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Verbascum bombyciferum Boiss. (Scrophulariaceae) as possible bio-indicator for the assessment of heavy metals in the environment of Bursa, Turkey

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Abstract In this study, we determined the heavy metal content (Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}) in the soil surrounding the roots and different organs of *Verbascum bombyciferum* Boiss. (Scrophulariaceae), which is endemic to Uludağ Mountain, Bursa, Turkey. Plant samples were collected from roadsides, and heavy metal accumulation capabilities were tested. This is one of the pioneer species of ruderal plant communities on roadsides, building sites, rubbish dumps, etc. Different organs of plant samples (roots, stems, leaves, and flowers) and their soils were analyzed by inductively couple plasma optical emission spectroscopy for their heavy metal contents. Some of the analyzed heavy metals (Cd^{2+}, Cr^{3+}, Pb^{2+}, and Zn^{2+}) were usually increased depending on the traffic in the sample sites, and this variation was also reflected in heavy metal content of plant samples. Our results show that this plant can be used as a bio-indicator species in the monitoring of increased Cd^{2+}, Cr^{3+}, Pb^{2+}, and Zn^{2+} in the environment. We also concluded that *V. bombyciferum* have the capability of Cd^{2+}, Cr^{3+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} accumulation.

Keywords Heavy metals · Biomonitoring · Ruderal plant · Roadside · *Verbascum bombyciferum*

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Introduction

Human being has increased the heavy metal contents of his environment by different ways such as industrial exhalations and wastes, agricultural applications, metalliferous mining and smelting, energy and fuel production, and vehicle emissions (Schwitzguebel 2001; Kim et al. 2003; Freitas et al. 2004; Swaileh et al. 2004; Sardans and Penuelas 2005; Zeidler 2005; González and González-Chávez 2006). There are many sites in the world polluted by heavy metals due to these activities. For instance, roadsides receive considerable amounts of traffic-generated pollutants, particularly lead (Sutherland and Tack 2000). Polluted sites in terrestrial and aquatic ecosystems have been monitored by using biological materials such as mosses (Rasmussen 1977; Yule and Lloyd 1984) and lichens (Seaward 1974; Seaward et al. 1981). In addition to these biological materials,

higher plants have been accepted as indicators for biomonitoring the heavy metal pollution in the environment (Aksoy and Öztürk 1997; Aksoy et al. 1999; Samecka-Cymerman and Kempers 2001; Pugh et al. 2002; Klump et al. 2002; Piczak et al. 2003; Swaileh et al. 2004; Zeidler 2005). Furthermore, the use of higher plants in the restoration of polluted sites and phytoremediation has recently become a tangible alternative to traditional clean up techniques (Schwitzguebel 2001; Chandra Sekhar et al. 2003; Pulford and Watson 2003; Freitas et al. 2004).

Verbascum is the second largest genus in the Turkish flora and includes numerous endemic species. *Verbascum bombyciferum* is one of the local endemic species of this genus and occurs at different altitudes of Uludağ Mountain (Davis 1978; Güleryüz and Malyer 1998). Seed of this species is offered for sale in Britain under the name of *Verbascum* "Bursa," "Brousa," or "Brussa" (Davis 1978) as an ornamental plant. Due to the high biomass production, it contributes to the soil organic material and to the following re-vegetation processes on destroyed areas (Güleryüz and Arslan 2001). In addition to high biomass production, high nitrate assimilation capacity of *V. bombyciferum* indicates the ruderal character of this species, especially on nitrate-rich environments (Güleryüz and Arslan 1999). In a previous study, Güleryüz et al. (2006) investigated the heavy metal contents of *V. olympicum* Boiss. and reported that this species can be considered as bio-indicator in the monitoring of some heavy metals in the environment. *V. olympicum* is the pioneer species of ruderal plant community on the disturbed areas in the sub-alpine and alpine belt of Uludağ Mountain (Rehder et al. 1994).

Plant composition or distribution in areas destroyed or contaminated by heavy metals may indicate a specific assemblage of plant species (Ellenberg 1988; Ernst 1990; Brown 1995; Brooks et al. 1998; Robinson et al. 1998). Some plants become dominant in the secondary sites, and they are pioneer species of ruderal plant communities (Ellenberg 1988). *V. bombyciferum* is widespread on destroyed areas such as roadsides, rubbish dumps, and picnic areas in Uludağ Mountain and public gardens, plantation areas, and archeological sites in Bursa city.

In this study, we aimed to identify the indicator value of *V. bombyciferum* collected from four populations on the roadsides, which are influenced by the different traffic intensities, by using heavy metal contents (Cd^{+2}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}) of this plant and their corresponding soils. Moreover, heavy metal contents in different organs (root, stem, leave, and flower) of the plant were also examined in order to obtain information about their distributions.

Material and methods

Species

V. bombyciferum is a biannual species and belongs to Scrophulariaceae. It is a hemicryptophyte with broad basal leaves. Stems are robust, terete, simple, or rarely with a few branches above. Basal leaves are ovate or obovate, obscurely crenate; cauline is similar but smaller, upper sessile, and entire. Inflorescence is usually simple and very dense; flowers immersed in long white indumenta. Flowering time is from May through June (Güleryüz and Malyer 1998; Davis 1978).

Sample sites

The research was performed in four sample sites (Soğukpınar village, Oldest Uludağ Road, Bursa–İstanbul Road, Bursa–Ankara Road), which were selected between 200- and 980-m altitudes from Uludağ Mountain. The bedrock of the sample sites is composed mainly of sandstone with the exception of the Oldest Uludağ Road, which lays on calcareous rock.

Site I (Soğukpınar village) The sample site has the highest altitude (980 m), and it was selected as a reference point for the study. Samples were collected around the buildings and along the village road with limited traffic activity.

Site II (Old Uludağ Road) The samples were collected along the road on which traffic inten-

sity is low. This site was selected approximately 10–20 m far from this road.

Site III (Bursa-İstanbul Road) and Site IV (Bursa-Ankara Road) The samples were collected on the disturbed areas within about 2 m of the road near the city of Bursa. These sites are the highways that connect Bursa to İstanbul (Bursa–İstanbul Road) and Ankara, the capital of Turkey (Bursa–Ankara Road), and traffic intensity is very high.

Sampling

Soil and plant samples were taken from five different places in each sampling site (10 × 10 m) on July 2006. Sampling of all plants was performed in the flowering phase. Plant samples were harvested together with aboveground and belowground parts. Soils were taken from 0 to 5 cm layer, sifted with a standard 4-mm sieve. Afterwards, samples of soil and plant were transferred to the laboratory in plastic bags. The soil samples were air-dried for heavy metal analyses. Plant samples were carefully separated into compartments (roots, stems, leaves, and flowers). They were washed with tap water and then with deionized water. Samples were dried in an oven (105°C) until their weight became constant. Then, all plant material was grounded using a mortar and pestle. Homogenized plant material and soil samples were stored in clear paper bags for heavy metal analyses.

Chemical and statistical analyses

Soil samples (0.5 g dry weight) were digested with 10 ml pure HNO_3 (65%), using a CEM-MARS 5 (CEM Corporation Mathews, NC, USA) microwave digestion system (digestion conditions are the following: maximum power, 1,200 W; power (%), 100; ramp. (min), 20:00; pressure (psi), 180; temperature (°C), 180; and hold time (min), 10:00). After digestion, the volume of each sample was adjusted to 25 ml using double deionized water (Yılmaz 2007). Homogenized plant samples (0.5 g dry weight) were also prepared using the same procedure for heavy metal analyses. The solution of soil and plant samples was analyzed (Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}) by inductively couple plasma optical emission spectroscopy (ICP-OES; Varian-Liberty II). All chemicals were analytical reagent grade. Detection limits of Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} are 0.3×10^{-3}, 0.3×10^{-3}, 0.5×10^{-3}, 0.2×10^{-3}, 0.8×10^{-3}, 2×10^{-3}, and 0.2×10^{-3} mg/kg, respectively.

The difference among sample sites regarding heavy metal contents of soils and plant organs (flowers, leaves, stems, and roots) were tested by analysis of variance. Subsequent pair-wise comparisons were performed using Tukey honestly significant difference post hoc tests. Simple correlations between heavy metal contents of the soils and plant organs were also tested. All tests were analyzed in the significance level of 0.05. Statistical analyses were carried out using the Statistica v. 5.0 software package (StatSoft, Inc., 1984–1995).

Table 1 Comparison of the sampling sites according to mean values of elements (Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}) determined in soil solution digested in HNO_3 (65%)

Elements (mg/kg DW)	Sampling sites			
	Site I	Site II	Site III	Site IV
Cd^{2+}	$0.18^{c} \pm 0.11$	$0.48^{b} \pm 0.08$	$0.59^{ab} \pm 0.14$	$0.78^{a} \pm 0.15$
Cr^{3+}	$23.6^{cb} \pm 10.1$	$33.7^{b} \pm 4.4$	$53.2^{ab} \pm 14.0$	$70.9^{a} \pm 19.6$
Cu^{2+}	$25.1^{a} \pm 13.3$	$36.4^{a} \pm 13.4$	$37.7^{a} \pm 19.9$	$46.5^{a} \pm 14.3$
Fe^{3+}	$45.2^{a} \pm 19.1$	$25.0^{a} \pm 4.0$	$80.9^{a} \pm 13.8$	$37.5^{a} \pm 6.0$
Ni^{2+}	$15.0^{a} \pm 4.2$	$13.4^{a} \pm 1.5$	$21.3^{a} \pm 4.8$	$20.0^{a} \pm 2.3$
Pb^{2+}	$10.2^{b} \pm 2.4$	$9.6^{b} \pm 1.8$	$18.4^{a} \pm 2.3$	$22.1^{a} \pm 4.1$
Zn^{2+}	$9.3^{b} \pm 1.2$	$9.7^{b} \pm 0.8$	$15.8^{a} \pm 3.1$	$14.6^{a} \pm 1.2$

For mean soil element values, different letters indicate significant differences between the sampling sites according to Tukey's HSD test (rejection level 0.05). $n = 5$, means ± standard deviation

Table 2 Mean values of Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} determined in organs and whole plant (mg/kg DW) of *V. bombyciferum* collected from different sites

Plant organ		Sampling sites			
		Site I	Site II	Site III	Site IV
Cd^{2+}	Flowers	$0.01^{b} \pm 0.00$	$0.01^{b} \pm 0.00$	$0.01^{b} \pm 0.00$	$0.33^{a} \pm 0.12$
	Leaves	$0.01^{b} \pm 0.00$	$0.01^{b} \pm 0.00$	$0.01^{b} \pm 0.00$	$0.24^{a} \pm 0.04$
	Stems	$0.01^{b} \pm 0.00$	$0.01^{b} \pm 0.00$	$0.01^{b} \pm 0.00$	$0.67^{a} \pm 0.18$
	Aboveground total	$0.03^{b} \pm 0.01$	$0.03^{b} \pm 0.01$	$0.03^{b} \pm 0.01$	$1.24^{a} \pm 0.25$
	Roots	$0.01^{c} \pm 0.00$	$0.01^{c} \pm 0.00$	$0.27^{b} \pm 0.06$	$0.65^{a} \pm 0.18$
	Whole plant	$0.04^{c} \pm 0.01$	$0.04^{c} \pm 0.01$	$0.30^{b} \pm 0.01$	$1.89^{a} \pm 0.34$
Cr^{3+}	Flowers	$0.05^{b} \pm 0.01$	$0.07^{b} \pm 0.02$	$0.25^{b} \pm 0.14$	$20.20^{a} \pm 2.92$
	Leaves	$0.07^{b} \pm 0.03$	$0.19^{b} \pm 0.02$	$0.37^{b} \pm 0.08$	$66.25^{a} \pm 13.46$
	Stems	$0.04^{c} \pm 0.02$	$1.44^{b} \pm 0.30$	$0.77^{bc} \pm 0.19$	$7.67^{a} \pm 1.15$
	Aboveground total	$0.16^{b} \pm 0.02$	$1.70^{b} \pm 0.28$	$1.39^{b} \pm 0.34$	$94.12^{a} \pm 13.79$
	Roots	$0.02^{b} \pm 0.02$	$1.21^{b} \pm 0.27$	$42.71^{a} \pm 9.06$	$36.75^{a} \pm 8.32$
	Whole plant	$0.18^{c} \pm 0.02$	$2.91^{c} \pm 0.26$	$44.10^{b} \pm 8.88$	$130.86^{a} \pm 6.51$
Cu^{2+}	Flowers	$1.08^{b} \pm 0.48$	$0.99^{b} \pm 0.01$	$0.98^{b} \pm 0.02$	$8.48^{a} \pm 1.37$
	Leaves	$0.30^{b} \pm 0.12$	$1.00^{b} \pm 0.03$	$1.00^{b} \pm 0.00$	$10.39^{a} \pm 0.69$
	Stems	$0.27^{c} \pm 0.19$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$2.15^{a} \pm 0.40$
	Aboveground total	$1.65^{c} \pm 0.46$	$2.99^{b} \pm 0.03$	$2.98^{b} \pm 0.02$	$21.02^{a} \pm 0.70$
	Roots	$1.00^{b} \pm 0.01$	$1.00^{b} \pm 0.01$	$3.47^{a} \pm 0.79$	$2.19^{a} \pm 0.52$
	Whole plant	$2.65^{c} \pm 0.46$	$3.99^{c} \pm 0.03$	$6.45^{b} \pm 0.81$	$23.21^{a} \pm 0.52$
Fe^{3+}	Flowers	$8.25^{a} \pm 0.53$	$9.00^{a} \pm 0.40$	$4.44^{c} \pm 0.17$	$11.37^{a} \pm 1.77$
	Leaves	$8.55^{a} \pm 0.85$	$8.85^{a} \pm 0.33$	$4.73^{b} \pm 0.01$	$7.32^{a} \pm 1.12$
	Stems	$7.27^{b} \pm 1.14$	$8.91^{ab} \pm 0.34$	$4.65^{bc} \pm 0.08$	$11.34^{a} \pm 2.92$
	Aboveground total	$24.07^{c} \pm 1.48$	$26.76^{b} \pm 0.60$	$13.82^{d} \pm 0.15$	$30.00^{a} \pm 2.03$
	Roots	$9.13^{b} \pm 0.28$	$8.16^{b} \pm 1.96$	$10.55^{b} \pm 2.29$	$16.75^{a} \pm 2.24$
	Whole plant	$33.20^{b} \pm 1.76$	$34.92^{b} \pm 2.13$	$24.37^{c} \pm 2.35$	$46.75^{a} \pm 1.18$
Ni^{2+}	Flowers	$1.33^{b} \pm 0.45$	$1.33^{b} \pm 0.02$	$1.33^{b} \pm 0.02$	$4.23^{a} \pm 0.25$
	Leaves	$0.41^{b} \pm 0.10$	$1.31^{b} \pm 0.03$	$1.32^{b} \pm 0.02$	$4.88^{a} \pm 0.33$
	Stems	$0.76^{b} \pm 0.40$	$1.30^{b} \pm 0.03$	$1.32^{b} \pm 0.03$	$14.27^{a} \pm 0.94$
	Aboveground total	$2.50^{c} \pm 0.94$	$3.94^{b} \pm 0.02$	$3.97^{b} \pm 0.03$	$23.38^{a} \pm 1.20$
	Roots	$1.33^{c} \pm 0.03$	$1.31^{c} \pm 0.01$	$21.01^{a} \pm 8.31$	$11.44^{b} \pm 1.44$
	Whole plant	$3.83^{c} \pm 0.92$	$5.25^{c} \pm 0.02$	$24.98^{b} \pm 8.31$	$34.82^{a} \pm 1.73$
Pb^{2+}	Flowers	$0.02^{b} \pm 0.01$	$0.15^{b} \pm 0.01$	$0.15^{b} \pm 0.00$	$12.19^{a} \pm 1.25$
	Leaves	$0.03^{b} \pm 0.02$	$0.15^{b} \pm 0.01$	$0.15^{b} \pm 0.01$	$7.09^{a} \pm 1.31$
	Stems	$0.05^{b} \pm 0.06$	$0.15^{b} \pm 0.00$	$0.15^{b} \pm 0.01$	$21.93^{a} \pm 8.00$
	Aboveground total	$0.10^{b} \pm 0.06$	$0.45^{b} \pm 0.01$	$0.45^{b} \pm 0.01$	$41.21^{a} \pm 8.35$
	Roots	$0.15^{c} \pm 0.01$	$0.15^{c} \pm 0.00$	$11.99^{b} \pm 2.43$	$34.98^{a} \pm 3.93$
	Whole plant	$0.25^{c} \pm 0.06$	$0.59^{c} \pm 0.01$	$12.44^{b} \pm 2.43$	$76.19^{a} \pm 8.64$
Zn^{2+}	Flowers	$5.08^{b} \pm 0.74$	$4.89^{b} \pm 0.02$	$4.87^{b} \pm 0.01$	$7.44^{a} \pm 0.20$
	Leaves	$2.17^{c} \pm 0.36$	$4.93^{b} \pm 0.02$	$4.81^{b} \pm 0.02$	$7.07^{a} \pm 0.23$
	Stems	$2.14^{c} \pm 0.57$	$4.95^{b} \pm 0.01$	$4.96^{b} \pm 0.00$	$7.68^{a} \pm 0.09$
	Aboveground total	$9.39^{c} \pm 0.92$	$14.77^{b} \pm 0.02$	$14.64^{b} \pm 0.02$	$22.19^{a} \pm 0.36$
	Roots	$4.92^{c} \pm 0.02$	$4.94^{c} \pm 0.01$	$6.89^{b} \pm 0.22$	$8.25^{a} \pm 0.23$
	Whole plant	$14.31^{d} \pm 0.93$	$19.71^{c} \pm 0.03$	$21.53^{b} \pm 0.21$	$30.43^{a} \pm 0.45$

For mean soil element values, different letters indicate significant differences between the sampling sites according to Tukey's HSD Test (rejection level 0.05). $n = 5$, means ± standard deviation

Results and discussion

The mean heavy metal (Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) contents of soils and different organs of *V. bombyciferum* are given in Tables 1 and 2. The mean Cd^{2+} contents in soils of all sample sites varied between 0.18 and 0.78 mg/kg dry weight. These values were lower

than the Cd^{2+} levels of an uncontaminated soil (Temmerman et al. 1984). However, there was a difference among sample sites regarding soil Cd^{2+} content ($P < 0.05$; Table 1). While the lowest Cd^{2+} content was found in the soil samples of site I (0.18 mg/kg dry weight), the highest Cd^{2+} content was determined in the soils of site IV (0.78 mg/kg dry weight). The increase in Cd^{2+} concentration in the soil of site IV sampling site may be attributed to higher traffic intensity in this site. Higher Cd^{2+} content were also determined in *V. bombyciferum* plants taken from this site (Table 2). The mean Cd^{2+} content of plant samples (1.89 mg/kg dry weight) taken from site IV was 47.3-fold higher than site I. This value indicates that *V. bombyciferum* can accumulate significant amount of Cd^{2+} concentrations. Because the mean Cd^{2+} content of *V. bombyciferum* plants taken from this site was higher than that of a plant taken from in non-polluted environment (0.01–0.3 mg/kg dry weight) (Allen 1989). The mean Cd^{2+} content of *V. bombyciferum* plants taken from site IV was higher than that of a plant taken from non-polluted environment (0.01–0.3 mg/kg dry weight; Allen 1989). Significant correlation was found between Cd^{2+} content of soil and all organs collected ($P < 0.05$) (Table 3). The correlation between Cd^{2+} content of roots and aboveground organs (stems, leaves, and flowers) is significant as well ($P < 0.05$; Table 4). These suggest that Cd^{2+} can be taken up to the roots of *V. bombyciferum*, and it can be transported to aboveground organs.

According to Temmerman et al. (1984), the upper limit of chromium in non-polluted soil is 15 mg/kg. Table 1 shows that soil Cr^{3+} contents of all sample sites were higher than that of non-polluted soils. There was significant difference between sample sites in terms of the soil chromium content ($P < 0.05$). The Cr^{3+} content was lowest in soils of site I (23.6 mg/kg dry weight). On the other hand, the highest Cr^{3+} content was determined in the soils of site IV (70.9 mg/kg dry weight). Cr^{3+} contents in different organs and whole plants of *V. bombyciferum* taken from this sample site were also higher than that of other sites (Table 2). This was a reflection of high soil Cr^{3+} concentration, and it indicates the Cr^{3+}-accumulating capacity of *V. bombyciferum*. Except for plant samples taken from site I, the mean Cr^{3+} content of *V. bombyciferum* was between 2.91 and 130.86 mg/kg dry weight (Table 2). These values were higher than the normal Cr^{3+} composition (1.5 mg/kg dry weight) in a plant (Markert 1994). Furthermore, according to Allen (1989), 0.5 mg/kg dry weight Cr^{3+} concentrations are considered as toxic to plants. Our results indicate that this species is a bio-monitor for Cr^{3+}. However, a Cr^{3+} distribution model among plant organs was not observed. For example, Cr^{3+} was accumulated in aboveground parts in plant samples taken from site IV (94.12 mg/kg dry weight), whereas it was accumulated in roots of plant samples taken from site III (42.71 mg/kg dry weight; Table 2).

Copper is one of the pollutants in the soils of all sample sites. The mean Cu^{2+} content in the soils of all sample sites was higher than the upper Cu^{2+} limit of a non-polluted soil (15 mg/kg) (Temmerman et al. 1984) reaching to 46.5 mg/kg dry weight in the soils of site IV. No significant difference in soil Cu^{2+} content was found among sample sites (Table 1). Although soil Cu^{2+} contents of all sample sites were high, the Cu^{2+} contents of *V. bombyciferum* taken from sites I, II, and III were lower than the normal Cu^{2+} concentration levels of a plant (10 mg/kg dry weight) (Markert 1994). High Cu^{2+} content was only determined in the plant samples taken from site IV (23.21 mg/kg dry weight). This value was also above the poisonous limits of Cu^{2+} (5–20 mg/kg dry weight; Allen 1989). Significantly positive correlations were only found between soil Cu^{2+} and the mean Cu^{2+} contents of stems and flowers ($P < 0.05$; Table 4). There was no significant correlation between the Cu^{2+} content of roots and other organs ($P > 0.05$). For this reason, the Cu^{2+} distribution model among plant organs is not clear.

Iron content in the soils of sample sites varied between 25.0 and 80.9 mg/kg dry weight, and the difference among sample sites regarding to soil Fe^{3+} content in soil samples was not significant ($P > 0.05$; Table 1). However, the difference among sample sites regarding the mean Fe^{3+} content in whole plant samples was significant ($P < 0.05$; Table 2). The highest Fe^{3+} content was determined in plant samples collected from site IV (46.75 mg/kg dry weight), whereas the lowest was

Table 3 Simple correlation coefficients (r^2), significant levels (possibility, P), and linear regression equations $Y = a + bx$ between the acid-soluble contents of elements in soil and different organs (mg/kg DW) of *V. bombyciferum* Boiss

Parameters	r^2	P	$Y = a + bx$
Soil–Cd^{2+}			
Root–Cd	0.562	0.000	Root − Cd = −0.2020 + 0.854x Soil − Cd
Stem–Cd	0.376	0.004	Stem − Cd = −0.2098 + 0.7580x Soil − Cd
Leaf–Cd	0.444	0.001	Leaf − Cd = −0.0774 + 0.2822x Soil − Cd
Flower–Cd	0.442	0.001	Flower − Cd = −0.1208 + 0.4102x Soil − Cd
Soil–Cr^{3+}			
Root–Cr	0.395	0.003	Root − Cr = − 6.6453 + 0.5915x Soil − Cr
Stem–Cr	0.553	0.000	Stem − Cr = − 2.3105 + 0.1056x Soil − Cr
Leaf–Cr	0.532	0.000	Leaf − Cr = − 27.7923 + 0.98162x Soil − Cr
Flower–Cr	0.427	0.002	Flower − Cr = − 6.8525 + 0.2645x Soil − Cr
Soil–Cu^{2+}			
Root–Cu	0.041	0.390	Root − Cu = 1.3941 + 0.0142x Soil − Cu
Stem–Cu	0.147	0.096	Stem − Cu = 0.4843 + 0.0171x Soil − Cu
Leaf–Cu	0.133	0.113	Leaf − Cu = − 0.3592 + 0.0969x Soil − Cu
Flower –Cu	0.199	0.048	Flower − Cu = − 0.5148 + 0.0933x Soil − Cu
Soil–Fe^{3+}			
Root–Fe	0.000	0.930	Root − Fe = 10.9882 + 0.0034x Soil − Fe
Stem–Fe	0.383	0.004	Stem − Fe = 11.5361 − 0.0741x Soil − Fe
Leaf –Fe	0.458	0.001	Leaf − Fe = 9.7332 − 0.0503x Soil − Fe
Flower –Fe	0.534	0.000	Flower − Fe = 12.0996 − 0.0815x Soil − Fe
Soil–Ni^{2+}			
Root–Ni	0.414	0.002	Root − Ni = − 4.7586 + 0.7298x Soil − Ni
Stem–Ni	0.015	0.612	Stem − Ni = 2.8086 + 0. 0867x Soil − Ni
Leaf–Ni	0.025	0.504	Leaf − Ni = 1.3433 + 0. 0344x Soil − Ni
Flower–Ni	0.019	0.565	Flower − Ni = 1.6486 + 0.0220x Soil − Ni
Soil–Pb^{2+}			
Root–Pb	0.695	0.000	Root − Pb = − 18.8603 + 2.0384x Soil − Pb
Stem–Pb	0.372	0.004	Stem − Pb = − 10.2103 + 1.0484x Soil − Pb
Leaf–Pb	0.434	0.002	Leaf − Pb = − 3.3490 + 0. 3458x Soil − Pb
Flower–Pb	0.473	0.001	Flower − Pb = − 6.1458 + 0.6160x Soil − Pb
Soil–Zn^{2+}			
Root–Zn	0.584	0.000	Root − Zn = 2.2099 + 0. 3265x Soil-Zn
Stem–Zn	0.302	0.021	Stem − Zn = 0.8681 + 0.3287xSoil − Zn
Leaf–Zn	0.319	0.009	Leaf − Zn = 1.0476 + 0.2987xSoil − Zn
Flower–Zn	0.107	0.159	Flower − Zn = 4.1723 + 0.1127x Soil − Zn

($n = 20$, $P < 0.05$ significant correlation)

determined in plant samples collected from site III (24.36 mg/kg dry weight). It is interesting that the mean Fe^{3+} contents of *V. bombyciferum* plants taken from all sample sites were lower than the normal Fe^{3+} composition of a plant (150 mg/kg dry weight; Markert 1994). Iron content of aboveground organs of all examined plant samples was higher than that of roots (Table 2). This suggests that Fe^{3+} can be transported to aboveground organs and agrees with its biochemical role (Marschner 1995). Significantly high positive correlation was only found between Fe^{3+} content of soil and aboveground organs ($P < 0.05$).

In addition to Cr^{3+} and Cu^{2+}, high Ni^{2+} contents were determined in soils of all sample sites. These values were many times higher than the Ni^{2+} content of a non-polluted soil (1 mg/kg dry weight; Temmerman et al. 1984), and they indicate that there was probably a Ni^{2+} pollution source. On the other hand, soil Ni^{2+} content were highest in sites III and IV due to the possible effects of traffic. As shown in the soil, the mean Ni^{2+} contents of plants at all sites were higher than that of a normal plant (1.5 mg/kg dry weight; Markert 1994). There was significant difference among sample sites regarding the Ni^{2+} content of whole plant and all organs ($P < 0.05$; Table 2). The mean Ni^{2+} content (3.83 mg/kg dry weight) of *V. bombyciferum* plants in site I was approximately twofold higher than the normal Ni^{2+}

Table 4 Correlation coefficients (r^2), significant levels (possibility, P), and linear regression equations ($Y = a + bx$) between the element contents (Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}) of roots and other organs ($n = 20$, $P < 0.05$ significant correlation)

Parameters	r^2	P	$Y = a + bx$
Root–Cd^{2+}			
Stem–Cd	0.675	0.000	Stem − Cd = − 0.0325 + 0.8880x Root − Cd
Leave–Cd	0.732	0.000	Leave − Cd = − 0.0081 + 0.3167x Root − Cd
Flower–Cd	0.816	0.000	Flower − Cd = − 0.0263 + 0.4867x Root − Cd
Root–Cr^{3+}			
Stem–Cr	0.193	0.053	Stem − Cr = 1.1436 + 0.0662x Root − Cr
Leave–Cr	0.184	0.059	Leave − Cr = 4.3365 + 0.6136x Root − Cr
Flower–Cr	0.214	0.040	Flower − Cr = 1.1253 + 0.19905x Root − Cr
Root–Cu^{2+}			
Stem–Cu	0.114	0.145	Stem − Cu = 0.6946 + 0.2151x Root − Cu
Leave–Cu	0.035	0.433	Leave − Cu = 1.8226 + 0.7044x Root − Cu
Flower –Cu	0.011	0.663	Flower − Cu = 2.2892 + 0.3098x Root − Cu
Root–Fe^{3+}			
Stem–Fe	0.133	0.114	Stem − Fe = 4.9905 + 0.2738x Root − Fe
Leave –Fe	0.027	0.488	Leave − Fe = 8.2183 − 0.0768x Root − Fe
Flower –Fe	0.203	0.046	Flower − Fe = 4.7373 + 0.3158x Root − Fe
Root–Ni^{2+}			
Stem–Ni	0.036	0.426	Stem − Ni = 3.3672 + 0. 1196x Root − Ni
Leave–Ni	0.068	0.269	Leave − Ni = 1.5455 + 0. 0496x Root − Ni
Flower–Ni	0.028	0.478	Flower − Ni = 1.8467 + 0.0238x Root − Ni
Root–Pb^{2+}			
Stem–Pb	0.750	0.000	Stem − Pb = − 1.6222 + 0.6085x Root − Pb
Leave–Pb	0.807	0.000	Leave − Pb = − 0.4186 + 0.1924x Root − Pb
Flower–Pb	0.872	0.000	Flower − Pb = − 0.9169 + 0.3420x Root − Pb
Root–Zn^{2+}			
Stem–Zn	0.679	0.000	Stem − Zn = − 2.2789 + 1.1541x Root − Zn
Leave–Zn	0.638	0.000	Leave − Zn = − 1.4411 + 0.9896x Root − Zn
Flower–Zn	0.566	0.000	Flower − Zn = 1.7792 + 0.6061x Root − Zn

content of a plant. This shows the Ni^{2+} accumulation capacity of *V. bombyciferum*, and this accumulation is seen prominently in the plant samples in sites III and IV sample sites that are exposed to more intensive traffic (Table 2). The mean Ni^{2+} contents of plant samples from these sites were many times higher than the poisonous Ni^{2+} level (5 mg/kg dry weight; Allen 1989). The significant positive correlation between nickel contents of soils and roots ($P < 0.05$) indicates the high contribution of roots in Ni^{2+} accumulation capacity. However, a significant nickel distribution model was not observed for this species (Table 2).

Soil Pb^{2+} contents of all sample sites were lower than the upper Pb^{2+} limit of non-polluted soil (50 mg/kg dry weight; Temmerman et al. 1984). The difference between Pb^{2+} content in soil samples from four sites was significant ($P < 0.05$; Table 1). The highest mean Pb^{2+} content was found in the soils of site IV (22.1 mg/kg dry weight). According to Markert (1994), the normal lead composition is 1.0 mg/kg dry weight in a plant, and the mean lead contents of *V. bombyciferum* plants taken from sites I and II were lower than this level (respectively, 0.25 and 0.60 mg/kg dry weight). On the other hand, the highest Pb^{2+} levels (76.19 mg/kg dry weight) were found in plant samples taken from site IV. There was a significant correlation between Pb^{2+} contents of soils and all organs of plants (Table 3). Furthermore, the correlation between Pb^{2+} content of roots and other organs was significant ($P < 0.05$; Table 4). This suggests that the capability of this species in taking and accumulating Pb^{2+} is correlated with the Pb^{2+} content in soils.

Zinc content in the soils of sample sites varied between 9.3 and 15.8 mg/kg dry weight. Significant difference was found among sample sites in terms of Zn^{2+} content in soil (Table 1). Although Zn^{2+} contents in soils of site III road and site IV were

higher than that of the other sites, they were lower than the upper Zn^{2+} limit of non-polluted site (100 mg/kg dry weight; Temmerman et al. 1984). In addition, the mean Zn^{2+} contents in *V. bombyciferum* plants were not higher than the normal value in a plant (50 mg/kg dry weight; Markert 1994) and within the values considered normal by Shaw et al. (2004) (8–100 mg/kg dry weight). For instance, the mean Zn^{2+} content in plants collected from site IV was in this limit (30.44 mg/kg dry weight; Table 2). These results indicate that *V. bombyciferum* has no Zn^{2+} accumulation capacity. However, the high positive correlation between soil Zn^{2+} content and different organs (roots, stems and leaves; $P < 0.05$) can reflect the bio-indicator characteristic of this species for Zn^{2+}.

Conclusion

V. bombyciferum can play an important role in the monitoring of Cd^{2+}, Cr^{3+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} in its environment. These heavy metals, except for Zn^{2+}, can effectively be accumulated by this species. Our findings were also supported by the previous studies made on the heavy metal accumulation capacities and bio-indicator characteristics of other *Verbascum* species (Kfayatullah et al. 2001; Freitas et al. 2004; Güleryüz et al. 2006).

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