Turk J Bot
28 (2004) 147-153

Rare Plants and Nickel Accumulators from Turkish Serpentine Soils, with Special Reference to Centaurea Species

Roger D. REEVES
Institute of Fundamental Sciences – Chemistry, Massey University, Palmerston North - NEW ZEALAND
Nezaket ADIGÜZEL
Yüzüncü Yıl University, Faculty of Science and Arts, Department of Biology, Van - TURKEY

Received: 29.08.2002
Accepted: 29.01.2003

Abstract: Recent exploration and collecting from areas of serpentine soils in western and central regions of Turkey have shown that there is still much to be discovered. This work has led to the identification of new species, the re-collection of very rare species, the recording of extensions to the known ranges of several species, and the discovery of new instances of Ni hyperaccumulation, in which plant species can accumulate this element to concentrations exceeding 0.1% of the dry weight of the plant. In Turkey, there has already been special interest in Ni accumulation by species of *Aethionema* R.Br., *Alyssum* L., *Bornmuellera* Hausskn., *Cochlearia* L. and *Thlaspi* L. (*Brassicaceae*). We now report instances of hyperaccumulation of Ni in some of the Turkish serpentine occurrences of *Centaurea* L. (*Asteraceae*).

There is worldwide interest in exploiting the property of hyperaccumulation, both for remediation of metal-contaminated soils ('phytoremediation') and for economic selective extraction of metal compounds by cropping hyperaccumulators ('phytomining'). The potential for these processes to be carried out in Turkey, the need for further exploration of the natural resource and the conservation issues involved are discussed.

Key Words: Hyperaccumulation, nickel, serpentine plants, Turkey

Introduction

Following the discovery of Ni accumulating species of *Alyssum* L. by researchers in Italy, the USSR and Portugal (Minguzzi & Vergnano, 1948; Doksopulo, 1961; Menezes de Sequeira, 1969), other European species of this genus were investigated by Brooks and Radford (1978). They found that 9 other such species in Mediterranean Europe behaved in the same way. These species typically contained Ni at concentrations in the range 1000-10,000 mg/kg in the dry matter when the plants grew on Ni-rich serpentine soils, a level about 100 times greater than that normally found in plants on serpentine, and 1000 times greater than those found in most plants on substrates with normal Ni concentrations. The term hyperaccumulation was introduced, and definitions refined, to describe this behaviour (Jaffré et al., 1976; Brooks et al., 1977; Reeves, 1992).

Recognising that the centre of diversity of the genus *Alyssum* was in Turkey, and that many of the reported locations were in areas of ultramafic (serpentine) geology, Brooks et al. (1979) surveyed Ni concentrations in almost all of the 170 species in the genus, including all of the 89 species listed by Dudley (1965) in the Flora of Turkey. The survey showed that about 48 taxa in the genus, including 30 from mainland Turkey, Cyprus and Lesbos, were hyperaccumulators, many of them with particularly high Ni concentrations – often exceeding 10,000 mg/kg (1%) in the dry matter.

Further investigation of herbarium specimens of the Mediterranean serpentine flora (Reeves et al., 1983; Reeves & Brooks, 1983; Reeves, 1988) showed the existence of Ni hyperaccumulators in other genera of the *Brassicaceae* of both Turkey and Mediterranean Europe, including *Bornmuellera* Hausskn., *Cochlearia* L. and *Thlaspi* L. Examples of the Ni levels found in these species are given in Table 1. In many cases the hyperaccumulator species are endemic to high-Ni serpentine soils, and invariably show high Ni concentrations. Some species,

however, can sometimes be found on other low-Ni substrates, and as a result sometimes show lower Ni concentrations.

Table 1. Examples of Turkish Ni Hyperaccumulators in *Alyssum, Bornmuellera, Cochlearia* and *Thlaspi*.

Species	Range of Ni conc. (mg/kg)	No. of analyses
Alyssum callichroum	33-10,900	4
A. cassium	5590-20,000	5
A. huber-morathii	1220-13,500	5
A. masmenaeum	5480-24,300	4
A. pinifolium	6670-12,600	3
A. pterocarpum	1190-6740	3
Bornmuellera glabrescens	14,800-19,200	3
Cochlearia aucheri	11,500-17,600	3
C. sempervivum	3140	1
Thlaspi elegans	8800-20,800	5
T. jaubertii	26900	1
T. oxyceras	3080-35,600	14

References: *Alyssum*: Brooks et al. (1979); *Bornmuellera*: Reeves et al. (1983); *Cochlearia*: Reeves (1988); *Thlaspi*: Reeves (1988).

Further field exploration in Turkey and analytical work by the present authors and their colleagues since 1996 has led to a number of other discoveries of interest. Some of this work has been discussed in recent publications (Kruckeberg et al., 1999; Reeves et al., 2001). A summary of Ni hyperaccumulators worldwide can also be found elsewhere (Reeves & Baker, 2000).

Significant exposures of ultramafic rocks and soils are found in many parts of Turkey (Figure 1), although they are not such important features of the geology of the eastern and south-eastern provinces. Notable areas include the central part of the North-west (Kütahya and Balıkesir provinces), the South-west between Antalya and Marmaris (Antalya and Muğla provinces), the Amanus Mountains (Hatay and Adana provinces), regions of the eastern Taurus (north and north-east of Mersin) and its extension into the Aladağ massif (Niğde and Adana provinces), and numerous areas in a band running generally north-eastwards for several hundred kilometres from near Adana to near Erzincan. Other significant

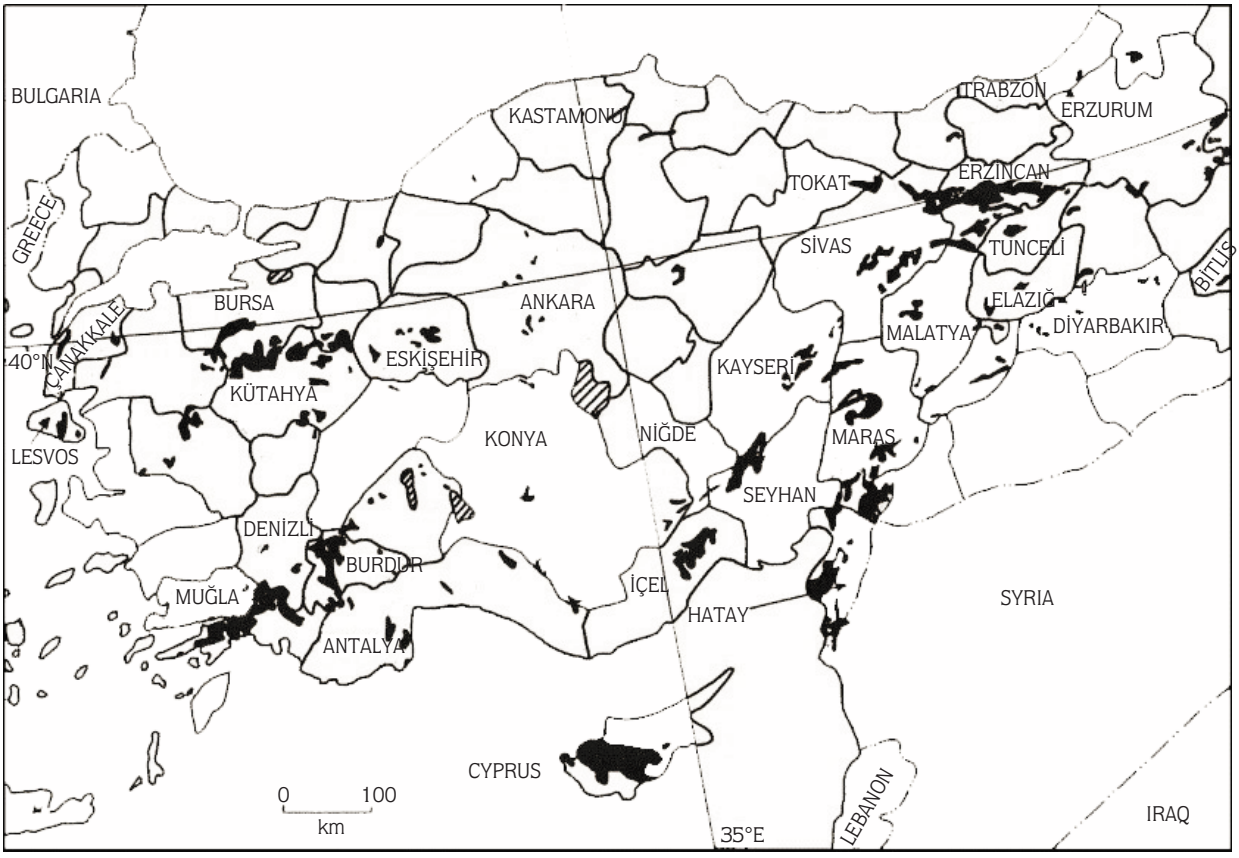

Figure 1. Map of Turkey showing areas of ultramafic geology (in black).

outcrops include several smaller areas near Ankara and in Çanakkale province.

The Turkish flora consists of more than 9000 species, with an endemism at the species or subspecies level that exceeds 25%. This included, in 1996, an extraordinary total of 500 species known only from their original collections (T. Ekim, pers. comm.); many others are known only from 2 or 3 specimens or locations. Due to the disproportionate contribution of serpentine floras to endemism elsewhere in the world, it appears possible that as many as 100 of these very rare species could be serpentine endemics. There is thus good reason to carry out further exploration of the Turkish serpentine flora. Our agenda has included: (i) finding out more about the distribution of known Turkish Ni hyperaccumulators, especially in *Alyssum* -- some of them are common, others are rare; some are serpentine endemic and others are not; (ii) trying to locate again species that have rarely been recorded; (iii) searching for new species; and (iv) searching for new Ni hyperaccumulators.

Materials and methods

The laboratory part of this investigation involved the analysis of plant material both from field collections by the authors and from herbarium specimens. Soil samples were also collected in the field and analysed by plasma emission spectroscopy (ICP) for about 20 elements to establish the general nature of the substrate. In particular, it is possible to confirm the ultramafic origin of the soils from a characteristic combination of high Mg, Fe, Ni, Cr and Co concentrations and a high Mg/Ca ratio. The plant and soil analysis procedures have been described in more detail elsewhere (Reeves et al., 1999).

Leaf samples of both field and herbarium specimens were washed by careful agitation in deionised water for 120 s, followed by air drying at 40 °C. In general, samples of 0.010-0.070 g were weighed out for analysis. The smaller samples were sometimes necessary to prevent undue damage to herbarium specimens. Larger samples were generally available from material collected directly from the field for analysis. Where data for a limited range of elements was sought (e.g. Ni, Co, Fe, Zn), the analysis was completed by atomic absorption spectrophotometry, following dry ashing for 4 h to a maximum temperature of 500 ºC, and dissolution of the ash in a suitable volume (usually 5.0 mL) of 2M HCl. Some specimens were analysed by inductively coupled plasma emission spectrometry (ICP) for a more comprehensive range of 12-20 elements.

Results and Discussion

During the 1996 sampling in Çanakkale province, the very rare *Alyssum pinifolium* (Nyár.) T.R.Dudley was found again. It was discovered by J. Kirk in April 1856, and collected again by P. Sintenis in April 1883 -- both when flowering had just started -- and was believed to have been collected again only by the late Frederike Sorger of Vienna in the 1960s, her July collections being the only ones known with fruit. Specimens of this species, occurring north of Ezine, exhibit both flowers and well developed fruit in May, and were found to have Ni concentrations of 4520-9330 mg/kg.

On a serpentine outcrop adjacent to the highway between Ankara and Kırıkkale, above the Kızılırmak, *Silene cserei* Baumg. subsp. *aeoniopsis* (Bornm.) Chowdhuri has been found, previously known only from its original discovery by J. Bornmüller in 1929, in a place 25 km further to the north. This species is not a Ni accumulator, but is notable for having 1.5-3.5% Mg in its leaves, very high even for a serpentine plant. Its initial discovery was near Kalecik, where it has recently been found again (Vural & Adıgüzel, 1996).

South of Ankara in the Beynam Forest area, studied extensively by Akman (1972; 1973), many specimens were collected, including the very rare and recently described *Aethionema dumanii* Vural & N.Adıgüzel, known previously from only 3 sites on carbonate-rich soil. Another unusual find in the Beynam Forest area was that *Thlaspi perfoliatum* L. here has >1000 mg/kg Ni in its leaves; this is the first time that an annual *Thlaspi* has been found accumulating Ni to this level.

Specimens from the serpentines of the coastal regions of south-western Turkey have confirmed the Ni accumulation by species such as *Alyssum pterocarpum* T.R.Dudley, *A. corsicum* Duby and *A. caricum* T.R.Dudley & Hub.-Mor. *Centaurium serpentinicola* Carlström, described only in 1986, was also found again. Near Marmaris is *Alyssum discolor* T.R.Dudley & Hub.-Mor., which occurs on and off serpentine, and is unusual in being a Ni accumulator that appears to grow better elsewhere, and *Thlaspi cariense*, first described by Carlström (1986), and known at present from only 3

Table 2. Ni accumulation by *Centaurea species* on serpentine in Turkey. (Species names are preceded by their number in Flora of Turkey, from which authorities can be obtained; the number of specimens analysed is given in parentheses after the concentrations).

Species	Localities	Ni conc. (mg/kg)
13. *C. cariensis*	Sandras da.; Fındıkpınarı; Değirmendere	18-31 (3)
14. *C. austro-anatolica*	Fethiye; Kumluca-Kemer	20-41 (3)
15. *C. dichroa*	Kale; Marmaris	27-32 (2)
17. *C. aggregata*	Pozantı; Tunceli; Mersin	6-17 (4)
46. *C. lycopifolia*	Dörtyol; Osmaniye	15-98 (2)
47. *C. cheirolopha*	Gülekboğazı; Arsus; Musa da.	2-1558 (6)
54. *C. drabifolia*	Beynam	89 (1)
59. *C. sericea*	Dursunbey	14830 (1)
79. *C. antitauri*	Karsantı	20280-24990 (2)
84. *C. haradjianii*	Amanus (Egbez)	4768 (1)
86. *C. aladaghensis*	Kamışlı	9899 (1)
89. *C. spicata*	Arsus; Erzin; Kuzucubelen	94-24040 (7)
90. *C. arifolia*	Amanus (Ordu)	17540 (1)
92. *C. tomentella*	Çardak-Göksun	59-1514 (2)
93. *C. cataonica*	Maraş-Narlı	7836 (1)
95. *C. amanicola*	Osmaniye	11050-11550 (3)
103. *C. ptosimopappa*	Amanus (Belen; Soğukoluk; Nur da.)	1075-23930 (10)
104. *C. ptosimopappoides*	Gerdibi; Karsantı	7320-11660 (6)
169. *C. ensiformis*	Sandras da.	19550-37750 (6)

locations, was collected and its predicted Ni hyperaccumulation confirmed.

Further east along the Mediterranean coast near Mersin, *A. murale* Waldst. & Kit. subsp. *murale* var. *haradjianii* (Rech.) T.R.Dudley was found, outside its previously recorded range; this taxon had not previously been analysed, but it is a Ni hyperaccumulator, and is probably serpentine endemic.

North-east of Pozantı there is a very extensive area of ultramafics constituting the western side of the Aladağ Massif. This supports Ni accumulating species such as *Alyssum floribundum* Boiss. & Bal. and *A. callichroum* Boiss. & Bal., the unusual crucifer *Cochlearia sempervivum* Boiss. & Bal. (a Ni accumulator with a Crassulaceous appearance), *Aethionema spicatum* Post and *Thlaspi elegans* Boiss. Adjacent to *T. elegans* in the open pine forest several kilometres south of Gerdibi was *Centaurea ptosimopappoides* (described by Wagenitz in 1974 from specimens collected by E. Yurdakulol), which contained the surprisingly high Ni concentration of 7320-11,660 mg/kg (Reeves et al., 2001).

Centaurea L. has over 600 species worldwide and is one of the major genera of the Turkish flora, with 179 species, 111 of which are endemic; as many as 15 may be serpentine endemic, but probably at least 20-30 others may occur at least some of the time on serpentine. Six other *Centaurea* species collected by the authors on serpentine in Turkey had previously been found to contain only 1-90 mg/kg Ni, with a median of 20 mg/kg; the behaviour of *C. ptosimopappoides* therefore set this species apart from the rest. This finding was particularly surprising because all other reliable Ni hyperaccumulators from Turkey and the Mediterranean have been in the *Brassicaceae*. A more detailed study of *Centaurea* in Turkey has now been carried out, to see if species related to *C. ptosimopappoides*, and others that may be serpentine endemic, share this property. The closest species, *C. ptosimopappa* Hayek, occurring in the Amanus Mountains, is certainly a hyperaccumulator, and is probably also serpentine endemic.

The results of this study indicate that there are at least 13 Ni hyperaccumulators in this genus; some appear to be serpentine endemic and are always found with high

Ni, while others occur on a variety of substrates and show a much wider range of Ni concentrations. Ni concentrations in selected *Centaurea* species from serpentine soils in Turkey are shown in Table 2.

Collections of *Alyssum* species in north-western Turkey in 1996 and 1998 have led to the discovery of a new Ni accumulating *Alyssum* species, recently published as *A. dudleyi* by Adıgüzel and Reeves (2002). This is one of several *Alyssum* species colonising the ultramafic overburden from the massive Tunçbilek open-cast coalmine, but has also been collected from several other locations from this area westwards towards Dursunbey.

A. sibiricum Willd. in this same region is unusual in that it accumulates 2000-9000 mg/kg Ni: this species has hitherto been regarded as anomalous in that it is a member of Sect. *Odontarrhena* (Meyer) Hook. containing all the 50 Ni hyperaccumulators known up to the present, but has itself not previously been found with high Ni, even on serpentine. Further detailed studies of this species, and its behaviour in relation to soil composition, are called for as various populations on serpentine are now known with 15-9000 mg/kg Ni.

Increasing interest is now being taken in potential practical applications of metal hyperaccumulation. Uses for Ni hyperaccumulators include the following.

(1) Since many Ni hyperaccumulators are endemic to soils of ultramafic origin, their distribution can be used as a guide in defining or delimiting the extent of such soils, even in the absence of soil analysis. (It is not necessary for a plant to be a Ni accumulator for this purpose: the use of any well-established serpentine-endemic species is sufficient. However, the existence of a rapid field test for Ni accumulation by a hyperaccumulator is especially helpful as a guide to soil composition.)

(2) There has been extensive recent work on phytoremediation. In the case of Ni, this potential is currently being demonstrated by the planting of *Alyssum murale* on several hectares of land around a former Ni smelter at Port Colborne, Ontario (Canada), where fallout from the smelter has raised soil Ni levels and made this land unsuitable for agricultural production.

(3) Work on the phytomining concept is also in progress. In the case of Ni, this implies that a Ni hyperaccumulator crop can be developed as a commercial proposition on areas of arable serpentine soils (Chaney et al., 2000; Angle et al., 2001), which occur in countries such as Turkey, Indonesia and the western USA. The soil may contain 0.1-0.3% Ni, which is both subeconomic for use as a Ni ore material and also makes the soil suboptimal for obtaining good yields of conventional crops. Growing a hyperaccumulator can produce 10-15 t/ha of dry matter, containing in favourable cases 1.5-2.0% Ni. Burning this dry matter can be used as an energy source, and also results in an inorganic ash containing 15-20% Ni (much richer than conventional Ni ores currently being exploited, most of which have 0.8-2.0% Ni). Such material will not replace conventional ore extraction, but could supplement it, representing a way of enriching the ore for further processing.

In Turkey, species such as *Alyssum corsicum* and *A. murale* already form almost a monoculture on some areas of waste land of serpentinitic soil composition, and could easily be developed as an agronomic crop. It is possible that some others of the best Ni hyperaccumulators, such as *Bornmuellera tymphaea* Hausskn. & Sint., *Centaurea ptosimopappa* and *C. ensiformis* P.H.Davis could also be developed in this way, although good information about their rate of biomass production is not available at present.

These observations should stimulate further exploration of the Turkish serpentines for other Ni accumulating species. In the wider scientific context, it is certain that more serpentine endemic species remain to be discovered in Turkey (whether Ni accumulators or not). Further exploration of some of the less well-known serpentine areas should be carried out before there is any further habitat modification for agriculture, forestry, conventional mining, urban development or other activities. Furthermore, it is essential that the exact distribution of all the Turkish Ni hyperaccumulators should be fully documented, to allow any necessary decisions to be made regarding their conservation, before their Ni accumulating property is exploited.

Conclusions

Recent collections from some of the serpentine areas of Turkey have led the following discoveries: (i) about 20 new Ni hyperaccumulators -- *Alyssum murale* subsp. *murale* var. *haradjianii, A. sibiricum,* A. aff. *cassium* Boiss. (probably a new taxon), *A. dudleyi*, *Aethionema spicatum, Thlaspi perfoliatum, T. cariense, Centaurea ptosimopappoides, C. ptosimopappa* and 11 other

Centaurea spp.; (ii) 1 new *Alyssum* species and the possibility that at least 1 other represents a new taxon; (iii) new records, and extensions to the recorded ranges, of rare plants such as *Alyssum pinifolium*, *Alyssum murale* subsp. *murale* var. *haradjianii, Aethionema dumanii, Silene cserei* subsp. *aeoniopsis*, *Cochlearia sempervivum* and *C. aucheri* Boiss. There is a real possibility that some of the Turkish Ni accumulating species will be useful in the future (not only in Turkey) for phytoremediation (removal of Ni from soils superficially contaminated by smelter fallout), and for phytomining (particularly some of the larger biomass *Alyssum* species such as *A. corsicum* and *A. murale*, and perhaps *C. ptosimopappa* also). More detailed field work in some of the Turkish ultramafic areas is therefore clearly justified.

Acknowledgements

The authors thank their colleagues from İstanbul and from Gazi University, Ankara (Prof. T. Ekim, Prof. M. Vural and Prof. H. Duman) for advice and for help with identification, and Mr. F. Karavelioğulları and Mr. Ö. Bingöl for assistance in the field. They are also grateful for help given by several persons connected with herbaria outside Turkey, especially Mr. I. Hedge, Prof. G. Wagenitz and Dr. M. Nydegger.

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