Anatolian Journal of Botany
5(2): 138-143 (2021)
Research article *doi:10.30616/ajb.999023*

Accumulation of cadmium, copper and zinc in selected natural *Viola* taxa in Turkish Mediterranean serpentine soils

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Received : 22.09.2021
Accepted : 13.10.2021
Online : 21.10.2021

Türkiye Akdeniz serpantin topraklarında seçilmiş doğal *Viola* taksonlarının kadmiyum, bakır ve çinko akümülasyonu

Abstract: Currently, 55 serpentine areas have been determined in Turkey. More than 60 Ni-accumulators and more than 43 serpentine-endemics are known from these areas. Within our field studies in the Mediterranean phytogeographic region, 8 *Viola* taxa distributed in serpentine areas and their respective soil samples were collected. Cd, Cu, and Zn concentrations of the soil and plant samples were investigated. After the plant and soil samples were digested in the microwave, metal measurements were made using a ICP-OES device. As a result of the Cd, Cu, and Zn measurements, none of the 8 *Viola* taxa collected from different localities were found to be hyperaccumulators. However, it was determined that *Viola kizildaghensis* has an accumulator feature in terms of Zn.

Key words: accumulator, serpentine soil, *Viola* taxa, Zinc

Özet: Ülkemizde 55 serpantin alan belirlenmiş ve bu alanlarda 60'dan fazla Ni akümülatörü ve 43'den fazla sayıda serpantin endemiği olduğu tespit edilmiştir. Akdeniz fitocoğrafik bölgesinde yapmış olduğumuz arazi çalışmalarında serpantin alanlarda yayılış gösteren 8 *Viola* taksonu ve bunların yetiştiği toprak örnekleri toplandı. Toplanan 8 *Viola* taksonunun yetiştiği topraklar ve bitkilerdeki Cd, Cu, ve Zn konsantrasyonları araştırılmıştır. Bitki ve toprak örnekleri mikrodalgada çözüldükten sonra metal ölçümleri ICP-OES cihazında yapılmıştır. Farklı lokalitelerden toplanan 8 *Viola* taksonunda yapılan Cd, Cu, ve Zn ölçümleri sonucunda hiçbir bitkide hiperakümülatör özelliği tespit edilememiştir. Ancak, *Viola kizildaghensis* bitkisinin Zn yönünden akümülator özelliğinin olduğu belirlenmiştir.

Anahtar Kelimeler: akümülatör, serpantin topraklar, *Viola* taksonları, çinko

Citation: Aksoy A (2021). Accumulation of cadmium, copper and zinc in selected natural *Viola* taxa in Turkish Mediterranean serpentine soils. Anatolian Journal of Botany 5(2): 138-143.

1. Introduction

It is becoming increasingly important to use hyperaccumulator or accumulator plants in stabilizing environments contaminated with heavy metals. Among heavy metals, Cd, Cu, and Zn are toxic elements that are easily taken up by plants and translocated to different parts of the plant.

Some of the earlier dry matter concentration thresholds for hyperaccumulation of trace elements in terrestrial plants have been revised recently. Recommendations by Krämer (2010), van der Ent et al. (2013), Remigio et al. (2020) and Peng et al. (2020), for plants that are growing in their natural habitat, are as follows: Cd > 100 mg kg^{-1}; Cu 300 mg kg^{-1}; Zn 3,000 mg kg^{-1}. The global hyperaccumulator database (www.hyperaccumulators.org) includes 721 hyperaccumulators, and their number is growing. Most hyperaccumulators species (532 species) are identified as Ni hyperaccumulators, while hyperaccumulators of Cd (7 species) and As (5 species), which currently pose the greatest threat to human health, are notably scarce (Reeves et al., 2018). At the same time, hyperaccumulator plants must manage to grow and to survive in an environment rich in metals.

Many studies have been carried out about serpentine areas in Turkey. Until now, 55 serpentine areas have been examined in Turkey and 62 Ni-accumulators and more than 43 serpentine-endemics have been reported from these areas (Reeves, 1998; Reeves et al., 1983, 2001, 2009; Reeves and Adıgüzel, 2004, 2008; Adıgüzel and Reeves, 2012; Altınözlü et al., 2012; Aksoy et al., 2015; Çelik et al., 2018). According to the Flora of Turkey, it is reported that there are 248 plants specific to serpentine areas, of which 119 are serpentinophytes and 129 are serpentinophages (Kurt et al., 2013; Özdeniz et al. 2017). According to Kurt et al. 2013 and Özdeniz et al., 2017, *Viola* species were not recorded in the serpentine flora of Turkey.

The hyperaccumulator/accumulator properties of some *Viola* species have been investigated by various researchers. Hyperaccumulator/accumulator properties have been determined for *Viola calaminaria* (Ging.) Nauenb., *V. guestphalica* Nauenb. (Zn and Pb), *V. baoshanensis* W.S.Shu, WLiu & CY.Lan (Cd), *V. principis* H.Boissieu (As, Cd and Pb), *V. kopaonikensis*, Pančić ex Tomović & Niketić, *V. elegantula* Schott and *V. beckiana* F.Fiala ex Beck (Ni, Cd, and Pb), as well as *V. arsenica* Beck, *V. allcharensis* G.Beck and *V. macedonica* Boiss. & Heldr. (As, Sb and Tl) (Brooks, 1998; Jedrzejczyk et al., 2002; Liu et al., 2004; Lei et al., 2008; Stevanović et al., 2010; Bačeva et al., 2014: Tomović et al., 2021).

The genus *Viola* L. is a member of the family *Violaceae* and is represented by approximately 600 taxa in the World (Marcussen et al., 2015; Düşen et al., 2018). In Turkey the genus *Viola* is represented by 36 taxa, of which 14 are endemic (Coode and Cullen, 1965; Davis et al., 1988; Yıldırımlı, 2000; Dinç, 2012; Adıgüzel and Reeves, 2012; Knoche and Marcussen, 2016; Düşen et al., 2018).

According to the current literature, there is no study that investigated the hyperaccumulator/accumulator properties of *Viola* taxa from Turkey. In this study, 8 *Viola* taxa were collected from serpentine areas of the Mediterranean Region and Cd, Cu and Zn concentrations of the soils in which these plants grow were determined. The aim of this study was to investigate whether some of the *Viola* taxa studied have hyperaccumulator/accumulator properties for Cd, Cu and Zn.

2. Materials and Methods

2.1. Materials

Soil samples and specimens of the investigated 8 *Viola* taxa were collected from serpentine areas in the Mediterranean phytogeographical region in 2016 (Table 1). For each taxon, three soil samples (24 in total) were collected from the areas where the *Viola* taxa (Table 1) were growing. Plant and soil samples were collected in 3 replicates for heavy metal analysis. Further, the collected specimens were stored in the Herbarium of Akdeniz University.

2.2. Sampling

Soils were taken from the 0-5 cm zone, brought to the laboratory, sieved with a standard 4 mm sieve and were than air-dried. At least 15-50 adult plants were randomly selected and collected from each site. Two to ten plants were retained as herbarium specimens. The remaining other plants from each site were divided into above- and below-ground parts. These were transferred to the laboratory in plastic bags. Plant samples were washed with tap water, followed by deionized water. They were dried in an oven (80 °C) until they reached constant weight and brittleness. The samples were subsequently ground with a pestle and mortar. Homogenized plant materials and soil samples were then stored in clean paper bags before heavy-metal analysis.

2.3. Chemical and statistical analyses

Soil samples (0.5 g dry weight) were digested with 10 ml of pure HNO_3 (65%), using a CEM-MARS 5 microwave digestion system (digestion conditions were the following: maximum power 1200 W; power 100%; ramp time 20 min, pressure 180 psi; temperature 180 °C; and hold time 10 min). After digestion, the volume of each sample was adjusted to 25 mL by using double-deionized water. Homogenized plant samples (0.5 g dry weight) were also prepared using the same procedure as for heavy-metal analysis. The soil and plant samples were analyzed for Cd, Cu, and Zn by ICP-OES; Varian-Liberty II. All chemicals were of analytical reagent grade. Standard peach leaves (NIST, SRM-1547) were used as a reference material. All analytical procedures were performed using this reference material. Soil and plant samples were digested in triplicate and analyzed. The means and standard deviation (SD) of the data were calculated using SPSS v15.0 (SPSS Inc., Chicago, IL, USA).

Table 1. Collecting sites and protection categories of *Viola* taxa.

No	Collected species	Turkish name	Collecting places	IUCN
1	*Viola alba* Besser subsp. *dehnhardtii* (Ten.) W. Becker	Meşe menekşesi	B5: Kayseri, Yahyalı Çamlıca village, Kayapınar location, 1410m, 11.08.2016, *Aksoy2645*	-
2	*V. dirimliensis* Blaxland	Dirmil menekşesi	C2: Burdur-Altınyayla, Dirmil Pass,1630 m, 21.04.2016. *Aksoy 2569*	CR
3	*V. heldreichiana* Boiss.	Gök menekşe	B5: Kayseri, Yahyalı Çamlıca village, Kayapınar location, 1410m, 04.04.2016, *Aksoy 2605*	
4	*V. kitaibeliana* Roem. & Schult.	Yabani menekşe	B5: Kayseri, Yahyalı Çamlıca to Ulupınar village, 2 km from Ulupınar village 1370 m, 04.04.2016, *Aksoy 2608*	-
5	*V. kizildaghensis* Dinç & Yıld.	Pembe menekşe	C3: Konya-Derebucak, Çamlık village, Kızıldağı, Akçeşme location 1450n, 26.00.2016. *Aksoy 2642*	CR
6	*V. modesta* Fenzl	Sahra menekşesi	C4: Konya-Bozkır, Üçpınar village, Tufan Deresi,1990, 28.04.2016. *Aksoy 2581*	-
7	*V. sandrasea* Melch. subsp. *sandrasea*	Sandras menekşesi	C2: Muğla-Köyceğiz, Sandras Dağı 1830 m, 21.04.2016. *Aksoy 2563*	EN
8	*V. suavis* M. Bieb.	Akgöz menekşe	C5: Hatay- Arsuz, above the village of Kale, Kızıldağ, 17.04.2016 *Aksoy 2586*	-

3. Results and Discussion

The minimum, maximum and mean concentrations of Cd, Cu, and Zn in the investigated soils and aboveground and underground parts of the *Viola* taxa are given in Table 2 and the mean concentrations of Cd, Cu, and Zn are further shown in Figure 1.

While the Cd concentrations ranged between 3.40-33.75 mg kg^{-1} in the studied soils, it was found that the values varied between 0.15-1.86 mg kg^{-1} in the underground parts and between 0.68-1.71 mg kg^{-1} in the aboveground parts of the *Viola* taxa. The highest Cd concentration (33.75 mg kg^{-1}) was detected in the soils where *V. heldreichiana* grew and the lowest Cd concentration (3.40 mg kg^{-1}) was found in the soils where *V. modesta* samples grew (Table 2). It has been reported that the Cd concentration of *V. baoshanensis*, which grows in the Baoshan lead / zinc mine field in Hunan, China, varies between 456-2310 mg kg^{-1} in the aboveground parts and between 233-1846 mg kg^{-1} in the underground parts (Wei et al., 2004).

Cu concentrations ranged between 7.51-33.30 mg kg^{-1} in the studied soils, between 1.21-6.31 mg kg^{-1} in the underground parts and between 2.57-4.69 mg kg^{-1} in the aboveground parts of the investigated *Viola* taxa. The highest Cu concentration (30.85 mg kg^{-1}) was detected in the soils where the sampled *V. heldreichiana* grew while the lowest Cu concentration (7.51 mg kg^{-1}) was found in the soils where *V. kizildaghensis* grew (Table 2).

While the Zn concentrations ranged between 5.65-95.25 mg kg^{-1} in the studied soils, Zn concentrations for the investigated *Viola* taxa varied between 4.65-69.25 mg kg^{-1} in the underground parts and between 12.15-64.65 mg kg^{-1} in the aboveground parts. The highest Zn concentration (33.30 mg kg^{-1}) was detected in the soils where the investigated *V. heldreichiana* samples grew and the lowest

Zn concentration (9.68 mg kg^{-1}) was found in the soils where *V. sandrasea* subsp. *sandrasea* samples grew (Table 2).

The investigated Cd and Cu concentrations were found to be higher in the soil compared to the underground and aboveground parts of the plants. However, Zn concentrations in *V. dirimliensis*, *V. kizildaghensis*, *V. suavis* and *V. sandrasea* subsp. *sandrasea* were found to be higher, both in the underground and aboveground parts of the plant, than the concentrations of the soil samples (Fig. 1).

Table 2. Minimum, maximum and mean concentration of Cd, Cu, and Zn in underground and aboveground parts of the investigated *Viola* species and soils collected from serpentine areas in the Mediterranean region of Turkey (mg kg^{-1} dry weight ± SD).

		Soil			**Underground**			**Aboveground**		
Plant species	Elements	**Cd**	**Cu**	**Zn**	**Cd**	**Cu**	**Zn**	**Cd**	**Cu**	**Zn**
V. alba* subsp. *dehnhardtii	min	10,68	16,82	30,94	1,44	5,74	42,99	1,00	2,95	23,72
	max	10,83	16,84	31,40	1,50	5,76	43,35	1,03	2,98	23,80
	mean	**10,75**	**16,83**	**31,17**	**1,47**	**5,75**	**43,17**	**1,02**	**2,96**	**23,76**
	SD	±0,11	±0,01	±0,32	±0,04	±0,01	±0,25	±0,02	±0,02	±0,06
V. dirimliensis	min	7,77	10,24	13,31	0,97	2,12	15,78	0,93	2,57	19,13
	max	7,80	10,35	13,49	1,06	2,16	15,80	0,96	2,60	19,49
	mean	**7,78**	**10,30**	**13,40**	**1,01**	**2,14**	**15,79**	**0,94**	**2,58**	**19,31**
	SD	±0,02	±0,08	±0,13	±0,06	±0,30	±0,01	±0,02	±0,02	±0,26
V. heldreichiana	min	33,65	32,70	94,45	0,15	1,20	4,65	1,61	3,50	38,20
	max	33,75	33,30	95,25	0,17	1,30	4,75	1,71	3,55	40,15
	mean	**33,70**	**32,91**	**94,88**	**0,16**	**1,25**	**4,71**	**1,66**	**3,51**	**38,90**
	SD	±0,13	±0,30	±0,40	±0,02	±0,05	±0,05	±0,32	±0,02	±0,98
V. kitaibeliana	min	30,51	23,70	86,05	0,57	2,30	17,15	1,44	3,05	34,10
	max	30,85	24,35	88,25	0,59	2,35	17,40	1,50	3,15	34,85
	mean	**30,68**	**24,00**	**86,96**	**0,58**	**2,31**	**17,25**	**1,47**	**3,10**	**34,56**
	SD	±0,36	±0,32	±1,10	±0,02	±0,26	±0,12	±0,12	±0,05	±0,37
V. kizildaghensis	min	11,46	7,51	29,67	1,84	5,05	68,25	1,08	2,77	64,36
	max	11,49	7,52	29,96	1,86	5,11	69,25	1,10	2,75	64,62
	mean	**11,47**	**7,52**	**29,82**	**1,85**	**5,08**	**68,75**	**1,09**	**2,76**	**64,49**
	SD	±0,27	±0,01	±0,21	±0,01	±0,05	±0,70	±0,16	±0,01	±0,18
V. modesta	min	3,40	10,72	35,50	1,11	5,17	17,50	1,11	4,04	21,20
	max	3,45	10,83	35,76	1,15	5,19	17,53	1,14	4,05	21,29
	mean	**3,42**	**10,78**	**35,63**	**1,13**	**5,18**	**17,53**	**1,12**	**4,04**	**21,25**
	SD	±0,04	±0,08	±0,18	±0,03	±0,01	±0,02	±0,02	±0,01	±0,06
V. sandrasea* subsp. *sandrasea	min	6,89	9,68	5,65	1,31	6,27	26,47	0,68	3,92	12,15
	max	6,93	9,76	5,87	1,33	6,31	26,91	0,70	3,96	12,48
	mean	**6,91**	**9,72**	**5,76**	**1,32**	**6,29**	**26,69**	**0,69**	**3,94**	**12,32**
	SD	±0,03	±0,06	±0,16	±0,01	±0,03	±0,32	±0,01	±0,03	±0,23
V. suavis	min	9,37	12,92	25,85	1,20	5,49	45,36	0,92	4,64	33,03
	max	9,55	13,17	26,09	1,22	5,51	45,85	0,94	4,69	33,43
	mean	**9,46**	**13,05**	**25,97**	**1,21**	**5,50**	**45,61**	**0,93**	**4,66**	**33,23**
	SD	±0,13	±0,18	±0,17	±0,01	±0,02	±0,34	±0,012	±0,04	±0,28

Among the eight *Viola* taxa studied, Zn concentrations of *V. dirimliensis* were highest in the aboveground (19.31 mg kg^{-1}) and slightly lower in the underground parts (15.79 mg kg^{-1}) and in the soil (13.40 mg kg^{-1}). The Zn concentrations in the aboveground (64.49 mg kg^{-1}) and underground (68.75 mg kg^{-1}) parts of *V. kizildaghensis* were found to be twice as high as in the soils (29.82 mg kg^{-1}). The fact that the Zn concentration in the aboveground parts of *V. kizildaghensis* is markedly higher than in the soil where the plants grew indicates that *V. kizildaghensis* has a Zn accumulator feature. Stevanovic et al. (2010) and Bačeva et al. (2014) examined whether some *Viola* species in the Alchar region of Macedonia are hyperaccumulators for several heavy metals including Zn. They found that the *Viola* species studied had an accumulator feature for Zn.

Different ecosystems in many parts of the world have been contaminated by various heavy metals such as Cd, Cr, Cu, Ni, Pb, Zn. In recent years, hyperaccumulator or accumulator plants have been used as a model in the cleaning of these contaminated environments, and thus to reduce this pollution (Aksoy and Öztürk, 1997; Baker et al., 2000; Prasad, 2005).

Many studies have been carried out about serpentine areas in Turkey with 62 Ni-hyperaccumulator plants being recorded in these areas. Most of these plants belong to the *Brassicaceae* and *Asteraceae* family. According to these studies, no plant species with Cd, Cu and Zn hyperaccumulator properties were found in Turkey. (Reeves, 1988; Reeves et al., 1983, 2001, 2009; Reeves and Adıgüzel, 2004, 2008; Adıgüzel and Reeves, 2012; Altınözlü et al., 2012; Aksoy et al., 2015; Çelik et al., 2018).

A large number of hyperaccumulators in *Viola* species have been reported in Asian and European countries, for example, *V. baoshanensis* for the hyperaccumulation of cadmium (Liu et al., 2004; Tonin et al., 2001) and *V. calaminaria* (Tonin et al., 2001) and *V. lutea* (Sychta et al., 2018) for the hyperaccumulation of zinc. In their study on *V. boashanensis* in China, Wu et al. (2010) found Cd concentrations of 1090 mg kg^{-1}, Pb concentrations of 1902

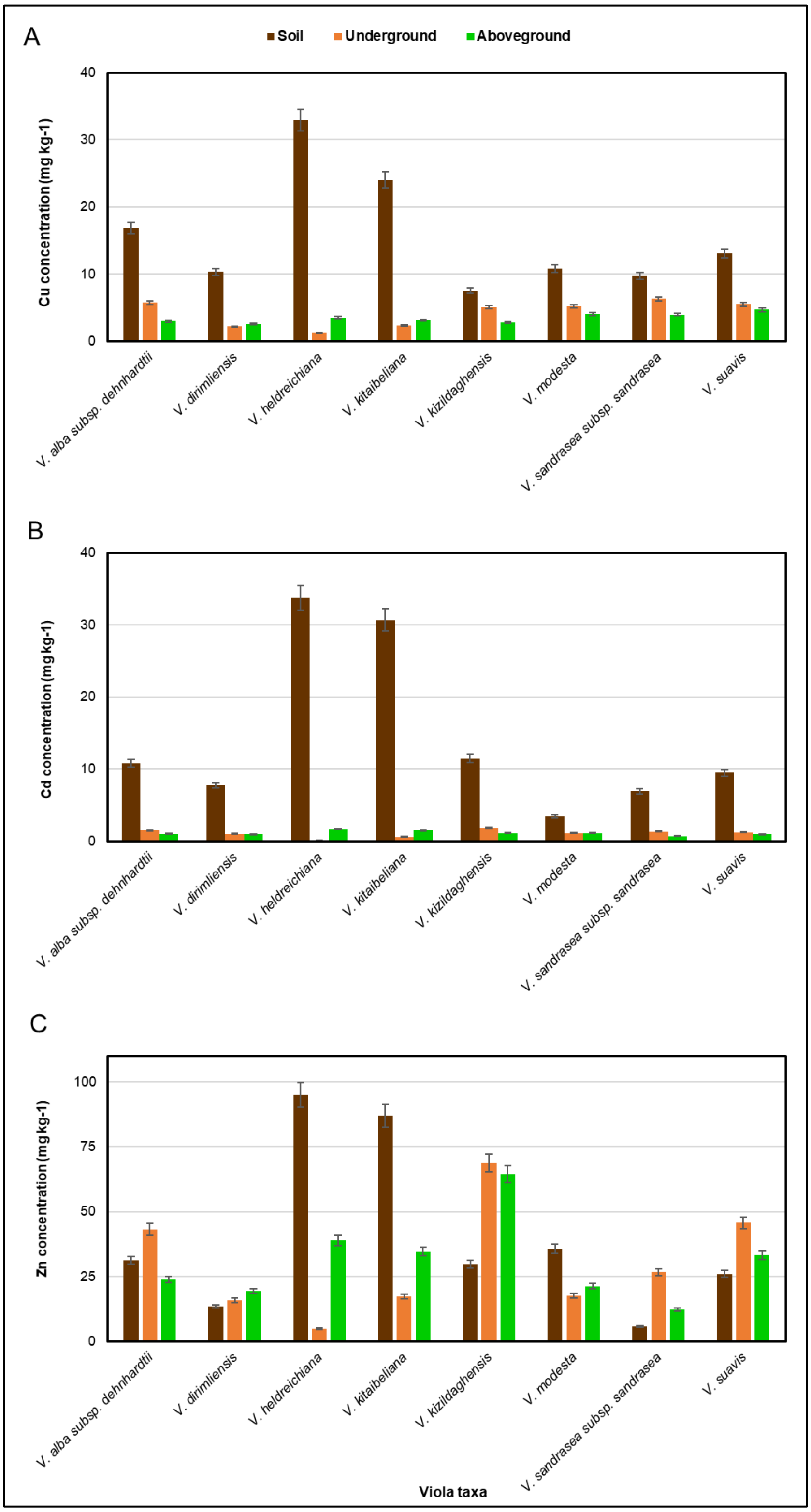

Figure 1. Mean concentrations of Cd (**A**), Cu (**B**) and Zn (**C**) in the soil and the aboveground and underground parts of the *Viola* taxa, together with standard errors (S.E.).

mg kg^{-1}, and Zn concentration of 3428 mg kg^{-1}. They reported that *V. boashanensis* was a hyperaccumulator plant for Pb and Zn as well as for Cd. As a result of this study, no hyperaccumulator properties could be detected for the investigated *Viola* taxa. However, it has been determined that *V. dirimliensis*, *V. kizildaghensis*, *V. suavis* and *V. sandrasea* subsp. *sandrasea* accumulate zinc to a certain extent.

Acknowledgments

This work was part of a project supported by the Scientific Research Projects Coordination Unit of Akdeniz University (Project number: FBA-2016-1982). I would like to thank Jale Çelik and Uğurcan Baran for their help and Aylin Güney for language editing.

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