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Nickel hyperaccumulation by natural plants in Turkish serpentine soils

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Abstract: Natural plants in Turkish serpentine soils were surveyed to determine their Ni accumulation capability. Geographic distribution and diethylene triamine pentaacetic acid (DTPA)-extractable nickel contents of the western Anatolian serpentine soils and their vegetative contents were studied to find the possible relationships between the phytoavailable Ni amount in the soil and the Ni content of potential accumulator plants. Over half of the surface area of Turkey was targeted in the study. Aboveground parts of 413 herbaceous plants and the surface soil (0-15 cm) of 192 serpentine samples were collected. A digital elevation model and ANUSPLIN and ArcGIS 8.1 software packages were employed for generation of climatic surfaces and analysis in preparation of comparative maps. Scientifically approved Ni hyperaccumulator plant taxa as well as readily emerging species were tested under greenhouse and climate chamber conditions. The varying magnitude of nickel determined in the aboveground parts of the test plants indicated that the resistance or vulnerability and Ni requirements of a plant species were species-specific and were more effective than the Ni amount in the soil on the uptake of the element. Significant differences were found between the amount of DTPA-extractable Ni in the soil and the Ni content of hyperaccumulator Brassicaceae plants grown in the same soil bodies. *Isatis pinnatiloba*, which is endemic to Turkey, was introduced as a nickel hyperaccumulator species. Bottlenecks and drawbacks of phytoremediation techniques for commercial use were discussed.

Key words: DTPA-extractable Ni, Brassicaceae, serpentine soils, *Isatis pinnatiloba*

Türk serpantin topraklarında doğal bitkilerce hiper nikel birikimi

Özet: Türk serpantin topraklarındaki doğal bitkiler, Ni biriktirme kapasitelerinin saptanması amacıyla etüt edilmiştir. Batı Anadolu'da serpantin topraklarının coğrafi dağılımı ve DTPA (dietilen triamin pentaasetik asit) ile ekstrakte edilebilir nikel kapsamları, üzerlerindeki bitki varlığıyla birlikte incelenerek, topraktaki bitkilerce alınabilir Ni miktarı ile potansiyel hiper toplayıcı bitkilerde biriken Ni kapsamı arasındaki ilişki ortaya konmaya çalışılmıştır. Araştırma kapsamında, Türkiye yüz ölçümünün yarısından fazlası taranmıştır. Dört yüz on üç adet otsu bitkinin toprak üstü aksamı

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ile bunların yaşadığı 192 serpantin alanından yüzey toprak örnekleri (0-15 cm) toplanmıştır. Karşılaştırmalı haritaların hazırlanmasında iklim yüzeylerinin ve analizlerinin elde olunabilmesi için, sayısal yükselti modeli (DEM), ANUSPLIN ve ArcGIS 8.1 yazılım paketlerinden yararlanılmıştır. Sera ve iklim odası denemelerinde, bilimsel açıdan kabul görmüş Ni hiper toplayıcı taksonların yanı sıra kolay çıkış yapan türler test edilmiştir. Sonuçlar, bir elementin alımında bitki türünün duyarlılık veya direncinin topraktaki miktarından daha önemli olduğunu göstermiştir. Topraktaki DTPA ile ekstrakte edilebilir Ni miktarı ile o toprakta yetişen bitkinin biriktirdiği Ni miktarı yönünden, hiper toplayıcı Brassicaceae türleri arasında önemli farklar olduğu belirlenmiştir. Türkiye endemiği olan *Isatis pinnatiloba*'nın, nikel hiper toplayıcısı türler arasına alınması gerektiği belirlenmiştir. Fitoremidasyon tekniklerinin uygulamadaki darboğazları ve sakıncaları tartışılmıştır.

Anahtar sözcükler: DTPA ile ekstrakte edilebilir Ni, Brassicaceae, serpantin toprakları, *Isatis pinnatiloba*

Introduction

Ultramafic igneous or metamorphic serpentine rocks containing less than 45% silica (SiO_2) and high concentrations of Fe, Mg, Cr, Ni, and Co, and the soils formed on these materials, may have hundreds of times more nickel than many other soils. Nickel content may be as high as 2% in some soils from which magnesium and silicon were leached (Golightly, 1981; Schellmann, 1983). Serpentine soils have invariably nickeliferous derivatives, particularly where environmental conditions are not favourable to nickel leaching. Therefore, some 85%-90% of nickel-accumulating plants are serpentine endemic (Reeves & Adıgüzel, 2008). The exchange of chemicals between plants and soil is an important part of the element cycling. Plants may easily uptake dissolved or exchangeable ions adsorbed at the colloidal surfaces of any elements (Branquinho et al., 2007). Thus, the total amount at the rhizosphere is not a good indicator of bioavailability (Adriano et al., 2004; Wang et al., 2004). Metal phytoavailability is controlled by a number of parameters, including biological and environmental conditions as well as soil properties, which makes it complicated to establish a link between metal availability in the soil and resulting plant uptake.

Nickel is an essential element for some plant species at a range of 0.01-5.00 µg g^{-1} dry matter. Urease, found in several higher plants, is a nickelloenzyme. However, nickel is not as important as copper and zinc in plant metabolism. Excess nickel is toxic to plants, like many other heavy metals (Seregin & Kozhevnikova, 2006), and the toxic threshold level of Ni in the soil is not certain (Baker & Proctor, 1990).

The phytoextraction technique targets the most dangerous, bioavailable fraction of a harmful metal that inhibits green plant use for the purpose of phytoextraction, because phytoavailability is more meaningful than the total amount of any heavy metal when studying the hyperaccumulator plants and phytoremediation phenomena. Chaney (1983) and Baker and Brooks (1989) were the pioneers who proposed the use of green plants as a remediation agent of contaminated soils. The term “hyperaccumulator” is restricted to describing the metal accumulation on a dry matter basis for aboveground tissues grown and picked from a natural field (Baker & Brooks, 1989; Baker et al., 2000; Baker & Whiting, 2002). This means that species grown in Ni-rich solutions or in soils amended with excess Ni should not be considered hyperaccumulators (Reeves & Adıgüzel, 2008). As naturally polluted soils have contained heavy metals for long periods of time, the emergence of several biological entities, including plants, that are adapted to the soil conditions may be expected. The genetic differences among the species and the soil properties are the main factors determining the uptake rate.

A nickel hyperaccumulator grown under natural conditions should contain 1000 mg kg^{-1} dry matter or more Ni in its aboveground tissues (Baker & Brooks, 1989; Reeves et al., 2001, Reeves et al., 2009). The nickel content of hyperaccumulators may range between 4200 and 20,400 mg kg^{-1} (Li et al., 2003). Nickel-accumulating species comprise about three-quarters of all hyperaccumulator plants (Baker et al., 2000; Reeves & Baker, 2000; Ghaderian et al., 2007). The genus *Alyssum* L. (Brassicaceae) has the highest number of such members, with 48 taxa accumulating up to 3% Ni in their foliage (Brooks et al., 1979; Reeves & Baker, 2000). The number of discovered Brassicaceae members, some of which are Ni hyperaccumulators, has increased day by day (Özhatay et al., 2009; Mutlu, 2010; Özhatay et al., 2011). Hyperaccumulators do not usually compete with normal flora (Baker et al., 2000). We observed some allelopathic characteristics of hyperaccumulators *Alyssum murale* M.Bieb. and *A. corsicum* Duby in the field; the former species was also reported by Zhang et al. (2007), and neither species allowed other genera to grow nearby.

Little is known about the genetic properties of hyperaccumulators. Some individuals of any species can be hyperaccumulators, while others have no signs of genetic polymorphism differences (Pollard et al., 2002). No plant species has so far been discovered or bred holding all desirable properties, including fast growth, high biomass, and high amounts of heavy metal accumulation in the foliage. Hyperaccumulators rarely have all desirable characteristics required by phytoremediation exercises. Any hyperaccumulator plants lacking some suitable traits could be used as gene sources for hybrid development, or as test plants for understanding the physiology of metal accumulation in model system studies (Roosens et al., 2003). They can be selected by conventional or more sophisticated breeding methods when desirable genotypes are defined.

Material and methods

The aboveground parts of 413 herbaceous plants and the surface soil (0-15 cm) of 192 serpentine samples were collected from western Asia Minor and eastern Thrace. Areas contaminated by human activities were excluded as not resembling the typical soil properties and for the rare possibility of containing genotypes well adapted to those soils. The total research area was over half of the entire surface area of Turkey, about 400,000 km^2, possibly covering all visible serpentine formations in the region. More soil samples were taken every 25-100 km^2 from extensive ultramafic bodies. A geological map at a scale of 1:100,000, prepared by the Turkish Mineral Research and Exploration General Directorate, was used to determine serpentine routes. The geographical coordinates and elevations of the sampling locations from sea level were determined using a handheld Magellan eXplorist XL GPS device with 3-6 m of accuracy. The plant species from natural vegetation and from virgin soils belonged to 31 families; among them, Brassicaceae was particularly common, with 62 genera and 174 defined species. The 3 serpentine soils containing the highest levels of diethylene triamine pentaacetic acid (DTPA)-extractable Ni (DTPA-Ni) were selected for the experiments under controlled conditions. Seeds of 153 plants were collected to check their germination ability, growth potential, and expected metallophyte abilities under controlled conditions.

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

Average annual rainfall in the research area ranges between 321 mm (Konya) and 1127 mm (Muğla). Precipitation increases from the continental region to the seashores towards the west. The lowest mean annual temperature is 10.4 °C (Kayseri) and the highest is 19.2 °C (Mersin). Temperate climate, characterised by seasonal rainfall, was greatly responsible for the formation of extensive neutral to alkaline soils.

Climatic data (1975-2004) from 185 stations in the research area were provided by the Turkish State Meteorological Service. A digital elevation model (DEM) and ANUSPLIN and ArcGIS 8.1 software packages for generation of climatic surfaces and analysis were employed to generate comparative maps (Tunçay et al., 2006). Procedures from the ANUSPLIN software were used to fit the thin plate spline functions, which were trivariate functions of longitude, latitude, and elevation (Hutchinson, 1991). The ANUSPLIN package arbitrarily provides many surfaces and introduces the concept of surface-independent variables that may be changed systematically from one surface to another.

The ready-to-use DEM is a map converted from a digital topographic map of 1:250,000 scale with a resolution of 0.01° covering the study area. After the small-scale DEM data were used in generating climatic surfaces, all climatic maps were reprojected to the geographic coordinates with 0.5° × 0.5° resolution. Extractable-Ni data were tabulated into 6 arbitrary range classes for fitting in the ArcGIS 8.1 software for mapping. Most of the meteorological stations were located in towns, whereas almost all of the sampling points were in remote rural areas. Thus, an additional GIS analysis was performed by matching the climatic data with the sampling locations. This part of the study facilitated estimation of the real climatic conditions at the sampling locations.

Hyperaccumulators and volunteer plants were preferably planted during the preliminary greenhouse and growth chamber trials. The serpentine soils containing the highest amount of DTPA-Ni were used in all experiments. During the control tests, the seedlings were germinated in a peat-perlite mixture and were then transplanted to a media containing 50 ppm nitrogen, 50 ppm phosphate, and 5% peat on a volumetric basis. To simulate natural conditions, another set of the seeds was directly planted in the test soils for evaluation of accumulating performances. No artificial applications were performed except for lighting, heating, and humidity control in the growth chamber and periodical watering in the greenhouse, which were inevitable practices differing from natural field conditions. The variable DTPA-Ni contents of each soil facilitated the comparison of the metal-accumulating abilities of several plants.

Surface soil samples (0-15 cm) were sieved to 2 mm in situ and put in polyethylene bags (Ure, 1996). Plant samples, carried in cotton sacks, were washed with tap water and deionised water and dried at 65 °C until a constant weight. Extracts were taken, ranging from air-dried soil equivalents of ≤2 mm in diameter to 50 g of oven-dried samples, with 100 mL of a DTPA solution consisting of 0.005 M DPTA, 0.1 M triethanolamine, and 0.01 M $CaCl_2$ at pH 7.3 (Lindsay & Norvell, 1978) at 25 ± 1 °C, then shaken for 2 h. Extracts were centrifuged at 5000 rpm and filtered on a 0.2-mm Whatman filter. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to determine the DTPA-Ni content of the soils (Miller, 1998).

Ground plant samples were digested with 5% nitric-perchloric acid in a Speedwave^{TM} MWS-3^{+} system from Berghof (Kalra & Maynard, 1998) and filtered on Whatman 42 for Ni analysis in ICP-OES (Miller, 1998).

The 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%. Leaves of several plants turned purple; this peculiar phenomenon was attributed to the deficiency of the solar spectrum. The following species were selected for the greenhouse experiments based on their stand establishment and heavy metal accumulation capabilities: *Alyssum caricum* Dudley, *Alyssum peltarioides* Boiss. subsp. *virgatiforme* (Nyar.) Dudley, *Alyssum pateri* Nyar. subsp. *pateri*, and *Isatis pinnatiloba* Davis.

Experiments were conducted with 8 parallels to reduce experimental errors in freely drained polyurethane pots. The majority of the test material consisted of perennial plants; therefore, they were harvested when they produced a sufficient amount of biomass for laboratory analyses, i.e. before they reached maturity.

Results

The polynomial curves drawn for the Ni content of the plants versus the extracted amount in the soil showed a great deal of relationship. This suggests that the accumulation rate of Ni in plant tissues increased with the increase of the DTPA-Ni content of the soil up to a certain tolerance level. Varying magnitudes of nickel accumulation indicated that resistance (tolerance or vulnerability) and requirements of a plant species were more effective than the Ni amount in the soil, whereas the terms “extractable” and “bioavailable” were often used interchangeably.

The soil pH in the research area ranged between 7.30 and 9.38, while the most common values were 7.50-7.80 (Table 1). None of the serpentine soils were saline. Their free carbonate content was normally less than 1%, with a few exceptions of up to 37.3%. The dominant soil texture was close to loam. A few soils containing high levels of organic matter were of woodland origin.

Total nickel contents in the research area ranged between 2000 and 3000 mg kg^{-1}. Most samples contained less than 0.1% extractable nickel (Table 2). Evidently, climatic conditions, soil formation processes and soil properties, elevation, aspect, topographic components, and vegetation history, as well as geological factors, played a role in the final extractable nickel contents. The first conclusion to be drawn was the existence of a negligible

Table 1. Ranges of selected properties in the serpentine soils.

Properties	Lower limit	Higher limit
pH, 1:2.5 suspension	7.30	9.38
EC, 1:2.5 suspension, dS m^{-1}	0.07	0.42
Clay, %	13.2	59.3
Silt, %	10.3	48.6
Sand, %	19.4	74.1
Free carbonates, %	0.0	37.3
Organic matter, %	0.65	9.30

Table 2. DTPA-extractable Ni content ranges of the serpentine soils.

Ni content range, ppm	<1	1-5	5-10	10-20	20-40	40-80	80-150
No. of samples	66	43	28	27	15	11	2

relationship between the total and DTPA-extractable nickel contents of the soils. If DTPA extraction is an indication of partial or full bioavailability, the establishment of any correlation between the total Ni amount in soils and the accumulated amount in the plant body is likely to be misleading.

It is clear that the terms "phytoremediation" and "phytomining" have different meanings. To date, no record has been displayed of the commercial or wide application of any phytoremediation techniques. Phytomining, in addition, requires more complicated, harsh, and rigid applications, including ease of access to the area, convenient particle size distribution, availability of nutrients and toxic substances, satisfactory rhizosphere-depth total root length, and a large amount of biomass. It is especially necessary to understand the uptake and transport mechanisms of heavy metals in soils and plants, as well as their possible threats in the food chain.

The phytoavailable Ni contents of the central Anatolian soils were rarely more than 10 mg kg^{-1} (Figures 1 and 2). The nickel content of the plant samples collected from that region never exceeded the hyperaccumulation limit. This consistency was taken as evidence of the relationship between the DTPA-Ni content of the soil and the amount of Ni accumulated in the plant. Where the DTPA-Ni content of the soil was low, the plants grown on that soil did not accumulate high amounts of Ni, even if they were a hyperaccumulating species. Another conclusion that can be drawn from Figures 1 and 2 is the apparent effect of annual precipitation and mean temperature on the weathering conditions of serpentines and the resulting Ni release. Figures 1 and 2 show that a number of soil samples collected from the rainy, hot regions contained more than 10 mg kg^{-1} of DTPA-Ni. This was not the case with cooler continental soils, which also receive less precipitation on average.

The greenhouse trials were conducted with 3 serpentine soils. Nickel contents of the aboveground parts of the plants were as follows: *Alyssum caricum*, 7576 mg kg^{-1}; *Alyssum peltarioides* subsp. *virgatiforme*, 4411 mg kg^{-1}; *Alyssum pateri* subsp. *pateri*, no growth; and *Isatis pinnatiloba*, 275 mg kg^{-1}.

All selected plants were perennial species due to the absence of any known annual Ni hyperaccumulators. If there had been any annual hyperaccumulator plants available, they would probably have grown more vigorously and more rapidly, and then the results could have been more easily interpreted. The heterogeneity of growing periods of the test material, limited or no plant

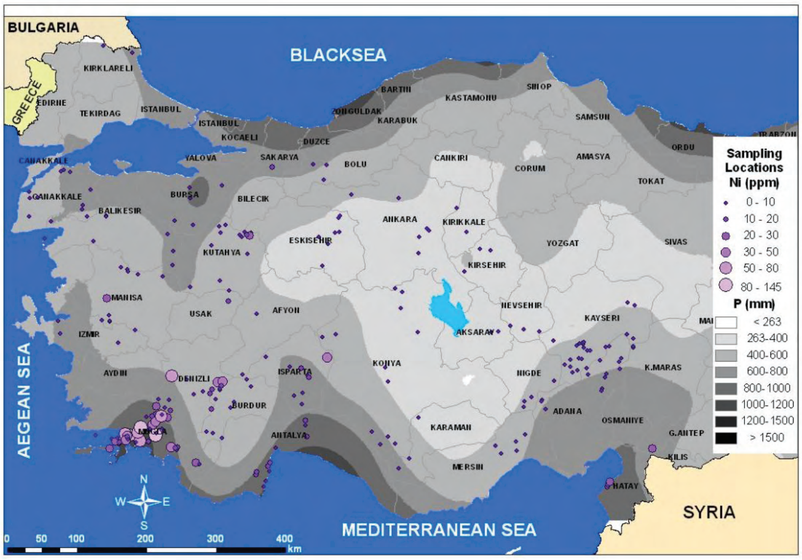

Figure 1. The relationship between annual rainfall (mm) and DTPA-extractable Ni content of the soils.

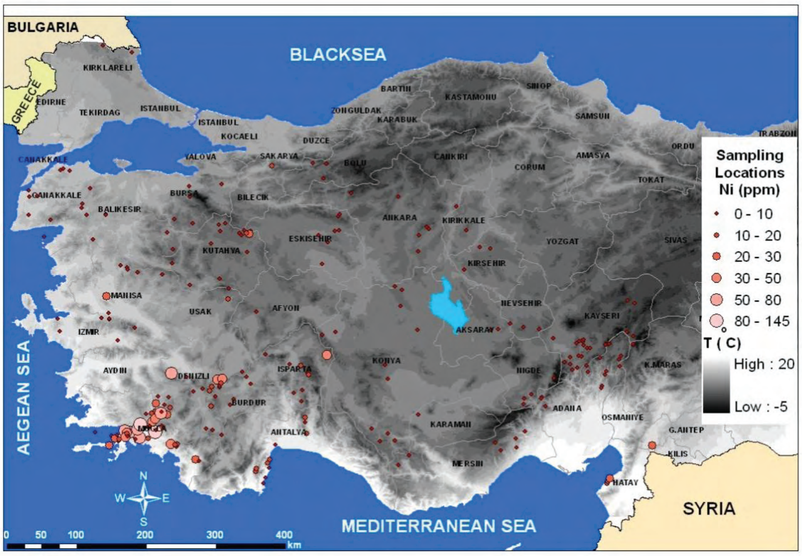

Figure 2. The relationship between mean annual temperature normal (°C) and DTPA-extractable Ni content of the soils.

emergence in some pots, inexplicable wilting and drying of some individuals, and delayed or no growth of some biennial and perennial species were among the difficulties encountered during the greenhouse experiments. In the absence of sufficient material from which to draw a reliable conclusion, the values obtained from the experiment need to be verified with further studies conducted under appropriate conditions with sufficiently large amounts of material and replications.

The total and DTPA-Ni contents of the serpentine soils were found to be much higher than those of the ordinary soils. The rate of DTPA-Ni to total Ni varied between 2.9% and 5.8% (Table 3). The narrow range of these percentages versus the great changes of any definite hyperaccumulator species indicated the partially dependency of the DTPA-Ni content of the soils on the total amount. Plant roots were able to uptake dissolved Ni as well as exchangeable ions at the colloidal surfaces, and the ions of any particles were evidently not available.

Table 3. DTPA-Ni and total nickel contents of the selected serpentine soils, mg kg^{-1}.

Soil	Total Ni	DTPA-Ni
A1	2484	143.35
A2	2821	105.40
A3	2603	75.68

The relationships between the DTPA-Ni content of the soils and *Alyssum minus* (L.) Rothm. var. *minus*, *Ricotia carnosula* Boiss. & Heldr., and *Sisymbrium altissimum* L. ($P < 0.05$); *Silene aegyptiaca* (L.) L.f. subsp. *aegyptiaca* ($P < 0.01$); and *Cruciata taurica* (Pallas ex Willd.) Ehrend. ($P < 0.001$) grown in those soils were found to be significant, although none of these species are Ni hyperaccumulators. Correlation analysis could be performed for the species that existed at 3 or more sampling locations. *A. sibiricum*, a well-known Ni hyperaccumulator collected from 10 different locations, was closely dependent on the DTPA-Ni content of the soil. The Ni content of the foliage of *A. masmenaeum* Boiss., another perennial Ni hyperaccumulator, was 1894-13,485 mg kg^{-1} in 6 samples. According to the currently accepted limits, steady Ni hyperaccumulation under the studied conditions suggests that the DTPA-Ni content of the soil (none contained more than 2.3 mg kg^{-1} DTPA-Ni) may hardly affect the Ni accumulation of the species. No prior research results were available to compare with our results as to whether those 2 species are restricted to soils in which DTPA-Ni content is limited or whether DTPA-Ni analysis is not a reliable indicator for determining their hyperaccumulation traits. *A. sibiricum* seeds germinating in a soil sample with 105.4 mg kg^{-1} DTPA-Ni did not grow vigorously in the growth chamber. Although the seedlings did not grow by more than a few centimetres, they accumulated 2334 mg kg^{-1} of Ni on average. The relationship between the DTPA-extractable Ni content of the serpentine soils and the Ni content of the foliage of the 4 most extensive hyperaccumulators showed that Ni uptake and accumulation dependency were as follows: *I. pinnatiloba* ($R^2 = 0.2927$, insignificant, negative), *A. masmenaeum* ($R^2 = 0.1441$, insignificant, negative), *A. murale* ($R^2 = 0.4661$), and *A. sibiricum* ($R^2 = 0.8054$) (Figure 3).

The curve showing the relationship between the DTPA-extractable nickel content of the soil and the nickel content of *I. pinnatiloba* Davis is unique, as this species was first introduced as a Ni hyperaccumulator in the present study. Of the 7 samples, 2 contained 1441 mg kg^{-1} and 1288 mg kg^{-1} in their aboveground parts in field conditions, values over the Ni-holding threshold value. Varying Ni contents in the plants (23-1441 mg kg^{-1}) may be partly attributed to the varying DTPA-Ni content of the soils or genotypic differences. The contents determined were relatively low compared to those of other nickel hyperaccumulators, but the large biomass and rapid growth of the species were 2 important advantages. *I. pinnatiloba* is a biennial or perennial plant and it is up to 70-80 cm tall. The germination and emergence rates of that species were more than 80% under controlled conditions. All those features suggest the potential of the plant to be introduced as a hyperaccumulator.

Geologically polluted soils may differ from those contaminated by human activities as the former may more frequently contain a number of heavy metals and the geological development era might have been shaped by peculiar physical, chemical, and biological properties. DTPA-extractable Fe, Mn, and Zn contents as well as DTPA-Ni contents were higher than usual in the studied serpentine soils. That is why studies carried out on artificially contaminated soils are of relatively limited use (Kirkham, 2006). The results obtained from those soils may be used for an understanding of the environmental and biological conditions that may play a role in the efficiency of

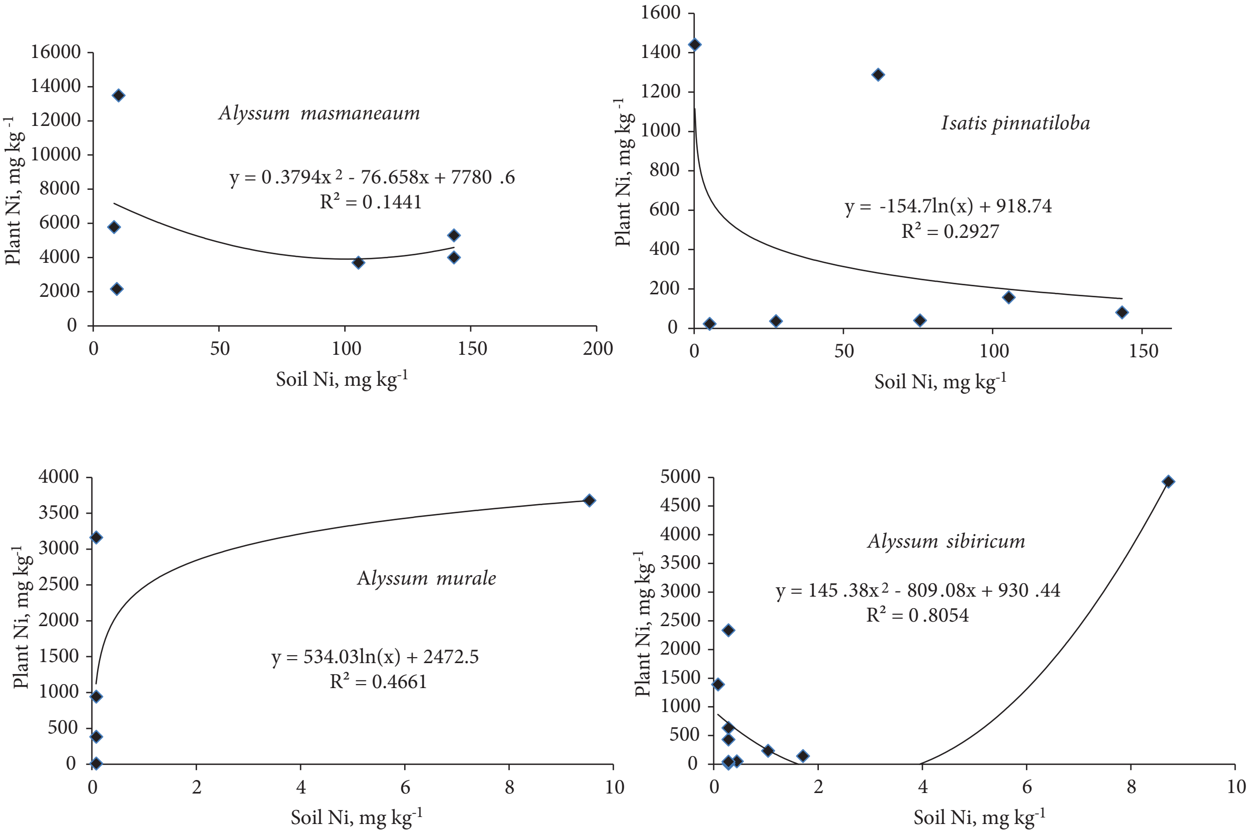

Figure 3. The relationship between the DTPA-extractable nickel content of the soil and nickel content of hyperaccumulator plants.

remediation. This information may also be used by plant breeders in clarifying the characteristics of the specific enzyme activities and genetic characteristics. However, field applications require more distinct results obtained from open-land studies under similar environmental and biological conditions.

DTPA extraction is an extensive technique used in determining the bioavailable Ni content in the soil (Lindsay & Norvell, 1978; Leita et al., 1999). Bioavailability is determined by a number of parameters, including soil organic matter content, texture, and pH. The concept of bioavailability loses its importance when the differences in the uptake ability between plant species and individuals are considered; in other words, an element might be readily available for some plants, but not for others. Misra and Pande (1974) compared 9 extraction techniques for the Ni uptake of sorghum in alluvial soils. They did not find any statistical correlations between Ni accumulation in the plants and EDTA, $K_4P_2O_7$, and citric acid, while Griggs reagent was the most effective method for removing Ni from the soil. The acetic acid extraction method evaluated with pH gave the best correlation among 9 techniques (Haq et al., 1980). In short, "the best-fitting method" is a hard term to define.

Effects of soil conditions and climate

The bioavailable Ni content in the soil generally increased with annual rainfall and mean temperature, while it was limited along newly cut roads. Brassicaceae and Poaceae members were the pioneer species at new roads. The DTPA-Ni content of the soils, or so-called bioavailable Ni, was less than 1 mg kg^{-1} at most locations, possibly allowing for a variety of species to grow with no visible symptoms and without accumulating large amounts of nickel. All research soils had neutral to slightly alkaline reactions, and the conditions were generally not favourable for heavy metal release.

Table 4. The relationship between nickel contents of the soils and nickel accumulation in the foliage.

Location	Coordinates[1]	Family	Plant species	PNi[2]	SNi
Adana, Feke, Mansurlu	37°49′462″N, 35°32′994″E	Brassicaceae	*Alyssum filiforme* Nyar.	2699	3.41
Ankara, Bala, Oğulbey	39°41′001″N, 32°49′583″E	Brassicaceae	*Alyssum sibiricum* Willd.	234	1.04
Denizli, Acıpayam, Çamköy	37°13′871″N, 29°31′400″E	Brassicaceae	*Alyssum pateri* Nyar. subsp. *pateri*	1560	16.20
		Rosaceae	*Potentilla reptans* L.	110	
		Caryophyllaceae	*Bolanthus thymoides* Hub.-Mor.	227	
Denizli, Altınyayla	36°57′620″N, 29°35′238″E	Brassicaceae	*Erysimum kotschyanum* Gay	755	8.72
		Brassicaceae	*Alyssum caricum* Dudley & Hub.-Mor.	9505	
Denizli, Tavas, Yahşiler	37°39′680″N, 28°52′228″E	Caryophyllaceae	*Silene aegyptiaca* (L.) L. fil. subsp. *aegyptiaca*	327	56.80
Gaziantep, İslahiye, Tahtalıköprü Dam	36°51′379″N, 36°41′144″E	Brassicaceae	*Hesperis bicuspidata* (Willd.) Poiret	533	27.30
		Brassicaceae	*Thlaspi perfoliatum* L.	357	
		Caryophyllaceae	*Silene aegyptiaca* (L.) L. fil. subsp. *aegyptiaca*	2337	
Hatay, İskenderun, Arsuz, Höyük village	36°21′670″N, 35°55′345″E	Brassicaceae	*Thlaspi oxyceras* (Boiss.) Hedge	7550	19.80
Hatay, İskenderun, Arsuz, Höyük village	36°21′870″N, 35°55′560″E	Brassicaceae	*Ricotia carnosula* Boiss. & Heldr.	147	21.00
		Brassicaceae	*Alyssum tortuosum* Willd.	1278	
		Brassicaceae	*Alyssum murale* Waldst. & Kit. var. *murale*	942	
		Brassicaceae	*Alyssum sibiricum* Willd.	431	
Kayseri, Yahyalı, Büyükçakır	37°46′932″N, 35°25′166″E	Brassicaceae	*Alyssum peltarioides* Boiss. subsp. *peltarioides*	4411	2.69
Kütahya, Harmancık	39°37′778″N, 28°47′107″E	Brassicaceae	*Alyssum peltarioides* Boiss. subsp. *virgatiforme* (Nyar.) Dudley	2505	74.20
Mersin, Fındıkpınarı	36°53′219″N, 34°23′864″E	Brassicaceae	*Erysimum smyrnaeum* Boiss. & Bal.	117	8.61
		Brassicaceae	*Erysimum crassipes* Fisch. & Mey.	114	
Muğla, Ekincik, Ova District	36°51′271″N, 28°32′396″E	Brassicaceae	*Erysimum kotschyanum* Gay	167	12.60
Muğla, Göcek-Fethiye highway	36°44′618″N, 29°00′230″E	Asteraceae	*Inula heterolepis* Boiss.	241	17.30
Muğla, Köyceğiz, Arıcılar, Geyik canyon road	37°06′786″N, 28°36′181″E	Brassicaceae	*Alyssum caricum* Dudley	13 778	14.50
		Geraniaceae	*Geranium lucidum* L.	112	
		Illecebraceae	*Scleranthus perennis* L.	178	
		Rubiaceae	*Galium tenuissimum* subsp. *tenuissimum*	108	
		Brassicaceae	*Thlaspi oxyceras* (Boiss.) Hedge	12 273	
		Brassicaceae	*Alyssum caricum* Dudley	5647	
		Brassicaceae	*Alyssum dasycarpum* Steph. ex Willd.	132	
Muğla, Marmaris, Ayınkoyu	36°55′283″N, 28°08′988″E	Asteraceae	*Crepis reuterana* subsp. *reuterana*	278	63.20
		Poaceae	*Avena wiestii* Steudel	100	
		Poaceae	*Poa annua* L.	124	

[1] GPS coordinates show the sampling location with 3-6 m of accuracy.

[2] PNi: Ni content of dried foliage, mg kg^{-1}; SNi: DTPA-extractable Ni content of the soil, mg kg^{-1}.

Prominent plant species containing over 100 mg kg^{-1} of Ni content are listed in Table 4 considering the hidden potential of any species possibly covered by a limited amount of phytoavailable nickel in the soil, although the lower limit for a Ni hyperaccumulator species is 1000 mg kg^{-1} dry matter (Baker et al., 2000). The only representative of Ni hyperaccumulators among the 46 plant samples from central Anatolia was *A. sibiricum* from the Oğulbey township of Ankara Province. This might be considered as a sign of the relatively small role of the DTPA-extractable Ni content of the soil on Ni uptake by this species.

The less favourable conditions for nickel release in the soil are seen in Table 4, with a high number of soils having DTPA-extractable Ni contents of more than 10 mg kg^{-1}. Table 4 suggests that nickel uptake and accumulation are related to the bioavailable amounts in the soil as well as the plant species.

Discussion

Some three-quarters of hyperaccumulator plants are Ni-accumulating species. The currently known number of Ni hyperaccumulators, more than 360 (Reeves, 2006), has been increasing day by day. Most of the Ni hyperaccumulators are naturally found on Ni-rich serpentine soils. Perennial species of the genus *Alyssum* (Brassicaceae) were most commonly found on the Ni-rich serpentine soils in terms of number and area covered, followed by members of the families Poaceae, Scrophulariaceae, Asteraceae, and Campanulaceae. Dominant allelopathic Brassicaceae species on the serpentine soils, including *A. corsicum* and *A. murale*, suggest the need to focus on the phytoremediation and phytomining of those specific plants in future studies.

The nickel content of *Psychotria douarrei* shrubs was not found to be related to the size of the plant or the total Ni content of the soil. In addition, old leaves might contain up to twice as much nickel as the younger ones, and nickel content varied among individual shrubs and branches within a single shrub (Boyd et al., 1999). Those findings suggest that nickel movement in plants could be slower and that the degree of accumulation of Ni could be less than that of other elements. This fact also indicates that the total Ni content of the soil was less important in the uptake mechanism, and that the genetic composition of individuals may play an important role in accumulation.

The varying dependency of plant species on the DTPA-Ni content of the relevant soil for Ni (hyper) accumulation abilities revealed the differences in Ni uptake and transport processes. This variability also showed the need for more research and discussion about which extraction methods can better simulate the processes in the field.

This research area may be considered large enough to draw some reliable conclusions about Ni availability and accumulation phenomena. The results from the field studies were reinforced by a series of experiments under controlled conditions. However, all soil samples were collected from temperate semiarid or subhumid regions; in other words, the conditions were favourable only for the development of neutral to slightly alkaline soils. The results obtained from this particular study are not sufficient to deduce general conclusions about the relationship between the Ni content of soils and Ni accumulation by plants. However, a hyperaccumulator species should accumulate hundreds of times more Ni than the other plants growing nearby. Nickel harvesting by artificially grown and picked hyperaccumulator plants is not an open-ended process. Root depth and extension are other factors governing the process, but they also limit nickel uptake from the soil. Other important goals to consider are the maintenance of the yield and proper management of the by-products without imposing any harmful effects on living organisms and the environment, and the enhancing of phytoremediation efficiency by combining 2 or more species or by the inclusion of other treatment practices. Some narrow or deep cracks, however, might not be penetrable by plant roots even if all of those measures are taken.

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