens* hyperaccumulation of Zn appears to be a favourable factor with an effective mechanism for its acquisition and accumulation, the tolerance is positively correlated with soil Zn content in the natural habitats, but that it is inversely related to the capacity for Zn hyperaccumulation (Assuncao *et al*., 2001). Normal concentrations of Zn in plants are in the range of 10 to 100 µgg^{-1} (Allen, 1989). The mean Zn concentrations in surface soils of different countries vary from 17 to 25 µgg^{-1}. In *A. hippocastanum* the Zn concentrations measured during this study were between 0.374 - 0.532 in the washed leaves, 0.391-0.591 in the unwashed leaves, 0.406-0.660 in the bark and 2.196 and 4.598 µg g^{-1} in soil. These values are lower than those cited above. By following the Zn pollution criteria levels of Dmuchowski and Bytnerowicz (1995), we can conclude that Thrace region does not show Zn pollution. In the soils Zn levels in general were higher than Cu, Cd and Pb except for the Pb in urban roadside soils. According to Iqbal *et al*. (1998) plant species show differential behaviour as regards the Zn content. Very high concentrations of Zn (9.290 µg g^{-1}) have been reported in the leaves of *Eucalyptus* species and *Ficus religiosa* (0.766 µg g^{-1}) (Ara *et al*., 1996). A similar study by other workers has revealed that Zn in the top layer of soil was 95 µg g^{-1} and in leaves it was 1061 µg g^{-1}. In the uncontaminated soils, the total Zn content is said to lie between 10-300 µg g^{-1} (Freedman & Hutchinson, 1981). In these soils, total Zn is constant in the soil profile, but extractable Zn generally decreases with depth because of decrease in organic matter. The higher amount of the Zn in our soils can be attributed to the high organic matter content.

The mean Cu content in soil world-wide is 2 to 150 µg g^{-1}. According to this author 5 to 20 µg g^{-1} Cu was normal, while less than 4 µgg^{-1} was considered deficient and above 20 µg g^{-1} was considered toxic for plant growth. Copper is necessary for the formation of growth substances. In the soils of industrial areas the level of Cu varies according to the plant cover and lies between 0.023-2.988 µg g^{-1} and even goes up to 400 µg g^{-1} (Iqbal *et al*., 1998). The soil Cu can vary greatly among the sites at the same location and in different locations. The Cu values of soil samples supporting *A. hippocastanum* lie between 0.5171 and 1.1165 µg g^{-1},in the unwashed leaves Cu content ranges between 0.322-0.466, in the washed leaves between 0.256 and 0.387 and in the bark between 0.3451-0.117 µg g^{-1}. In normal uncontaminated soils Cu content varies between 2-100 µg g^{-1} (Freedman & Hutchinson, 1981). Our values are low.

Washing the leaves significantly reduced the lead, cadmium, zinc and copper concentrations. The reduction for the lead was 57.14 percent for the urban roadside, 27.66 for the city centre and 13.04 for the suburban area, whereas for cadmium these values were 97.06, 50.0 and 0.0 % respectively. In the case of zinc the reduction was 10.43 % in the urban roadside, 3.36 % in the city centre and 4.35 % in the suburban areas, and for copper it was 16.95 % in the urban roadside, 17.39 % in the city centre and 20.50 % in the suburban areas. The percentage removal was the highest in the urban roadside (57.14 %) as compared to suburban. The reason for this is differences in the atmospheric deposition of these metals and high and low traffic densities. This data coincides with that of Al-Shayeb *et al*. (1995) who reported a removal of 26-68 % of Pb by washing. Aksoy & Ozturk (1996, 1997) have also reported higher removal percentages of Pb from urban roadside in *P. dactylifera* (32.66 %) and *Nerium oleander* (56.21 %).

Generally, Cd and Pb are removed most as compared to copper and zinc. The higher heavy metal content in the soils, bark and leaves of urban roadside is mostly due to the density of the traffic which is considered as one of the major sources of heavy metal contamination, especially Pb, because, unleaded petrol is expensive and people instead use leaded petrol. Lead is one of the major sources of heavy metal pollution which enters the environment mainly by aerial deposition alongside the roads in proportion to the density of traffic and distance from the roadside. High levels of Pb and Cu have been reported in the needles of *Taxus baccata* coming from vehicular emission. Tam *et al*. (1987) 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. Tam *et al*. (1987) also studied metal contamination of *Bauhinia variegata* leaves, surface soil and dust collected in urban park, near roadside in Hong Kong. The results of their comparisons for unwashed and washed leaves suggested that the main source of Pb pollutant was aerial deposition from the vehicles. Same holds true for Thrace region. The atmospheric deposition of heavy metals in the present study area is not very high, the concentration of heavy metals in *A. hippocastanum* did not exceed the upper limit.

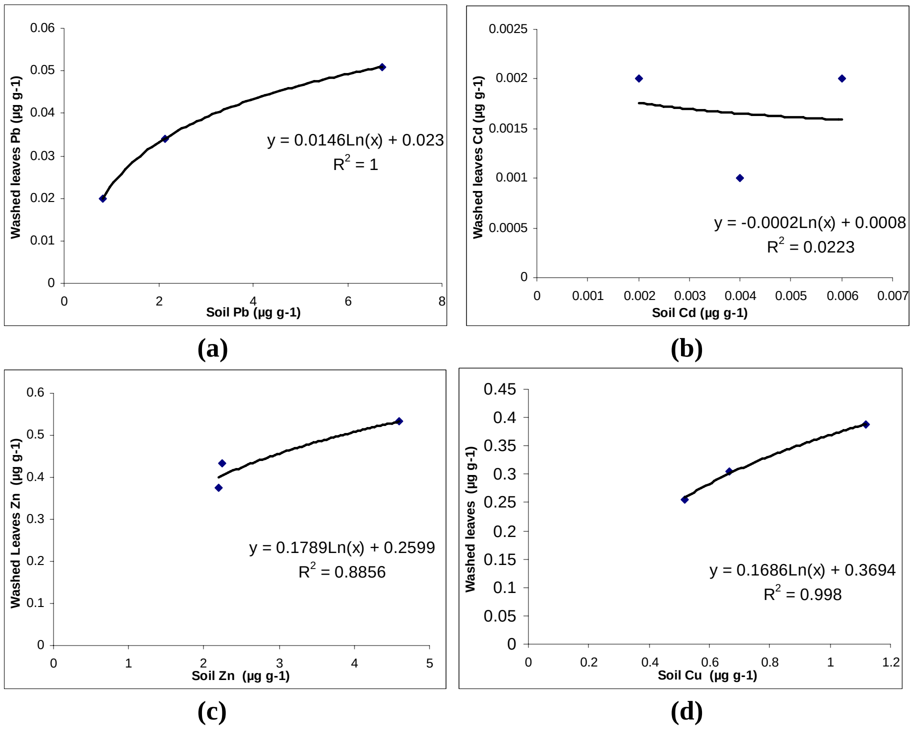


Fig. 6. Regression analysis showing Pb (a), Cd (b), Zn(c) and Cu (d) concentrations in the soil and washed leaves of *A. hipocastanum.*

The regression analysis data shows (Fig. 6) that our values for soils and plants are significant for Pb, Zn and Cu, but not for Cd. We can thus conclude that with an increase in the amount of the heavy metals in the soil, their uptake by plants also increased. Aksoy *et al*. (1999) have investigated the effect of soil pollution by heavy metals on *Capsella bursa-pastoris*. They found a linear regression between the soil and plants. The values of r^2 obtained by us are 1 (Pb), 0.0223 (Cd), 0.8856 (Zn) and 0.998 (Cu). *A. hippocastanum* is widely distributed in Europe and is used as a roadside ornamental tree. In accordance with the data presented here this tree possesses all the characteristics for its selection as a biomonitor.

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