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Extractability and phytoavailability of cadmium in Cd-rich pedogenic soils

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Abstract: Conspicuous vegetation entities of pedogenic soils rich in cadmium and their diethylene triamine pentaacetic acid (DTPA)-extractable Cd content (DCd) under the temperate continental and Mediterranean climatic conditions of western Anatolia were determined. Foliage of herbaceous plants and nearby soil samples was collected from 51 zinc mining areas. DCd content was found to reach 11.6%–16.2% of the total Cd content, indicating more extractability than in many other heavy metals. Growth chamber and nursery experiments were conducted with the seeds collected. *Dactylis glomerata* and *Galium tenuissimum* subsp. *tenuissimum*, both common in grazing lands, were found to be accumulating 23.5 mg Cd kg^{-1} and 22.3 mg Cd kg^{-1} dry matter, respectively, which are highly phytotoxic levels. *Thlaspi praecox*, known as a Cd-hyperaccumulating plant species, could not be successfully introduced into the Cd-rich mining soils under controlled conditions, suggesting genetic variation or inability to adapt. Volunteer species under controlled conditions included *Poa bulbosa* and *Plantago lanceolata*, both of which were potential Cd-hyperaccumulating candidates. *Silene aegyptiaca* and *Silene vulgaris* were other species commonly found around zinc mines and they were grown under controlled conditions without difficulty. However, Cd concentrations in the above-ground tissues of all species mentioned above were not over the 100 mg kg^{-1} dry matter threshold value for hyperaccumulating cadmium.

Key words: *Dactylis glomerata* L., DTPA extraction, phytoavailable cadmium, *Poa bulbosa* L., zinc mining soils

1. Introduction

The principal environmental concern over high concentrations of cadmium (Cd) in natural ecosystems relates to toxicity since Cd, like many other heavy metals, hardly decomposes in soils and it takes part in cycling. The contamination risk of Cd has been widely studied recently, as it is one of the most mobile and toxic heavy metals (Kumar Sharma et al., 2007; Li et al., 2009; Wei and Yang, 2010). Recent growing interest in environmental Cd contamination may likely come from increased information about the potentially harmful effects of the element associated with the increasing contamination of resources from human activities (Tran and Popova, 2013). Cadmium is a nonessential element that negatively affects plant growth (Benavides et al., 2005). It may be naturally found, usually with zinc- and lead-containing igneous rocks, or may be released by several anthropologic activities including power plants, phosphate fertilizers, urban traffic, and metal-working industries. Availability for plants, or, more properly, phytotoxicity concepts are aimed at the plants grown in the soils rich in Cd compounds.

Virgin soils containing Cd are not as widespread as many others, as this element is mostly found in narrow intrusive veins and deep galleries. On the other hand, the 4 species currently known as Cd hyperaccumulators are all perennial plants with relatively low biomass. Thus, it is hard, if not impossible, to remediate the soils contaminated by Cd compounds by growing Cd accumulator plant species. Finding new Cd (hyper)accumulators that grow fast, have a large volume of above ground tissues, and root deeply is on the agenda of related studies.

Some plants can accumulate high concentrations of Cd without any visible plant symptoms (Lehoczky et al., 1998). Existence of easily soluble Cd compounds in plant tissues indicates the importance of sap in Cd studies (Roosens et al., 2003). Onweremadu and Duruigbo (2007) reported that Cd is considered one of the most important environmental pollutants in agricultural soils because of the potential harmful effects it may have on food quality

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and health of soil. Cd toxicity may be highly seen at low concentrations due to its mobility and bioavailability (Brunetti et al., 2009). All crops may contain Cd, and the harvested portion will serve to remove some Cd from soil (Page et al., 1987). Amounts removed from soil in any harvested crops, however, are quite small compared with total amounts.

Many soil properties may influence Cd uptake. The major factors governing cadmium speciation, absorption, and distribution in soils are pH, soluble organic matter content, hydrous metal oxide content, clay content and type, presence of organic and inorganic ligands, and competition from other metal ions (OECD, 1994). For example, low pH increases the uptake of Cd (Lehoczky et al., 1998), while the presence of phosphate and zinc reduces its phytoavailability (Kirkham, 2006) and removal of other cations promotes Cd uptake (Salt et al., 1997). Cadmium content of a typical crop is about 0.1 mg kg^{-1} dry matter or a little more (Brooks, 1998). The uptake, transport, and accumulation processes of Cd in plants are not fully understood yet. It is assumed that Cd is transported similarly to Zn^{2+}, Cu^{2+}, or Fe^{2+}, or through some channels serving for Ca^{2+} or Mg^{2+} (Welch and Norvell, 1999). Xylem transportation and transpiration may be the main factors leading to Cd accumulating in foliage (Hart et al., 1998). Root-to-shoot Cd translocation via their specific xylem system is the major and common physiological process determining the Cd accumulation level in the shoots and grains of rice plants (Uraguchi et al., 2009). The varietal translocation rate of Cd from roots to shoots was found variable (Niaz et al., 2010).

The objective of this study is to determine diethylene triamine pentaacetic acid (DTPA)-extractable Cd content (DCd) of pedogenic soils rich in Cd compounds and their conspicuous vegetation entities under the temperate continental and Mediterranean climatic conditions of western Anatolia. For this purpose, the study was carried out in 4 phases: 1) field surveys and lab analyses, 2) growth chamber and nursery experiments with natural mine soils rich in Cd, 3) controlled experiments using promising Cd accumulator plants, and 4) experiments on artificially contaminated soils with $CdCl_2$ and defined accumulator plants.

2. Materials and methods

2.1. Sampling

Foliage of herbaceous plants and nearby surface soil samples (0–15 cm) were collected from 51 active and abandoned zinc mines and mine tailings. The coordinates and elevation of the sampling locations were determined by using a handheld Magellan eXplorist XL receiver, with 3–6 m of accuracy. Areas contaminated by human activities (e.g., dump sites or arable lands) were rejected for the rare possibility of well-adapted genotypes. A couple samples of plant tissues were mixed at each location to obtain randomized uniform samples, which were transported to the laboratory in cotton bags. Soil subsamples from a mining location were mixed, homogenized, passed through a 4-mm plastic sieve at the site, and put into polyethylene bags.

Another set of the plant specimens were sampled following the methodology outlined by Davis and Heywood (1973). They were identified by referring to the *Flora of Turkey and the East Aegean Islands* (Davis 1965–1985; Davis et al., 1988) and the material was added to that at the HUB herbarium (Holmgren et al., 1990).

2.2. Analytical procedures

The DTPA test was employed in determining so-called phytoavailable Cd contents of the soils. For this purpose, 20 g of soil sample crushed to 80 mesh was shaken with 40 mL of extract solution (0.005 M DTPA, 0.01 M $CaCl_2$, and 0.1 M triethanolamine, pH 7.3) for 2 h, centrifuged at 5000 rpm in Janetzki T-23 equipment, and gravimetrically filtered through a Whatman cellulose filter of grade 42 (Lindsay and Norvell, 1978; Leita et al., 1999). Phytoavailable Cd in the soil extracts (DCd, mg kg^{-1}) was determined with a Varian 720-ES inductively coupled plasma optical emission spectrometer (ICP-OES). Oven-dried soil samples at 60 °C were used for total Cd analysis. Each replicate of 0.5 g of soil (A1, A2, and A3, the 3 highest DCd-containing soils) was treated with aqua regia (ultrapure mixture of concentrated HNO_3/HCl, 1/3 (v/v)) at 95 °C to obtain aliquots. The highest DCd-containing 3 soils were selected as growth media for the greenhouse and growth chamber experiments. Analyses of those soils included total and DTPA-extractable heavy metal contents. Total elements of acid-digested samples were analyzed by means of a PE SCIEX ELAN 6100 ICP-mass spectrometer (Roelandts, 1990; Hossner, 1996). Wet digestion in 3.5 mL of aqua regia was used for mineralization of 0.1 g of soil sample on an aluminum block; the resulting compound was then diluted with 5 mL of 0.2% HNO_3.

Plant samples were washed with 0.01 mol L^{-1} H_2SO_4, tap water, and deionized water, respectively. Then they were dried at 65 °C until constant weight and were ground in an ASTM mill. Ground samples were digested with 5 mL of 5% nitric-perchloric acid in a Speedwave MWS-3$^+$ Berghof microwave digestion system (Kalra and Maynard, 1998) and filtered from Whatman 42 for Cd analyses using the ICP-OES (Miller, 1998).

2.3. Growth chamber and nursery studies

Preliminary greenhouse and growth chamber trials were established with (hyper)accumulator and volunteer plants. During the control tests, the seedlings were germinated in peat-perlite mixture. Young plants with 2–3 leaves were transplanted to a medium containing 50 mg kg^{-1} nitrogen, 50 mg kg^{-1} phosphate, and 5% peat on a volumetric basis. *Dactylis glomerata* L., *Galium tenuissimum* M.Bieb. subsp. *tenuissimum*, *Thlaspi praecox* Wulf., *Hymenocarpus circinnatus* L. Savi, *Poa bulbosa* L., *Plantago lanceolata* L., *Silene aegyptiaca* L.f. *aegyptiaca*, and *Silene vulgaris* (Moench.) Garcke. were selected for the growth chamber and greenhouse experiments, relying on their stand establishment and Cd accumulation capabilities. They were then grown under controlled conditions on 3 mine soils (A1, A2, and A3) that contained the highest amounts of DCd. Another set of the seeds were directly planted into the test soils for evaluating their accumulating performances under simulated natural conditions. Artificial applications, which were inevitably different from natural field conditions, were limited to lighting, heating, and humidity control in the growth chamber and periodical watering in the greenhouse. Variable DCd content of each soil facilitated comparison of the metal-accumulating abilities of test plants. Greenhouse experiments were conducted either under plastic covers or in open nurseries, depending on the growing season. Day and night temperature of the growth chamber was set to 21–24 °C and 16–18 °C, respectively. Relative humidity ranged between 35% and 55%. The peculiar phenomenon of several plants turning purple was attributed to the deficiency of the solar spectrum. Experiments were conducted in freely drained polyurethane pots with 8 parallel runs to minimize experimental errors. The majority of the test material consisted of perennial or biannual plants; therefore, they were harvested when they produced a sufficient amount of biomass for laboratory analyses, i.e. before they reached the maturity stage.

An Aridisol surface soil containing no traceable Cd was used in the experiments, which were carried out with artificially contaminated soils. Selected parameters of this control soil were as follows: moisture content at saturation: 56%, electrical conductivity: 1.04 dS m^{-1}, pH: 7.87, cation-exchange capacity: 55.2 cmol 100 g^{-1}, free carbonates: 9.82%, and texture: clay loam, with all properties within the ranges of normal soils under Anatolian conditions. To obtain the desired 0, 10, 20, and 40 mg kg^{-1} approximate DCd concentrations, the soils were spiked with 2-fold concentrated $CdCl_2$. These growth media were saturated, covered, mixed thoroughly twice a day, and left to sit for a fortnight before they were planted. These last series of experiments were established with *H. circinnatus* and *T. praecox* seeds, which were not pretreated. The experiments were designed as completely randomized plots with 4 replications. Plants were grown in freely drained 400-mL polyurethane pots. They were weighed daily to avoid excessive watering.

2.4. Evaluation procedures

Coordinates of the sampling locations and the DCd contents were fixed on a regional map by using ARC GIS 8.1 software. Package programs were employed for statistical analyses where required. Symbols of statistical significance are *: $P < 0.05$ and **: $P < 0.01$.

3. Results

3.1. General properties of the soils

Soil pH at the research area was between 6.57 and 8.20 (Table 1). With the exception of the soil sample from Bağırkaç, Çanakkale Province, the pH value of all soils studied exceeded 7.0, as is generally characteristic of the regional soils. Dominant soil texture classes were within medium ranges. Relatively high sand content of the soils may be attributed to the existence of detrital parent material. Higher free carbonate contents at a few locations might likely arise from the past geological or anthropological mixtures with local limestone formations, because carbonate is not an autochthonous geogenic component of those intrusive residuals. Sparse and patchy flora around resulted in generally low organic matter accumulation in the soils, whereas a few soils rich in organic matter were almost always covered with coniferous woods.

3.2. Phytoavailable Cd contents

Considering that phytoavailable cadmium, even when found in low concentrations, may be hazardous from the point of view of environmental health (Kabata-Pendias, 2001; Kirkham, 2006), a detection limit of 0.1 mg kg^{-1} DCd was selected. The DCd content of 13 of the 51 soils surrounding the zinc mines was under this limit (Figure 1). Six soils contained 10–20 mg kg^{-1} and the DCd content of 1 sample was over 30 mg kg^{-1} (Figure 2). Collecting any plant samples was hardly possible at many locations due to sparse or no surrounding vegetation. High elevation, toxic effect of any heavy metals in mine soils and surroundings, or too short a time for formation of mature soil may be possible causes of this poor biomass. A couple of locations where ore existed at the soil surface, however, were relatively rich in herbaceous plants. This exceptional case may deserve closer study.

3.3. Extractable-to-total Cd ratios

DCd content range of the selected soils was 11.6%–16.2% of the total amount (Table 2). Higher extractability indicated easier release of Cd salts. Solubility (or bioavailability) of Cd may be expected to increase at acidic conditions and the extractable-to-total Cd ratio may reach 50% under favorable conditions (Ramos et al., 1994). All of our studied soils were developed under temperate continental or Mediterranean climate, and the soil reactions varied between neutral and slightly alkaline as a usual consequence of the regional soil formation

Table 1. Ranges of selected parameters in the zinc mining soils.

Coordinates	Elevation, m	DCd	pH, 1:2.5 susp.	EC, 1:2.5 susp., dS m^{-1}	Clay, %	Silt, %	Sand, %	Free carbonates, %
38°04.862′N, 27°01.118′E	70	5.97	7.78	0.20	17.9	20.4	61.7	18.8
38°18.707′N, 27°41.928′E	507	3.49	7.44	0.18	11.7	22.1	66.2	1.3
38°20.562′N, 27°41.152′E	847	2.65	7.06	0.16	16.1	37.2	46.7	<1
38°21.860′N, 27°41.800′E	902	2.21	7.93	0.29	12.0	37.4	50.6	<1
39°45.877′N, 27°13.200′E	304	0.12	7.31	0.14	26.6	46.7	26.7	<1
39°44.104′N, 27°13.667′E	318	0.89	6.57	0.09	24.0	24.4	51.6	<1
39°26.952′N, 28°35.695′E	887	38.90	7.77	0.12	26.4	29.5	44.1	12.1
37°38.442′N, 35°50.506′E	880	14.50	7.90	0.16	11.1	32.9	56.0	14.8
37°46.764′N, 35°51.865′E	671	4.30	8.07	0.17	13.8	30.5	55.7	61.8
38°09.998′N, 35°31.698′E	1546	14.8	8.20	0.14	32.0	24.4	43.6	1.46
38°06.034′N, 35°32.107′E	1452	6.06	7.61	0.18	46.1	31.8	22.1	<1
38°07.983′N, 35°33.410′E	1450	0.99	7.85	0.14	39.1	27.0	33.9	21.1
37°00.899′N, 32°10.097′E	2100	14.11	7.46	0.06	48.1	26.7	25.2	<1
36°58.146′N, 32°13.649′E	2133	3.71	7.90	0.12	36.5	20.6	42.9	1.46
37°28.519′N, 34°36.351′E	1621	<0.06	7.06	0.14	7.7	8.0	84.3	1.62

processes. Evidently, the high bioavailability degree of Cd under these conditions indicated great toxicity potential of that element.

The zinc mining soils were commonly high in Fe and Mn contents and low in Al and Ca contents. Tables 3 and 4 indicate total metal contents of the 3 selected mining soils with the highest DCd content detected. However, total Cd amounts in the zinc mine surroundings, where the soils are geologically polluted, were lower than many other heavy metals.

3.4. Accumulator plants

It was difficult to identify dominant plant species on the zinc mine soils, as most soils had almost no vegetative cover. Dominant species were Poaceae, Scrophulariaceae, and Brassicaceae members (Table 5). Reeves et al. (2001) reported that only a few species were able to grow around the Balıkesir Balya zinc mine some 60 years after it ceased operations. The only exceptional case that we observed was Güğü village, Dursunbey, Balıkesir Province (soil A1), where low-grade ore was spread at the surface in spite

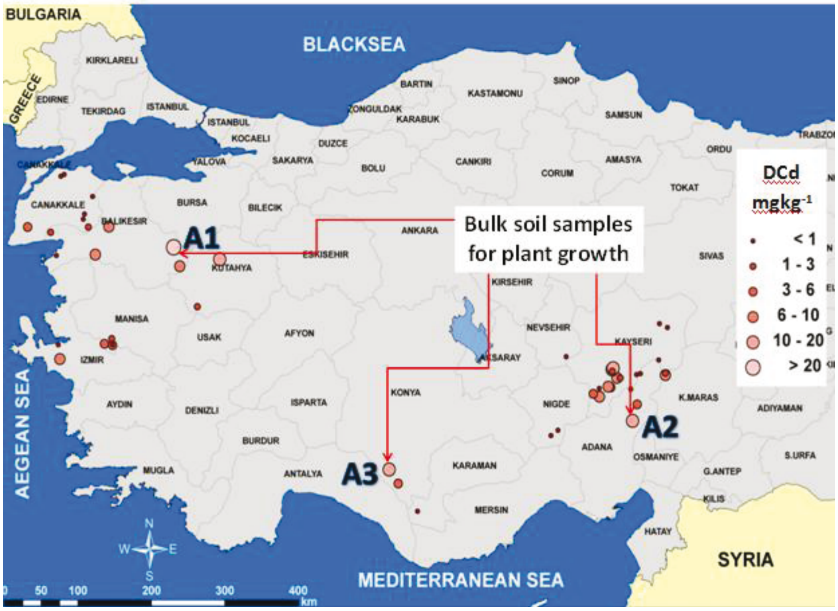

Figure 1. Sampling locations and DTPA-extractable Cd content ranges of their soils.

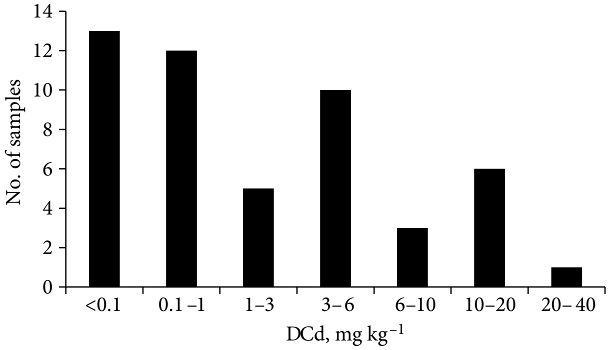

Figure 2. DTPA-extractable cadmium content ranges at zinc mining areas and tailings.

Table 2. Total and DTPA extractable Cd contents of the selected mine soils, mg kg^{-1}.

Soil	Total Cd	Extractable Cd
A1	232	38.9
A2	129	14.5
A3	94	14.1

Table 3. Total metal and semimetal contents of the soil macroelements, %.

Soil	A1	A2	A3
Fe	5.53	7.87	10.8
Ca	6.97	2.73	0.27
Mg	1.77	0.47	0.13
Na	udl	udl	udl
K	0.11	0.18	0.12
P	0.04	0.22	0.06
S	0.50	<0.05	<0.05
Al	1.57	2.42	0.02
Ti	0.05	0.02	0.00
Ni	0.27	0.25	0.27

udl: under detection limit of 0.003 mg kg^{-1}.

Table 4. Total metal and semimetal contents of the soil microelements, mg kg^{-1}.

Soil	A1	A2	A3
Mo	38.40	1.4	15.0
Cu	1.97	114	87.6
Pb	1620	1300	30
Zn	724	458	12.7
Ag	15.9	1.1	36.9
Co	28.1	17.2	15.4
Mn	9997	2970	710
La	5	23	16
As	58.1	60.5	73.2
U	1.7	1.4	2.6
Cr	24	38	39
Th	2.1	3.8	7.4
Sr	43	24	11
Sb	2.5	2.3	0.5
Bi	38.7	0.4	0.3
V	50	41	47
Ba	35	98	62
B	<20	<20	<20
W	30.4	<0.1	<0.1
Hg	0.03	0.48	1.63
Sc	3.0	4.1	7.0
Tl	0.3	0.2	0.4
Ga	10	7	19
Se	4.7	3.8	4.8
Au × 10^{-3}	4.2	1.8	4.7

Table 5. Number of plant species identified around zinc mining areas; some species may grow at more than one location.

Family	Quantity of plant species	Family	Quantity of plant species
Apiaceae	1	Lamiaceae	2
Boraginaceae	2	Liliaceae	1
Brassicaceae	22	Plantaginaceae	1
Caryophyllaceae	7	Poaceae	2
Cistaceae	1	Polygonaceae	1
Convolvulaceae	1	Resedaceae	1
Euphorbiaceae	1	Rubiaceae	3
Fabaceae	3	Scrophulariaceae	6
Hypericaceae	1	Valerianaceae	1

of frequent narrow, deep veins at the other mining areas studied. Volunteer species in the growth chamber were *P. bulbosa* (Kin, 2008), *Silene compacta* Fisher (Hagemeyer, 2004), and *P. lanceolata* (Cook et al., 1972), which were all reported as metalloresistant species. Those species were all selected as test plants for further experiments, as well as *H. circinnatus* as a potential hyperaccumulator after the initial experiments and *T. praecox* as a well-known Cd hyperaccumulator plant species.

A couple individuals of Cd-accumulator species *P. lanceolata* (Hutchinson et al., 2004) exceeded the hyperaccumulation limit, accumulating 171 mg kg^{-1} in the foliage on average. This result revealed the potential of *P. lanceolata* in uptake, translocation, and accumulation of Cd in Cd-rich virgin mine soils, since the analyzed amount was the highest record known to date. Those plants, though growing without any symptoms, may not be accepted as Cd hyperaccumulators because of their poor growth on artificial growth chamber medium.

D. glomerata and *G. tenuissimum* subsp. *tenuissimum* were found to be accumulating Cd at highly phytotoxic levels in grazing lands, with 23.5 mg kg^{-1} and 22.3 mg kg^{-1} dry matter, respectively. Cadmium accumulation in the foliage of 3 cultivated species, *Alyssum filiforme* Nyar (R^2: 0.994; $P < 0.01$), *S. aegyptiaca* (L.) L.f. subsp. *aegyptiaca* (R^2: 0.987; $P < 0.01$), and *H. circinnatus* (R^2: 1; $P < 0.01$), was closely related to the DCd concentration in the soil (Figure 3). Statistical analyses showed that all those relationships, all of which were best fitted by polynomial curves, were highly significant. Similar results were reported by Lehoczky et al. (2002) and Millis et al. (2004).

Cardaria draba L. Desv. subsp. *draba* was found growing without any malnutrition or toxicity symptoms on mining soils with the highest Cd content of 5.76 mg kg^{-1} dry matter. *S. aegyptiaca* and *A. filiforme* grew in mine soils accumulating some Cd according to the medium without any apparent deficiencies or symptoms. The latter is a perennial species and thus grows slowly.

3.5. *Hymenocarpus circinnatus* trials

H. circinnatus was the only species accumulating more than 100 mg kg^{-1} Cd of the hyperaccumulation limit in foliage in soil A1 in the growth chamber. Nevertheless, none of the few shoots analyzed altogether was within a normal growth range, so the species did not seem to deserve the title of "hyperaccumulator plant".

An initial experiment had been already conducted as follows: it was determined in the growth chamber that *H. circinnatus* accumulated more Cd as DCd increased in the

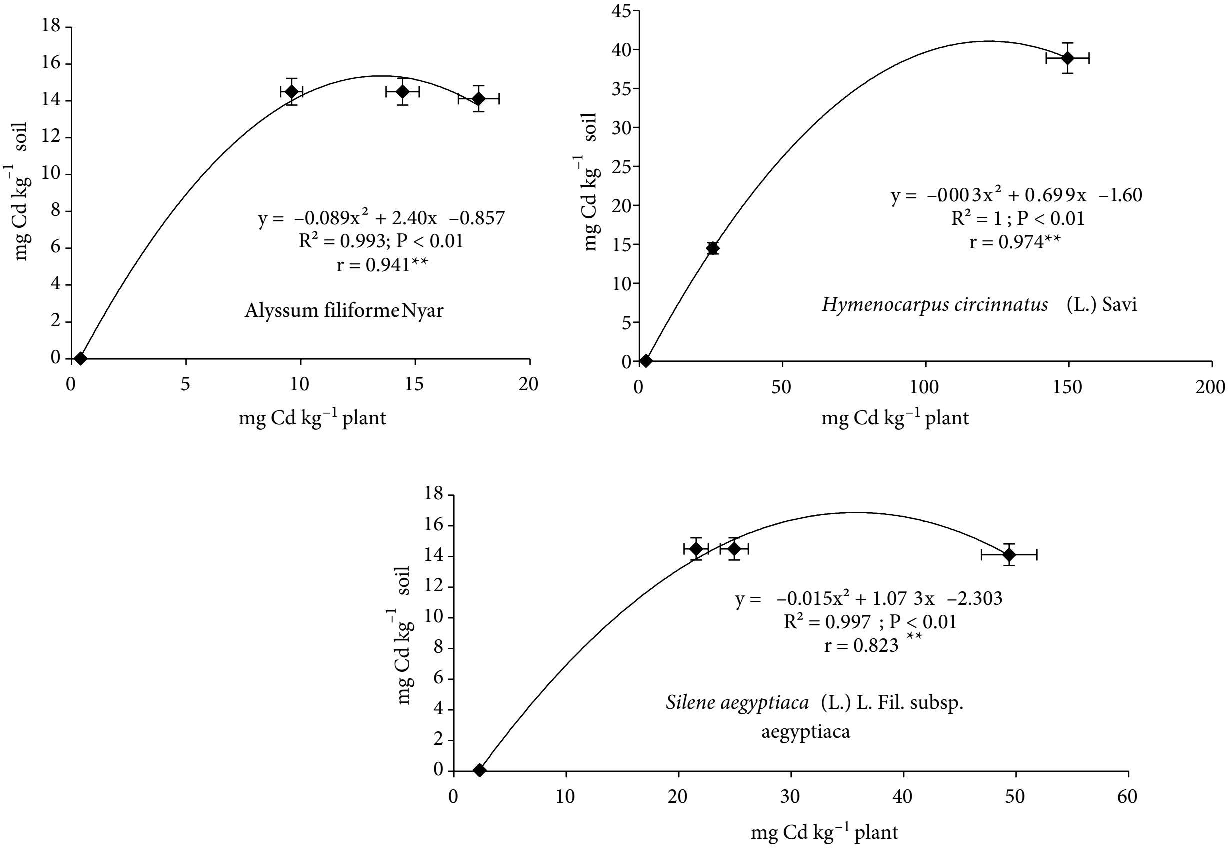

Figure 3. Relationship between the soil DTPA-extractable Cd and plant Cd contents.

soil. A greenhouse experiment was then conducted with the normal soil enriched with Cd by adding 20 mg kg^{-1} phytoavailable Cd which was obtained with a fortnight's equilibrium of 40 mg Cd kg^{-1} $CdCl_2$ mixed into a regular mist soil. The plants were grown at a similar rate to the control pots; both groups did not exhibit any symptoms. It was determined that *H. circinnatus* could uptake Cd at high doses, but translocation to the above-ground tissues was limited (Table 6). This species was noted as a promising Cd accumulator or a crossing agent in breeding studies. As a member of Fabaceae and a preferred legume for grazing animals, *H. circinnatus* may accumulate dangerous levels of Cd and thus it requires special attention, particularly on Cd-rich soils.

3.6. *Thlaspi praecox* trial

The only known Cd hyperaccumulator species in Turkey, *T. praecox* is restricted to Thrace, around Dereköy, where no Cd-rich soils have been detected so far. Pretests showed that the rates of germination and emergence were low, although every pot was seeded with plenty of seed grains. In addition, undergrown shoots dried in a couple of days, facilitating no analyses or evaluations. Another trial was then established, eliminating possible interferences of other toxic components, where varying doses of Cd (0, 5, 10, and 20 mg kg^{-1}) were applied as $CdCl_2$ to normal soil. Shoots only emerged from the control soil (0 Cd), suggesting that Cd was harmful at least at germination stage. Cadmium toxicity to a Cd-accumulating species can be perhaps attributed to the genotypic variation, as the seeds did not historically grow under Cd-rich conditions. Further studies were carried out under specific conditions: seedlings grown in the peat-perlite mixture were transplanted to the naturally Cd-rich soil (DCd content: 38.9 mg kg^{-1} soil), with no reportable results again. As a summary, only the difficulties in establishing a good stand of *T. praecox* could be mentioned.

Table 6. Cd contents of *Hymenocarpus circinnatus* grown in the soil polluted with $CdCl_2$.

Soil, mg kg^{-1} Cd DTPA-extractable	Plant, mg Cd kg^{-1} dry matter
0, control, foliage	<0.40
0, control, roots	<0.70
20 - 1, foliage	<1.56
20 - 1, roots	111
20 - 2, foliage	4.68
20 - 2, roots	40.00
20 - 3, foliage	7.21

4. Discussion

Depending on soil properties, phytoavailability and toxicity of Cd readily influence many environmental components (Sady and Kowalska, 2006). The permissible limit for Cd is stricter than those of most other heavy metals, as this element is highly toxic in living organisms and lacks any known vital functions (Tran and Popova, 2013). The major cause of cadmium toxicity is within the food chain, of which the initial link is grazing or edible plants. If some organisms, including earthworms, caterpillars, rodents, or sheep, seem likely to ingest vegetation rich in Cd, then harmful bioconcentrations up to the food chain become a serious concern. Close chemical properties and similar behavior between Cd and Zn, an essential element, indicates the importance of the potential threat in the food chain (NCM, 2003).

One of the bottlenecks in studying the geogenic pollution of soils is the interrelationship of a number of toxic components. Occurrence of 2 or more heavy metals in geologically contaminated soils usually results in deviation of physical, chemical, and biological properties of those soils from arable lands as well as preventing the growth of accumulator plants susceptible against some elements. These strongly expected interferences might likely limit the growing rate of the plant species, as well as the uptake and translocation of nutrients and nonessential and toxic elements. In this study, high amounts of DTPA extractable (so-called phytoavailable) Cd in the zinc mine soils were determined. These geogenic Cd-rich soils were also used in establishing plant growth experiments. Kirkham (2006) probably considered this when commenting on the reduced usefulness of studies conducted with artificially polluted soils. He pointed out that reduced Cd toxicity may be expected in the field as compared to a pot experiment carried out using $CdCl_2$, due to the interference of other ions. Human-made pollution may or may not involve this multielement increase. Cadmium accumulation in phosphate-fertilized soils or increases in lead content around highway soils are pronounced examples. Hydroponics or artificially salted soils may provide some good information. Moreover, information about genetic traits may assist in crossing studies, and information about enzymatic activities and environmental and biological features is of great importance. Studies with natural soils under natural conditions may become virtually essential in commercial practice.

A few shoots of *T. praecox* grown in the growth chamber could never blossom beyond a couple of undersized leaves. This failure was noted as signaling possible future difficulties in adaptation studies of the species. *P. bulbosa* and *P. lanceolata* were among the volunteer species under controlled conditions. These species seemed like potential Cd-hyperaccumulating candidates. However, Cd concentrations in their above-ground tissues were not over the 100 mg kg^{-1} dry matter threshold value for cadmium. They both may be annual, biannual, or perennial, and they require further study. *P. lanceolata* is a slow-growing perennial plant, but it may have more biomass than the known 4 Cd-hyperaccumulating plants. Another positive characteristic of *P. lanceolata* is a wider range of growing conditions. In summary, this species deserves closer study in areas of Cd accumulation and phytoremediation.

S. aegyptiaca and *S. vulgaris* were the other species commonly found around the zinc mines. They were grown in a growth chamber without difficulty. *S. aegyptiaca* accumulated remarkable amounts of Cd in its foliage. *P. bulbosa* and *H. circinnatus* were the first records known on Cd accumulation. *H. circinnatus* may be included in the promising species in Cd accumulation studies. Poor growth of *H. circinnatus* may be attributed to the following: any excess amount of phytoavailable lead, zinc, and possibly other heavy metals in the natural soil may prevent growth under the current study conditions; incomplete radio spectrum in the growth chamber might affect species to varying degrees; and/or poor physical conditions of the test soils might impede good growing. Any attempts at eliminating the ambiguities above may be expected to be more successful with that species.

Particular care should be paid to *Micromeria myrtifolia* Boiss. & Hohen because of its natural growth around zinc mining areas and tailings, Cd accumulation characteristics, and common consumption as an aromatic herb by local people.

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References

Benavides MP, Gallego SM, Tomaro ML (2005). Cadmium toxicity in plants. Braz J Plant Physiol 17: 21–34.

Brooks RR (1998). Geobotany and hyperaccumulators. In: Brooks RR, editor. Plants that Hyperaccumulate Heavy Metals. Wallingford, UK: CAB International, pp. 55–94.

Brunetti G, Soler-Rovira P, Farrag K, Senesi N (2009). Tolerance and accumulation of heavy metals by wild plant species grown in contaminated soils in Apulia region, Southern Italy. Plant Soil 318: 285–298.

Cook SCH, Lefébvre C, McNeilly T (1972). Competition between metal tolerant and normal plant populations on normal soil. Evolution 26: 366–372.

Davis PH (1965–1985). Flora of Turkey and the East Aegean Islands. Vols. 1–9. Edinburgh, United Kingdom: Edinburgh University Press.

Davis PH, Heywood VH (1973). Principles of Angiosperm Taxonomy. Huntington, NY, USA: Robert E. Kieger Publishing Co.

Davis PH, Mill RR, Tan K (1988). Flora of Turkey and the East Aegean Islands (Suppl. 1), Vol 10. Edinburgh, UK: Edinburgh University Press.

Hagemeyer J (2004). Ecophysiology of plant growth under heavy metal stress. In: MNV Prasad, editor. Heavy Metal Stress in Plants, from Biomolecules to Ecosystems, 2nd ed. Berlin, Germany: Springer, pp. 157–181.

Hart JJ, Welch RM, Norvell WA, Sullivan LA, Kochian LV (1998). Characterization of cadmium binding, uptake, and translocation in intact seedlings of bread and durum wheat cultivars. Plant Physiol 116: 1413–1420.

Holmgren PK, Holmgren NH, Barnett LC (eds.) (1990). Index Herbariorum. Part I: The Herbaria of the World. Vol 120. New York: New York Botanical Garden.

Hossner LR (1996). Dissolution for total elemental analysis. In: Sparks DL, editor. Methods of Soil Analysis Part 3 - Chemical Methods. Madison, WI, USA: Soil Science Society of America, pp. 49–64.

Hutchinson JJ, Young SD, Black CR, West HM (2004). Determining uptake of radio-labile soil cadmium by arbuscular mycorrhizal hyphae using isotopic dilution in a compartmented-pot system. New Phytol 164: 477–484.

Kabata-Pendias A (2001). Trace Elements in Soils and Plants, 3rd ed. Boca Raton, FL, USA: CRC Press, pp. 143–156.

Kalra YP, Maynard DG (1998). Microwave digestion of plant tissue in an open vessel. In: Kalra YP, editor. Handbook of Reference Methods for Plant Analysis. Windsor, CO, USA: Soil and Plant Analysis Council, Inc., pp. 63–67.

Kin NO (2008). Plant communities in the zone of gas field development and heavy metal accumulation by them. Russ J Ecol 39: 254–260.

Kirkham MB (2006). Cadmium in plants on polluted soils: effects of soil factors, hyperaccumulation, and amendments. Geoderma 137: 19–32.

Kumar Sharma R, Agrawal M, Marshall F (2007). Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. Ecotox Environ Safe 66: 258–266.

Lehoczky E, Loth I, Kiss Z (2002). Cadmium and lead uptake by white mustard (*Sinapis alba* L.) grown in different soils. Commun Soil Sci Plan 33: 3167–3176.

Lehoczky E, Szabo L, Horvath S (1998). Cadmium uptake by lettuce in different soils. Commun Soil Sci Plan 29: 1903–1912.

Leita L, De Nobili M, Mondini C, Muhlbachova G, Marchiol L, Bragato G, Contin M (1999). Influence of inorganic and organic fertilization on soil microbial biomass, metabolic quotient and heavy metal bioavailability. Biol Fert Soils 28: 371–376.

Li P, Wang X, Allinson G, Li X, Xiong X (2009). Risk assessment of heavy metals in soil previously irrigated with industrial wastewater in Shenyang, China. J Hazard Mater 161: 516–521.

Lindsay WL, Norvell WA (1978). Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sci Soc Am J 42: 421–428.

Miller OR (1998). Nitric perchloric acid wet digestion in an open vessel. In: Kalra YP, editor. Handbook of Reference Methods for Plant Analysis. Windsor, CO, USA: Soil and Plant Analysis Council, Inc., pp. 57–59.

Millis PR, Ramsey MH, John EA (2004). Heterogeneity of cadmium concentration in soil as a source of uncertainty in plant uptake and its implications for human health risk assessment. Sci Total Environ 326: 49–53.

NCM (2003). Cadmium Review. Report No. 1, Issue No. 04. Copenhagen, Denmark: Nordic Council of Ministers

Niaz A, Ghafoor A, Murtaza G (2010). Tissue concentration and translocation responses of rice cultivars to applied Cd in hydroponic studies. J Agric Biol Sci 5: 14–19.

Onweremadu EU, Duruigbo CI (2007). Assessment of Cd concentration of crude oil pollution arable soils. Int J Environ Sci Technol 4: 409–412.

Page AL, Chang AC, El-Amamy M (1987). Cadmium levels in soils and crops in the United States. In: Hutchinson TC and Meema KM, editors. Lead, Mercury, Cadmium and Arsenic in the Environment. New York: John Wiley & Sons, pp. 119–146.

Ramos L, Hernandez LM, Gonzalez MJ (1994). Sequential fractionation of copper, lead, cadmium and zinc in soils from or near Doñana National Park. J Environ Qual 23: 50–57.

Reeves RD, Kruckeberg AR, Adıgüzel N, Krämer U (2001). Studies on the flora of serpentine and other metalliferous areas of Western Turkey. S Afr J Sci 97: 513–517.

Roelandts I (1990). Inductively coupled plasma determination of nine rare-earth elements in sixty international geochemical reference samples. Geostand Geoanal Res 14: 137–147.

Roosens N, Vebruggen N, Meerts P, Ximénez-Embún P, Smith JAC (2003). Natural variation in cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from Western Europe. Plant Cell Environ 26: 1657–1672.

OECD (1994). Risk Reduction Monograph No. 5: Cadmium. Paris, France: OECD Environment Directorate.

Sady W, Kowalska I (2006). Effects of nitrogen form or solution pH on cadmium content and quality of spinach. ISHS Acta Hortic 700: 133–136.

Salt DE, Pickering IJ, Prince RC, Gleba D, Dushenkov S, Smith RD, Raskin I (1997). Metal accumulation by aquacultured seedlings of Indian mustard. Environ Sci Technol 31: 1636–1644.

Tran AT, Popova LP (2013). Functions and toxicity of cadmium in plants: recent advances and future prospects. Turk J Bot 37: 1–13.

Uraguchi S, Mori S, Kuramata M, Kawasaki A, Arao T, Ishikawa S (2009). Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. J Exp Bot 60: 2677–2688.

Wei B, Yang L (2010). A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. Microchem J 94: 99–107.

Welch RM, Norvell WA (1999). Mechanisms of cadmium uptake, translocation and deposition in plants. In: McLaughlin MJ, Singh BR, editors. Cadmium in Soils and Plants. Dordrecht, the Netherlands: Kluwer Academic Publishers, pp. 125–150.