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Research Article

Gypsophila sphaerocephala Fenzl ex Tchihat.: A Boron Hyperaccumulator Plant Species That May Phytoremediate Soils with Toxic B Levels

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Abstract: Analyses were carried out to identify boron (B) hyperaccumulating plant species in an actively B- mined area of Kırka, Eskişehir, Turkey. Only 4 plant species, *Gypsophila sphaerocephala* Fenzl ex Tchihat. var. *sphaerocephala* (*Caryophyllaceae*), *Gypsophila perfoliata* L. (*Caryophyllaceae*), *Puccinellia distans* (Jacq.) Parl. subsp. *distans* (*Gramineae*) and *Elymus elongatus* (Host) Runemark subsp. *turcicus* (McGuire) Melderis (*Gramineae*), were identified in the highest B- containing sections of the mine. The species were found growing successfully under high total (8900 mg kg^{-1}) and available (277 mg kg^{-1}) soil B concentrations. Among these plant species, *G. sphaerocephala* contained considerably higher B concentrations in its above-ground parts (2093 ± 199 SD mg kg^{-1}, seeds; 3345 ± 341 SD mg kg^{-1}, leaves), compared to the roots (51 ± 11SD mg kg^{-1}) and organs of the other species as revealed by analyses using an ICP-AES (Varian, Vista model) instrument. This species was followed by *G. perfoliata* with respect to B concentrations in its various organs. This study shows that *G. sphaerocephala* was not only able to grow on heavily B-contaminated soils, but was also able to accumulate extraordinarily high concentrations of B. This provides a new plant genotype to explore the mechanism(s) of B hyperaccumulation which may lead to identifying the gene(s) conferring B-resistance and to phytomining of contaminated soils, especially where B-toxicity symptoms occur. To our knowledge, there are no reports available on the hyperaccumulation of B, although many reports are available on the phytoremediation of metalliferous soils that contain excess amounts of Zn, Mn, Cu, Co, Pb, Al and Ni.

Key Words: Boron mine, *Elymus*, ICP-AES, phytoremediation, *Puccinellia*, tolerance

Gypsophila sphaerocephala Fenzl ex Tchihat: Toksik Seviyede Bor İçeren Toprakların Bitkisel Yolla Temizlenmesinde Kullanılabilecek Hiper Akümüla Bir Bitki Türü

Özet: Eskişehir İli, Kırka İlçesi'nde halen faaliyette bulunan bir bor (B) madeni alanında doğal olarak yetişen potansiyel hiper akümülatör (aşırı biriktirici) bitki türleri araştırılmıştır. Maden alanında *Gypsophila sphaerocephala* Fenzl ex Tchihat. var. *sphaerocephala* (*Caryophyllaceae*), *Gypsophila perfoliata* L. (*Caryophyllaceae*), *Puccinellia distans* (Jacq.) Parl. subsp. *distans* (*Gramineae*) ve *Elymus elongatus* (Host) Runemark subsp. *turcicus* (McGuire) Melderis (*Gramineae*) olmak üzere sadece 4 bitki türü tespit edilmiştir. Bu türler yüksek toplam toprak boru (8900 mg kg^{-1}) ve elverişli toprak boru (277 mg kg^{-1}) konsantrasyonlarında başarılı bir şekilde yetişmektedirler. ICP-Atomik Emisyon Spektrofotometre (Varian Vista Model) ile yapılan analizler sonucu, diğer türlerle karşılaştırıldığında, *G.a sphaerocephala*'nın toprak üstü aksamında oldukça yüksek konsantrasyonlarda (2093 ± 199 SD mg kg^{-1}, tohum; 3345 ± 341 SD mg kg^{-1}, yapraklar) B içerdiği, köklerinde ise B konsantrasyonu daha düşük (51 ± 11 SD mg kg^{-1}) bulunmuştur. B içeriği bakımından bu türü *G. perfoliata* takip etmiştir. *G. sphaerocephala*'nın yüksek B toksite belirtilerinin görüldüğü topraklarda yetiştirilmesiyle hiper akümülasyon yoluyla bitkisel madencilik yapılabileceği gözükmektedir. Literatürde diğer ağır metal veya elementlerle (Zn, Mn, Cu, Co, Pb, Al ve Ni) ilgili bir çok bildirim olmasına kar?ın B elementi ile ilgili herhangi bir bildirime rastlanmamıştır. Bu çalışma bu yönden bir ilk niteliğindedir.

Anahtar Sözcükler: Bor madeni, *Elymus*, ICP-AES, bitkisel temizleme, *Puccinellia*, hiper akümülatör

Introduction

Phytoremediation is the use of plants to make soil contaminants non-toxic and is one form of bioremediation. Plants which uptake high levels of an element from soil are called hyperaccumulators; these are now being closely investigated, both by molecular techniques and by soil/plant analyses, at the sites where they occur. The term phytoremediation generally refers to phytostabilization and phytoextraction. In phytostabilisation, soil amendments and plants are used to alter the chemical and physical state of the heavy metal contaminants in the soil. In phytoextraction, plants are used to remove contaminants from the soil and are then harvested for processing (Karenlampi et al., 2000).

The term hyperaccumulator was first used in relation to plants containing more than 1000 µg g^{-1} (0.1%) Ni in dry tissue (Jaffre et al., 1976; Brooks et al., 1977). A later publication (Baker & Brooks, 1989) extended the use of the term to include plants containing more than 1% Zn or Mn, or more than 0.1% Cu, Co, Cr and Pb. The ability of *Thlaspi caerulescens* L. to accumulate Zn to more than 10,000 µg g^{-1} (1%) in dry tissue has been known since the 1860s, but it has become apparent from more recent work that several species of this genus can also hyperaccumulate (Reeves & Brooks, 1983; Reeves, 1988) from metal-rich soils and can hyperaccumulate a wider variety of metals (including Cd, Mn and Co) from amended nutrient solutions (Baker et al., 1994). There has also been recent interest in high-Cd populations of *T. caerulescens* from mine soils (Robinson et al., 1998; Reeves et al., 2001).

Plants suitable for phytoremediation should possess (a) an ability to accumulate the targeted metal(s), preferably in the aerial parts; (b) tolerance to the metal concentrations accumulated; (c) fast growth of the metal accumulating biomass; and (d) ease of cultivation and harvesting (Baker & Brooks, 1989). Chaney et al. (1997) have argued that metal tolerance and hyperaccumulation are more important factors than high biomass production.

A recent list of hyperaccumulators for several metals (Zn, Cd, Pb, Ni, Cu, Se and Mn) has been published (Reeves & Baker, 2000). This work did not consider several other elements, such as B, As and Al. There has been recent interest in As accumulation by ferns (Ma et al., 2001), and a plant which accumulates 3000 mg kg^{-1} Al has also been identified (Kochian et al., 2002), but very little is known to date about abnormal accumulation of B by plants.

Both deficiencies and toxicities of micro-elements can suppress plant growth. When present at increased levels of bio-availability, both essential micronutrients (Cu, Zn, Mn, Fe, Ni, Mo and B) and non-essential metals (micronutrient analogues, e.g. Cd, Pb, Hg and Cr) are toxic (Baker & Brooks, 1989). B occurs in many rocks and soils at total concentrations of 5-50 mg kg^{-1}, and is normally present in plant leaf tissue at concentrations of 10-50 mg kg^{-1}. However, many species, including important cereals such as wheat, are quite sensitive to elevated B in their tissues, and show severe toxicity symptoms at tissue levels of about 50 mg kg^{-1}. Such levels can be found in tissues when the available soil B exceeds 3 mg kg^{-1}.

Recently, Gezgin et al. (2002) surveyed the B contents of 898 soil samples from 7 provinces in Turkey; Konya, Afyon, Karaman, Aksaray, Niğde, Nevşehir and Kayseri. These regions encompass 3.5 million ha of cultivated land in Central Southern Anatolia. According to the survey, nearly 50% of soils in these provinces contained low levels of available B which can be corrected by external B applications in the form of borax or boric acid. However, another 18% of soils in this region contain B at more than the critical upper level for available soil B, which is considered to be 3 mg kg^{-1} (Keren & Bingham, 1985) for most crops. Accordingly, strategies should be developed either by breeding B- tolerant genotypes (which may take many years to achieve), or by phytoremediation with B-accumulating species. This could offer enormous advantages at such sites by helping to widen the areas in which cereals could be cultivated without suffering yield reductions. Soil amendments by conventional techniques such as leaching or increasing pH by liming (Nable et al., 1997) for increased B adsorption on soil seem not to suit Central Anatolian conditions due to its low annual rainfall and water shortages, and the high lime content of the soils. For this reason, B-accumulating species have been sought through a study of plants growing in a B-mining area.

Materials and Methods

Plants species were collected on 21 August 2001 from Etibor Co., Turkey a B- mining area of Kırka, Eskişehir (lat 43° 19' 23'' long 28° 28' 24'' at an altitude of 1125 m). This is one of the richest B mines in the world, with a borax yield of 25% (w/w), 3 times richer than any other B mine in the world. In addition, 65% of world B reserves are in Turkey, with an estimated value of nearly 700 billion USD (personal communication, Mr. B. M. Temizkalp).

Plant samples along with their representative soils (0-50 cm deep) were collected from the area. Samples of surface soils were collected from pits measuring 20 x 20 x 20 cm. All samples were individually put into plastic bags, which were directly brought to the laboratory for descriptions and analyses. Roots, stems, leaves, seeds and spikes where appropriate were separated.

All plant samples were carefully washed with water to remove any traces of soil and were then oven-dried at 70 °C for 48 h before dry weights were measured. Samples (0.5 g) of finely ground plant material were digested with concentrated HNO_3 in a microwave system (CEM). The B in the extracts was analysed by ICP-AES (Varian-Vista model) (Nyomora et al., 1997) in at least 4 plant samples with 3 replicates. The B standard used was from Merck, Germany.

In addition to plant samples from the Kırka mine area, *Gypsophila perfoliata* L. (*Caryophyllaceae*) plants were also collected from Çomaklı, Konya for comparison purposes. These samples naturally grow on soils near the experimental area of the Faculty of Agriculture. This was the only species growing in both this area and in the Kırka mine area.

Extractable B concentrations in soil were determined according to the method of Cartwright et al. (1983) by extraction with 0.01 M mannitol plus 0.01 M $CaCl_2$ using a soil:solution ratio of 1:5 and a shaking time of 16 h. The B extracted was determined by ICP-AES. Total B in the soil was determined by both mixed acid digestion and sodium carbonate (Na_2CO_3) fusion (Bingham, 1982).

Results and Discussion

The B contents of the plant species described are given in the Table. In the mining area, the predominantly occurring plants were *Gypsophila sphaerocephala* Fenzl ex Tchihat. var. *sphaerocephala* (*Caryophyllaceae*) (Figure), *Gypsophila perfoliata* L., *Puccinellia distans* (Jacq.) Parl. subsp. *distans* (*Gramineae*) and *Elymus elongatus* (Host) Runemark subsp. *turcicus* (McGuire) Melderis (*Gramineae*). *G. sphaerocephala* is a perennial species, is 20-70 cm tall, propagates through rhizomes and has a strong woody stem at its base (Figure) (Huber-

Table 1. Plant species collected from boron mining area, Kırka, Eskişehir and the distribution of boron in various organs.

Plant species	Distribution of boron contents (mg kg dry matter^{-1})			
	Roots	Stem	Leaves	Seed/Spike
Gypsophila sphaerocephala Fenzl. ex Tchihat. var. *sphaerocephala*	51 ± 11	232 ± 40	3345 ± 341	2093 ± 199*
Gypsophila perfoliata L.	57 ± 16	64 ± 22	1490 ± 172	N/A
Gypsophila perfoliata L.**	9.3 ± 3.8	27.6 ± 9.1	342 ± 3.4	N/A
Puccinellia distans (Jacq.) Parl. subsp. *distans*	241 ± 25	117 ± 50	802 ± 61	501 ± 65***
Elymus elongatus (Host) Runemark subsp. *turcicus* (McGuire) Melderis	N/A	98 ± 44	587 ± 104	280 ± 44***

N/A: Not available, * Seed, ** The plants were collected from the Çomaklı area (Konya) that contains 10 mg kg^{-1} available soil B, *** Spike. Values are mean ± standard deviation (SD) of 4 plant samples in 3 replicates each.

Figure 1. A boron hyperaccumulator *Gypsophila sphaerocephala* plant at the boron mining area of Kırka, Eskişehir, Turkey.

Morath, 1967). According to the same author, this species usually grows on dry slopes and limestone rocks at elevations of 500-2000 m.

G. perfoliata is a perennial species 30-120 cm tall, and can regenerate and grow through rhizomes on saline soils, steppe, slopes and cultivated lands at elevations between 1000 and 1500 m (Huber-Morath, 1967).

P. distans is a perennial species, 30-75 cm tall. Stems are solitary or tufted and erect. This species is reported to usually be found around saline areas (Tan, 1985).

E. elongatus is a caespitose perennial species. Stems are 35-75 cm tall, robust, and usually glabrous. According to Melderis (1985), this species is usually found around dry calcareous and saline sites.

Soil samples recovered from roots and collected from the sampling area contained an average of 277 mg kg^{-1} available B and 0.89% total B as analysed by ICP-AES.

Leaves of *G. sphaerocephala* contained the highest B concentrations (3345 ± 341 SD mg kg^{-1}) compared to other organs of this species and organs of the other 3 species, followed by seeds of the same species (2093 ± 199 SD mg kg^{-1}) and leaves of *G. perfoliata* (1490 ± 172 SD mg kg^{-1}), *P. distans* (802 ± 61 SD mg kg^{-1}) and *E. elongatus* (587 ± 104 SD mg kg^{-1}). Roots and stems were generally lower in B content than leaves and seeds or spikes (Table). This is in line with Baker and Brooks (1989) and Chaney et al. (1997), who stated that high accumulator plants should exhibit such responses.

G. perfoliata plants collected from Çomaklı, Konya naturally grown on a soil with 10 mg B kg^{-1} contained 339 ± 7 SD mg B kg^{-1} in their leaves with relatively lower B concentrations (9 ± 5 SD) in their roots, and with similar distribution patterns of B content in various organs to the plants from the Kırka mining area. Considering the B content of the soil in Çomaklı, the amount of B accumulated in the leaves of this species was relatively high but was some 4 to 6 times lower than in the same plant species from the Kırka B mining area and nearly 10 times lower than in *G. sphaerocephala*. These findings agree with those of Baker & Brooks (1989), who suggested that populations of metal-tolerant, hyperaccumulating plants should be sought in naturally occurring metal-rich sites, although these plants are not ideal for phytoremediation since they are usually small and have a low biomass production. However, plants of both *Gypsophila* L. species from the mining area grew vigorously (nearly 0.8 m canopy diameter per plant, reaching as high as 90 cm) with high biomasses. The drawbacks of both species were their perennial growth habits and strong tap roots (*G.*

sphaerocephala) and rhizomic underground roots (*G. perfoliata*) that may discourage their cultivation for phytoremediation. However these plants can serve as excellent experimental materials for molecular investigations of B hyperaccumulation mechanisms.

Strains or ecotypes in strongly metal-enriched environments have usually evolved exceptionally high levels of heavy metal tolerance (Baker & Brooks, 1989; Kochian et al., 2002), as appeared to be the case in the plant species collected in the present study. Considering that more than 5 mg kg^{-1} of available soil B is toxic to most crop plants (Nable et al., 1997), the 277 mg kg^{-1} in the soil of the mining area should not have allowed any plants to survive.

Gypsophila species match the criterion of Baker et al. (2000) for a hyperaccumulator plant containing high levels of B, mainly in its leaves. If the plant is used for phytoremediation, B-rich plant material from the remediated areas can be transported to sites requiring B fertilisation. Thus the waste generated by phytoremediation may not be a problem since both deficiency and toxicity of B are present within the same provinces of Turkey, as reported by Gezgin et al. (2002). The behaviour of this species requires further testing, especially on soils with a range of lower available and total B concentrations. However, the concentration reported here will remain as a minimum concentration/criterion until further reports are available on the subject.

Conclusion

There are many reports available describing plant species for use in the phytoremediation of metalliferous soils that contain excess amounts of Zn, Mn, Cu, Co, Pb, Al and Ni. To our knowledge, this is the first report of a plant species possessing the potential for B hyperaccumulation, especially in a region where B toxicity symptoms occur.

According to Chaney et al. (1997) a hyperaccumulator plant should possess tolerance to high levels of a particular micro-element in root and shoot cells by means of vacuolar compartmentalisation and chelation and the ability to translocate an element from roots to shoots at high rates. In addition, such plants should produce high biomass (Robinson et al., 1998). In normal cases, root Zn, Cd or Ni concentrations are 10 or more times higher than shoot concentrations, but in hyperaccumulators, shoot metal concentrations in most cases exceed root levels (Chaney et al., 1997). Accordingly, the *Gypsophila* species reported here can be considered as hyperaccumulators because of their tolerance to high concentrations of both available and total soil B and their relatively higher (as much as 60 times) B concentrations in leaves and seeds than in roots. However, the mechanism(s) of B uptake and translocation as well as the genetic basis of B accumulation (for the isolation of genes conferring B toxicity tolerance) in *Gypsophila* require further investigation. The possibility of cultivation of the plant species should also be investigated for use in phytoremediation studies.

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