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Judas tree (*Cercis siliquastrum* L. subsp. *siliquastrum*) as a possible biomonitor for Cr, Fe and Ni in Istanbul (Turkey)

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Abstract

In this study, leaf (washed and unwashed) and bark samples of Cercis siliquastrum L. subsp. *siliquastrum and soil samples collected from 59 different localities, belonged to five stations (urban, urban park, urban roadside, The Bosphorus Coast and the control area) were used to determine heavy metal pollution in Istanbul between 2006-2007 years. In addition, the usability of this plant as a heavy metal pollution monitor to define the pollutant types and to calculate the ratio of airborne and soil borne contaminations in Istanbul was investigated.*

The Cr, Fe and Ni concentrations of our collected samples were measured by using ICP-OES. The obtained data were analyzed with SPSS statistics program. As a result of the measurements, the average highest and lowest values of heavy metal concentration on the basis of sample locations were as follows: The maximum value of Cr accumulation was gained by bark samples taken from urban park; the value was 6.12±0.59 µg/g dw. The lowest value was detected to be 1.63±0.20 µg/g dw in washed leaf samples from the control area. The highest Fe amount was measured in unwashed leaf samples collected from urban roadside, while the lowest was observed in washed leaf samples from urban area. The values were 134.74±35.06 µg/g dw and 44.97±5.15 µg/g dw, respectively. The average highest Ni value was determined in unwashed leaf samples gathered from urban roadside; the value was 4.47±0.82 µg/g dw. The lowest value was measured in washed leaf samples taken from control area, and this value was determined to be 2.19±0.39 µg/g dw.

Our results revealed that Cercis siliquastrum L. subsp. *siliquastrum is a useful biomonitor to determine the amount of heavy metal deposits except for Fe. In addition, the tree barks can be used for long-term measurements of heavy metal depositions for this species.*

Key words: *Cercis siliquastrum*, biomonitor, Cr, Fe, Ni, ICP-OES, Istanbul

Introduction

Ecologists and biologists realized that some organisms have more ability of accumulating certain metals and/or toxic elements and studied these organisms as biomonitors to obtain information on the quality of their environments. Many organisms (cyanobacteria, lichens, mosses, trees, mollusks, fishes, birds and small mammalians) and some of their parts (tree barks, tree rings, pine needles, grasses, leaves, blood, urine hair and nails) have been used as biomonitors [1-4]. After 1950s, the use of higher plants as biomonitors of heavy metal pollution was advanced and their washed and unwashed leaves were mostly used to detect deposition, accumulation and distribution of heavy metal pollution [5, 6].

In last decade, the following studies; Aksoy et al 1999, *Capsella bursa-pastoris* (Pb, Cd, Zn, Cu) [5]; Aksoy and Şahin 1999, *Elaeagnus angustifolia* (Pb, Cd and Zn) [7]; Öztürk et al 2000, *Cynodon dactylon*, *Nerium oleander* and *Pinus brutia* (Cd and Pb) [8]; Czarnowska and Milewska 2000, *Taraxacum officinale* (Fe, Mn, Zn, Cu, Pb, Ni and Cd) [9]; Badora 2002, *Pisum sativum* (Zn, Cd, Al and Mn) [10]; Çelik et al 2005, *Robinia pseudo-acacia* L. (Fe, Zn, Pb, Cu, Mn and Cd) [11]; Williams et al 2005, *Phoenix dactylifera* (Ag, Al, Ba, Be, Ga, La, Mo, Se, Si, Tl and V) [12]; Aksoy and Demirezen 2006, *Fraxinus excelsior* (Pb, Cd, Cu, Zn, Ni and Cr) [6]; Fernandez Espinosa and Rossini Oliva 2006, *Nerium oleander* L. and *Lantana camara* (Ba, Cu, Fe, Mn, Pb, Ti and V) [13]; Khan and Joergensen 2006, *Urtica dioica* L. (Zn, Pb, Cu and Cd) [14]; Atiq-Ur-Rehman and Iqbal 2008, *Prosopis juliflora* Swartz, *Abutilon indicum* (L.) Sweet and *Sena holosericea* (Fresen.) Greuter (Fe, Pb, Cu, Cr and Zn) [4]; Akgüç et al 2008, *Pyracantha coccinea* Roem. (Cd, Pb and Zn) [2] were carried out as good samples for biomonitoring studies by using vascular plants.

There are some common features of biomonitor organisms, which used for reflection of heavy metal pollution. They should be represented in large numbers all over the monitoring area, they should have wide geographical ranges, it should be possible to differentiate between airborne and soil borne heavy metals when used and they should be easily sampled and identified by the scientists [15].

The study area, Istanbul is located in the north-west part of Turkey (41° 01.2' N, 28° 58.2' E) and it is one of the biggest metropolitans of the world [16, 17]. There are 40 administrative districts in Istanbul now, but there were 32 administrative districts while we were collecting plant samples in 2006 and 2007. Istanbul is the only city that has served as the capital of a 1000-year-old Christian Empire (Roman, Byzantine, Latin) and a 500-year-old Muslim Caliphate (Ottoman Empire) and thus it has a long history and rich population density. Although the area of Istanbul is around 5750 km^2, the population is 12.697.164 (at the end of 2008) and the city is providing around 40% of the Turkey's tax revenue, is home to around 38% of the industrial companies and around 55% of the commercial companies [16, 18]. After technologic revolution in 19. century, an effective migration rate of Turkey's Anatolian population into Istanbul had taken place. This migration rate also increased after 80s and this situation led to the enlargement of the city's boundaries. Because of increased human activities; building, traffic and industrial production affected the air, water and soil quality like other fast-developed cities [19].

In this study, washed-unwashed leaf and unwashed bark samples of *C. siliquastrum* L. subsp. *siliquastrum* (Judas tree) and soil samples collected from 59 different localities, which classified as urban, urban park, urban roadside, The Bosphorus Coast and the control area (Islands) were used to determine heavy metal pollution in Istanbul between the years 2006-2007. In addition, the usability of *C. siliquastrum* L. subsp. *siliquastrum* as a biomonitor to define the pollutant types and to calculate the airborne and soil borne contaminations in Istanbul was investigated.

Materials and Methods

Plant Material

Cercis L.: Deciduous shrubs or small trees. Leaves simple, suborbicular, cordate, petiolate; stipules herbaceous, caducous. Flowers ± precocious, pink, fasciculate, pedicellate on short shoots borne on the old wood. Calyx broadly campanulate with 5 equal teeth. Corolla pseudo-papilionaceous, the 3 upper petals shorter than the two lower. Stamens on, free. Fruit a linear-oblong, laterally compressed legume.

C. siliquastrum L.: Shrub or tree, 2-10 m. Leaf lamina 7-12 cm diam.; petioles 2-4 cm. Flowers c. 10 to a fascicle. Pedicels 1-2 cm. Calyx c. 5 mm, pinkish, the teeth c. 1 mm.

Corolla 15-20 mm, bright pinkish- purple. Legume 6-10×1.5-2 cm, narrowly winged on the ventral suture, brown, 6-15- seeded. *Fl. 4-5 Macchie and deciduous forest, s.l.- 1370m.*

Cercis siliquastrum L. subsp. *siliquastrum*: Calyx, pedicels and legumes glabrous.

This taxon is distributed in central and south Europe, especially widespread in Spain and Italy. In Turkey, it is distributed in west part of Anatolia and Islands, especially in Istanbul, Kocaeli, Bursa, Zonguldak, Amasya, Balıkesir, Muğla, Antalya and Adana Cities. It is also present in Syria [20].

Ecologic Characteristics of Study Area

Istanbul's main topographical structure is a low plateau (100-200 m elevation) besides; there are many hills and a few streams. The geological structure consists of the Silurian, Devonian, Carboniferous and Tertiary Ages-originated formations. The rock types of the city are granitic plutons, quartzes, grovacs, clayed schsists and radiolarites. Although many different types of soils are present in Istanbul, the brown forest soils cover the largest area. The non-calcareous brown soils are the second, and the rendzinas, which cover especially the European Side of the city, are also present in the city [21].

Istanbul is a kind of transition zone between less rainy Mediterranean (south) and Oceanic (Bosphorus and north) climates. In the summer, less precipitation and high temperature are usually observed and the annual mean temperature was measured as 14.5^{o}C in last two decades. Between May and September, the temperature is generally above 30^{o}C and between November and April; it is rarely below 0^{o}C. In the vegetation period, the daily mean temperature is 8^{o}C and this period is about 280 days (between 15 March and 20 December) [22]. The total precipitation for Istanbul averages 640 mm per year. The minimal rain falls in July and August and its ratio is about 8%. The rain regime is Winter-Autumn-Spring-Summer (W.A.Sp.Su) and the rain type is "Central Mediterranean Rain Type" [23]. The ratio of relative humidity is between 73-77% in the city and this ratio decreases to 65 - 68% in summer despite of the effect of the seas. The dominant wind in the city is the northeast-originated winds [22].

Sample preparation

The leaves and barks of *C. siliquastrum* L. subsp. *siliquastrum* and soil samples were collected from 59 different localities in Istanbul between 2006-2007 years (Figure 1). The numbers of samples from different sites for each category were as follows: urban (fifteen), urban park (eleven), urban roadside (twelve), The Bosphorus Coast (seventeen) and control area (four). About 200 g (fresh weight) of leaves from *C. siliquastrum* L. subsp. *siliquastrum* were collected from the middle section of the main leafy area of the plant and divided into two sub-samples. One sub-sample was thoroughly washed with deionized distilled water to remove dust particles, and the other remained was untreated. Bark samples (10×2 cm) were taken from main stem, about 1 m high from the soil and wounded sites were covered with grafting wax against microbial contamination. Soil samples (about 500 g) were collected from a deep of about 10 cm with a stainless steel shovel. The samples were dried and then fed through a 2-mm sieve. All plant samples were oven-dried at 80^{o}C for 24h, milled in micro-hammer cutter and fed through a 1.5-mm sieve. Plant samples were then stored in labeled clean self-sealing plastic bags in silica gel. Contamination from the micro-hammer cutter was negligible while grinding, since it was washed with first absolute alcohol or acetone, then with sterile deionized water after each use.

Figure 1. The satellite view of Istanbul-Turkey. The study areas are circled. Right corner bar is indicated 10 miles (The picture was prepared by using The Google Earth Programme)

Analytical techniques

After drying, approximately 0.5 g of plant and soil samples were weighed and transferred into polytetrafluoroethylene (PTFE) vessels (XP-1500) and then 10 ml 65% HNO_3 (Merck) was added. Samples were mineralized in microwave oven (CEM MARS5) as follows: 20 min at 1200 W maximum power, 300-PSI pressure, 210°C and 15 min stand by. After cooling, the samples were filtered by Whattman filters, and made up to 25 ml with double distilled water in volumetric flasks and then stored in clean self-sealing plastic bags in silica gel.

Standard calibration techniques were used and Cr, Fe and Ni measurements were done by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The wavelengths used for determination were 267.716 nm for Cr, 259.940 for Fe and 221.647 nm for Ni. The standard error values of the means were calculated to compare the 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 plant samples for each type of site. F-test (ANOVA) was performed to compare different localities.

Results and discussion

1. Chromium

The mean Cr concentrations in leaves (washed and unwashed) and barks (unwashed) of *C. siliquastrum* L. subsp. *siliquastrum* and the soil samples are shown in Figure 2.

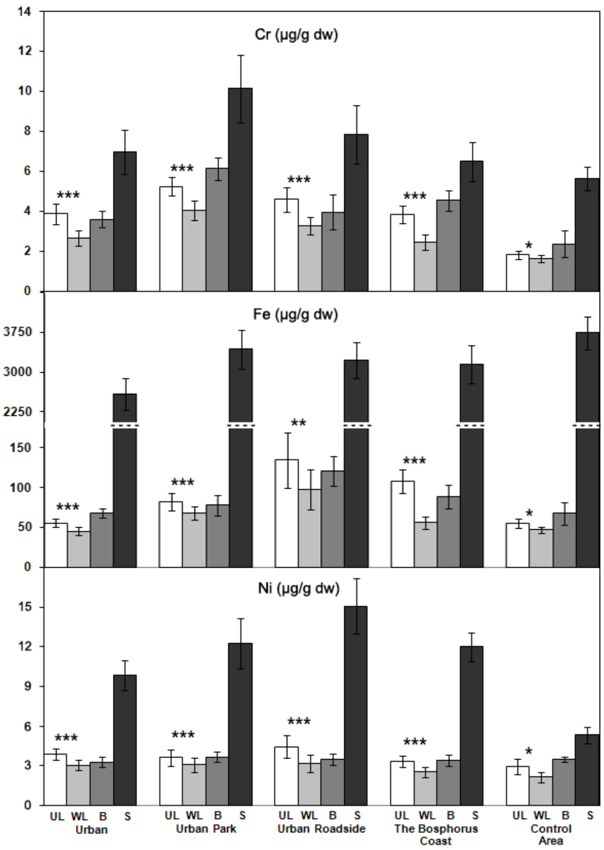

Figure 2. Mean Cr, Fe and Ni concentrations (µg/g dw) of unwashed leaf (UL), washed leaf (WL), bark (B) and soil (S) samples in five different stations together with S.E. bars. Significances of differences between washed and unwashed plants, from t-test, are indicated above the columns (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significant).

As a result of measurements, the average highest value of Cr accumulation was gained in bark samples taken from urban park with 6.12±0.59 µg/g dw. The lowest value was detected as 1.63±0.20 µg/g dw in washed leaf samples from the control area. Similar to the plant samples, the average highest soil Cr level was found in urban park (10.13±1.71 µg/g dw) while the lowest in control area with 5.65±0.59 µg/g dw. The second highest values were in unwashed and washed leaf samples respectively. In urban roadside, the average highest value was measured in unwashed leaf samples. It was also observed that, washing the leaves significantly reduced the Cr concentrations in leaves given in Table 1 as % removal of heavy metals in leaf samples after the washing procedure in different stations.

Table 1. Total percentage of Cr, Fe and Ni removed from the leaf samples of *Cercis siliquastrum* L. subsp. *siliquastrum* through washing procedure in five different stations. Significance of comparison means by ANOVA (F -test) are indicated (* p <0.05, *** p <0.001 significant).

Station Type	Cr % Removal	Fe % Removal	Ni % Removal
Urban	31.01	19.29	20.41
Urban Park	22.75	17.35	14.84
Urban Roadside	28.76	27.27	28.41
The Bosphorus Coast	36.10	48.20	24.04
Control Area	10.44	14.57	26.26
F **-test**	***	***	*

In nature, Cr is found in all phases (air, soil and water) of the environment depending on the parental material and it is considered a serious environmental pollutant. Soil Cr values range from 10 to 50 µg/g dw [24]. Cr is present in two oxidation states, +3 (is less toxic for plants) and +6 (highly toxic and mobile) and both Cr forms and their compounds have multifarious industrial uses, especially in metallurgical, refractory and chemical industries [24, 25]. They are extensively employed in leather processing and finishing, in the production of refractory steel, drilling muds, electroplating cleaning agents, catalytic manufacture and in the production of chromic acid and specialty chemicals [24-26]. The main source of Cr pollution is considered to be from dyestuffs and leather tanning when wastes are discharged directly into waste streams, either as liquids or as solids [24, 25].

Cr compounds are highly toxic to plants and are detrimental to their growth and development [24, 27]. Although some crops are not affected by low Cr concentration [27], Cr is toxic to higher plants at 100 µg/g dw [28]. Cr amounts of plants in the environment vary between 0.006-18 µg/g dw [29]. According to these values, the plant and soil samples in our study are between normal values and there is not Cr pollution in Istanbul, especially in our studied areas.

In a study, acorns belonged to 16 *Quercus* taxa were studied in different regions of Turkey and Cr concentrations were found between 0-27.5 µg/g whereas 117.5 µg/g in *Q. trojana* [30]. Aksoy and Demirezen 2006, in Kayseri found the highest Cr values in industrial area with 1.70 µg/g with *Fraxinus excelsior* and the highest Cr (124.42 µg/g) was found in soils in the urban roadside [6]. The results above showed variations in Cr amounts in Turkey. In our study, the increasing and decreasing Cr concentrations in plant and soil samples showed parallelism in different stations and as a result, it can be accepted that *C. siliquastrum* L. subsp. *siliquastrum* can be used as a good biomonitor plant for Cr.

2. Iron

Fe amounts in washed and unwashed leaves and barks of *C. siliquastrum* L. subsp. *siliquastrum* and the soil samples in five different station types are shown in Figure 2. The results were as follows: the highest Fe (134.74±35.06 µg/g dw) was measured in the unwashed leaf samples collected in urban roadside, while the lowest was measured in the washed leaf samples (44.97±5.15 µg/g dw) collected in urban area. The average highest soil Fe level was found in control area (3825.42±311.50 µg/g dw) while the lowest in urban area with 2589.35±306.39 µg/g dw.

Fe is the most important metal and one of the major constituents of the lithosphere. In soils, Fe occurs mainly in forms of oxides and hydroxides, as amorphous compounds, small particles, fillings in cracks and veins and coatings on other minerals or particles. Fe plays a special role in the behavior of several trace elements and is in the intermediate position between macro- and micronutrients in plants, animals and humans [31]. The Fe uptake by plants is metabolically controlled, although it may be absorbed as Fe^{+3} and Fe^{+2} or as Fe chelates [25]. 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 [32].

In the literature, the normal values of Fe concentrations in some plants vary between 43-376 µg/g dw in grasses and between 74-400 µg/g dw in clover [25]. According to Levy et al 1999, Fe contents which are higher than 500 µg/g dw are poisonous [33]. However, Romheld and Marschner 1991, suggested that Fe phytotoxicity is 400-1000 µg/g dw [34]. In this situation, the Fe contents of our plant samples are within normal limits. It was also observed that, washing the leaves reduced the Fe concentrations of leaves in all stations (Table 1). In our study, the difference between Fe levels of plant samples was in fewer amounts while in some other works, the difference between Fe values varied too much. In a study, Czarnowska and Milewska 2000 measured leaf Fe amounts between 121-1056 µg/g dw by using *Taraxacum officinale* in Warsaw Polonia related with the distance to the motorways [9]. In another study, Çelik et al 2005 used *Robinia pseudoacacia* L. and observed mean Fe values between 100.2-3087 µg/g dw in Denizli Turkey [11]. These results show that their plants have high accumulation capacity of Fe related with environmental conditions. Nevertheless, in our study we could not observe a correlation between Fe levels of soil and plant samples, especially between the soil and unwashed leaves. According to the values in this study, it can be explained that *C. siliquastrum* L. subsp. *siliquastrum* could not be a good biomonitor for Fe metal.

3. Nickel

Ni concentrations of three different samples (unwashed-washed leaves and barks) of *C. siliquastrum* L. subsp. *siliquastrum* and soil samples are shown in Figure 2. As a result of measurements, the average highest levels of Ni were in urban roadside (unwashed leaves) while the lowest was determined in control area (washed leaves). The values were 4.47 ± 0.82 μg/g and 2.19 ± 0.39 μg/g dw respectively. The average highest soil Ni values were measured in urban roadside with 15.07 ± 2.06 μg/g dw while the lowest were measured in control area with 5.34 ± 0.65 μg/g dw. The normal accepted Ni values in plant tissues are between 0.5-5 μg/g dw [35]. Ni is readily taken by plants from soil and the normal Ni values in soil are between 5-150 μg/g dw [25]. According to the results of this study, our plant and soil values are within normal limits. In addition, washing the leaves significantly reduced the Ni values (Table 1).

In nature, Ni is naturally found in soils, waters, and foods, and is emitted from volcanoes [2]. Environmental Ni pollution influences the concentrations of this metal in plants [25]. It is found in the environment mainly in combination with arsenic, antimony and sulfur. Metallic Ni is found alloyed with iron in many meteors and the Earth core is believed to contain substantial quantities [36, 37]. Therefore, a redistribution of this metal in the environment from the burning of fossil fuel, application of sludges to agricultural lands, and by industrial emissions should be of concern [25]. Commercially important Ni ores are garnierite, pyrrhotite and millerite. Moreover, consumer products that may contain Ni and compounds are Ni-Cd batteries, jewellery, coins, computer components, some paints and ceramics, goods containing stainless steel [36, 37].

There are some similar works carried out using different plant taxa and variable results were obtained. Elik and Akçay 2000, carried out a study in Sivas by using *Pinus sylvestris* and *Robinia pseudoacacia*'s unwashed leaves and they found out the Ni values 50 μg/g dw and 93 μg/g dw respectively [38]. Tuna et al 2005, used *Pinus* sp. and *Olea europaea* as biomonitors for Ni in Mugla [39]. The values of unwashed leaves were 3.2 μg/g dw for *Pinus* sp. and 3.9 μg/g dw for *Olea europaea*. These different results showed that in Turkey, Ni values are variable in different areas related to the types of source for this element.

In this study, it was observed that the concentrations of those three elements (Cr, Fe and Ni) showed variability in different station types related to type of their sources. The distinguishing ability between airborne and soil-borne contamination was assessed by washing the leaves with deionized distilled water. The highest % removal for Cr and Fe obtained in The Bosphorus Coast, while for Ni in urban roadside. Moreover, the lowest % removal for Cr and Fe was in control area, while for Ni in urban park.

Although we categorized the study area as five different stations, especially in many metropolitans like Istanbul, they are not separated with distinct borders and they are usually overlapped. Therefore, the results in different stations have showed similarity like in urban and urban park samples. High metal levels in bark samples observed in all types of stations could be the result of long time metal deposition capacity of *C. siliquastrum* L. subsp. *siliquastrum* and this is an advantage for this plant species as a biomonitor plant. In addition, we observed a strong correlation between Cr and Ni levels of soil and plant samples. Nevertheless, we could not observe a relation between Fe levels of soil and unwashed leaf samples. Table 2 reveals correlation coefficient (r) for each heavy metal and these are all highly significant at $p < 0.001$, except iron washed leaf-soil ($p < 0.01$) and iron bark-soil ($p < 0.05$). In soil, many reactions are involved in the solubility of Fe, among them hydrolysis and complexed compounds appear to be the most significant ones. It was reported by Lindsay 1979, the mobility of Fe in soils is largely controlled by the solubility of Fe^{+3} and Fe^{+2} amorphous hydrous oxides [40]. However, the formation of other Fe compounds, such as phosphates, sulfides, and carbonates, may greatly modify Fe solubilities. The content of soluble Fe in soil is extremely low in comparison with the total Fe content. In well-aerated areas, however, Fe^{+2} contributes little to the total soluble inorganic Fe, except under high soil pH conditions [25]. These situations may prevent the Fe uptake in many plant species, *C. siliquastrum* L. subsp. *siliquastrum* could be affected these conditions. According to the values in this study, it can be concluded that *C. siliquastrum* L. subsp. *siliquastrum* is a good biomonitor for Cr and Ni metals. On the other hand, for Fe metal, it cannot be used as a biomonitor or other supportive studies should be carried out in different station types with *C. siliquastrum* L. subsp. *siliquastrum*.

Table 2. Relationships between heavy metal concentration in Unwashed Leaf-Washed Leaf, Unwashed Leaf-Soil, Washed Leaf-Soil and Bark-Soil for *Cercis siliquastrum* L. subsp. *siliquastrum* (Correlation coefficient (r); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significant).

Element	Sample Number	Unwashed Leaf - Washed Leaf (r)	Unwashed Leaf - Soil (r)	Washed Leaf - Soil (r)	Bark - Soil (r)
Cr	59	0.81***	0.50***	0.64***	0.52***
Fe	59	0.86***	0.32**	0.43***	0.22*
Ni	59	0.91***	0.56***	0.54***	0.41***

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