RESEARCH ARTICLE

Boron and Molybdenum Contents of *Verbascum olympicum* boiss. Growing Around an Abandoned Tungsten Mine: A Case Study for Ecological Problem Solving

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Abstract: Interactions among micro-elements can affect their uptake by plants. Thus, it is important to evaluate nutrient interactions when designing phytoremediation strategies. In this study, the competitive or complementary effects between B and Cd, Cu, Fe, Mn, Mo, Pb, Zn, and W elements in leaf tissues of *Verbascum olympicum* Boiss. (Scrophulariaceae) were evaluated. This species is one of the main endemic species growing around the abandoned Etibank tungsten mine at Uludağ Mountain (Bursa, Turkey). Leaf and soil samples were collected from unpolluted and polluted areas around the mine. Inductively coupled plasma-mass spectrometry was used to determine the levels of selected elements in soil samples and in leaves of *V. olympicum.* Classical open wet and Kjeldahl digestion methods were used to process the samples. The analytical accuracy was guaranteed using two kinds of certified reference materials. Complementary behaviors of B and Mo were observed in leaves of *V. olympicum,* but not in the soil. This result provides information on the adaptation properties of this species in a polluted area.

INTRODUCTION

In plants, mineral nutrition is important for physiological and biochemical processes (1). Boron (B) is one of the mineral nutrients required for plant growth, and it is essential for cell structures (1, 2). The importance of B as an essential element for higher plant growth has been demonstrated (1). It is involved in many important processes in plants, including cell wall strength, cell wall synthesis and development, cell wall structural integrity, lignification, respiration, cell division, carbohydrate metabolism, fruit and seed formation and development, petal and leaf bud formation, vascular tissue repair, sugar transport, hormone development, metabolism of ribose nucleic acids, indoleacetic acid, and phenol, membrane stability, cytokinin production and transfer, pollen budding, and stimulation or inhibition of specific metabolic pathways (1–3). Elevated B levels may affect the metabolism of higher plants via its effects on enzyme activities (4). It has been suggested that B may generate oxidative stress in higher plants via the production of reactive oxygen species as a result of changes in the photosynthetic system (5). Total B levels in soils have been reported to range from 20 to 200 mg B kg^{-1} with less than 5%–10% in a form available to plants (2). Nutrient availability mainly depends on soil texture, organic matter content, and especially pH (6). The availability of B is also affected by interrelationships with other elements and environmental factors. For example, moderate to heavy rainfall, dry weather, and high light intensity are important factors for B availability to plants (2).

In plants, B interacts with macro-elements including nitrogen, phosphorus, potassium, and calcium (1). Compared with other elements, B reaches a toxic concentration at a lower level (4). Also, its mobility in plants differs from that of other elements, and this affects its function (7). Molybdenum (Mo) and B have complementary functions in the biological processes of soybean, which has higher Mo requirements than other plants. It is considered that Mo is an essential micro-nutrient for plants, while B is an essential micro-element for higher plants. The functions of B, the mechanisms of Mo uptake in plants, and the combined effects of B and Mo have been studied (8). While Mo is actively absorbed into the cell from the soil by roots mainly in the forms of MoO_4^{2-} and $HMoO_4^{-}$, B is passively absorbed in the form of H_3BO_3 (8), and there is a co-supplementary interaction between Mo and B (8, 9). On the other hand, tungsten (W) inhibits molybdoenzymes in plants (10); therefore, the interactions among B, Mo, and W elements in contaminated soils are significant in terms of their uptake and function.

The aim of this study was to evaluate the interactions between B and other elements in a plant growing around a polluted site. *Verbascum olympicum* Boiss. (Scrophulariaceae) was

selected as the plant material in this study, since it is one of the dominant endemic species growing around an abandoned tungsten mine on Uludağ Mountain (Bursa, Turkey), and its restoration and/or remediation properties in this area have been studied previously (11, 12). The main objective of this study was to investigate the influence of the mine and its residual wastes on *V. olympicum* growing at the mine site, and to collect data on its uptake of B and other related elements in this environment. Analyses of the contents of many elements in this plant can reveal competitive or complementary effects. Understanding the mutual, antagonistic, or synergistic interactions among elements is important for designing fertilization and/or phytoremediation strategies. Inductively coupled plasma-mass spectrometry (ICP-MS) was used to detect and quantify elements in soil and plant samples because of its multi elemental determination capability, low detection limits, and wide linear ranges.

MATERIALS AND METHODS

Sampling site and sample preparation

Verbascum olympicum Boiss. (Scrophulariaceae), one of the main species growing around the abandoned Etibank tungsten mine on Uludağ Mountain in Turkey (Figure 1), was selected for analysis in this study. The mine is located at an altitude between 2100 and 2487 m and lies at the intersection of 40°N latitude and 29°E longitude. It was active for approximately 20 years from 1969 to 1989. The ore generated tungsten oxide (40%), concentrated magnetite, pyrite, and by-products. The substratum of the waste removal pools and waste canals is granite at lower altitudes, and a calcareous substrate at upper altitudes (13). Samples were collected around the waste removal pools of this abandoned mine. The canals (on the right side) and waste-removal pools (on the left side) in the area are shown in Figure 2. Five soil samples were collected from each of three sites (sites I, II, and III) at 0- to 15-cm depth. Sites I and II were unpolluted, and site III was a polluted site at the waste removal pools. Soil samples were sieved (0.5 mm mesh) and air-dried under laboratory conditions. Plant materials collected at the same sites were washed and then dried to constant weight in an oven at 80°C. The dried plant materials were ground with a mortar and pestle, and the ground plant material and soil samples were stored in clear paper bags until element analyses. Leaf samples (100–500 mg) were subjected to open wet digestion in a borosilicate glass vessel with HNO_3 (5 mL) and H_2O_2 (3 mL). Soil samples (0.5 g) were digested by phosphoric acid, nitric acid, and hydrogen peroxide as described by Bednar *et al*. (14) using a DK 20 Kjeldahl digestion unit (VELP Scientifica, Milan, Italy).

Certified reference materials and blank samples were prepared in the same way as samples (*n*=3).

Figure 1. *Verbascum olympicum* Boiss. (Scrophulariaceae) (Photo by G. Güleryüz).

Figure 2. Sample collection area in the abandoned tungsten mine on Uludağ Mountain (Photo by G. Güleryüz).

Chemicals and Apparatus

A single-element standard solution of W at a concentration of 1000 µg mL^{-1} (Perkin Elmer, Shelton, CT, USA) and a multi-element standard solution containing 100 mg L^{-1} B and 10 mg L^{-1} Mo in a mixture of 30 elements (Merck 110580; Darmstadt, Germany) were used to prepare calibration standards. Nitric acid (65%) was of "suprapur" quality and was obtained

from Merck, as were the other reagents. The calibration curves for all elements were constructed from 0.5 to 1000 µg L^{-1}. The ^{11}B, ^{98}Mo, and ^{184}W isotope levels together with the concentrations of ^{111}Cd, ^{63}Cu, ^{57}Fe, ^{55}Mn, ^{208}Pn, and ^{64}Zn isotopes were determined in soil and plant samples by inductively coupled plasma-mass spectrometry (ICP-MS) using an Elan 9000 (Perkin Elmer SCIEX) instrument. The ICP-MS system comprised a Ryton cross-flow nebulizer, a Scott-type double-pass spray chamber, a standard glass torch, and a nickel sampler and skimmer cones (1.1 and 0.9 mm i.d., respectively). The conditions for ICP-MS were as follows: RF power, 1000 W; plasma argon flow rate, 17.0 L min^{-1}; nebulizer gas flow rate, 0.85 L min^{-1}; sample uptake rate, 1.5 mL min^{-1}; dwell time, 50 ms; scanning mode, peak hopping; and detector mode, dual. Soil samples were digested using the DK 20 model Kjeldahl digestion unit (VELP Scientifica). Digests were filtered through PVDF (polyvinylidene fluoride) hydrophilic syringe filters (0.45-µm pore size, Millex-HV, Millipore Corp., Bedford, MA, USA) and analyzed after appropriate dilution. The water used was ultrapure grade (18.3 MΩ.cm^{-1}, Zeneer Power I, Human Corporation, Seoul, South Korea). Argon (99.999% purity) was purchased from Asalgaz (Bursa, Turkey). The method was validated by analyses of GBW07605 tea leaves (National Research Center for Certified Reference Materials, Beijing, China) and NIST 1570 spinach leaves (National Institute of Standards and Technology, Gaithersburg, MD, USA). Data were analyzed by one-way ANOVA and Tukey's HSD test using Statistica 5.0 software. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The contents of elements at mg kg^{-1} levels were determined in above-ground parts (leaves) of *V. olympicum* and in soils at sampling sites I and II (unpolluted) and site III (polluted) (Table 1). The measured levels of all elements were in good agreement with the certified values of mentioned reference materials at the 95% confidence level, indicating that the total element determinations were accurate. For all elements, the limits of quantitation were below the measured values.

Table 1. Mean contents (mg kg_{dw}^{-1}) of elements in soil and plant parts of *Verbascum olympicum* at sites (non-polluted, sites I and II; polluted, within the waste removal pool, site III) of the abandoned tungsten mine on Uludağ Mountain, Bursa Turkey.

Elements	Element Levels* in sites					
	Site I		Site II		Site III	
	Soil	Leaf	Soil	Leaf	Soil	Leaf
B	78.7[a] ± 19.9	40.2[a] ± 7.7	9.2[a] ± 17.2	22.8[ab] ± 10.7	63.1[a] ± 12.6	17.1[b] ± 6.5
W	20.6[b] ± 2.4	1.3[b] ± 0.4	26.7[b] ± 0.8	0.4[b] ± 0.1	1868.8[a] ± 215.3	44.2[a] ± 25.7
Fe	39486[b] ±20580	48.2[a] ± 23.9	25425[b]± 799	8.5[b] ± 5.7	184346[a] ± 26822	35.2[ab] ± 4.3
Mo	14.0[a] ± 6.8	0.6[a] ± 0.2	7.2[a] ± 0.4	0.5[a] ± 0.3	5.7[a] ± 1.4	0.3[a] ± 0.1
Zn	169.9[b] ± 17.1	34.3[b] ± 5.1	220.6[b] ± 11.8	19.3[b] ± 4.6	3224.5[a] ± 368.5	116.4[a] ± 35.8
Cu	211.1[a] ± 4.3	11.8[ab] ± 2.4	242.0[a] ± 5.5	5.4[b] ± 1.7	595.4[a] ± 386.3	20.6[a] ± 7.1
Cd	0.22[b] ± 0.02	0.02[b] ± 0.00	0.62[b] ± 0.09	0.04[b] ± 0.03	12.94[a] ± 1.86	0.22[a]± 0.09
Mn	970[b] ± 388	538[a] ± 207	1721[b] ± 169	1237[a] ± 458	8689[a] ± 1565	1289[a] ± 718
Pb	27.97[b] ± 1.90	0.75[b] ± 0.16	48.81[a] ± 3.07	0.66[b] ± 0.19	49.34[a] ± 0.84	1.39[a] ± 0.29

Mean values are shown (n = 3) ± standard deviation. Different letters indicate significant differences between sampling sites (Tukey's HSD test; $p < 0.05$).

The B levels in leaf and soil samples from sites I and II ranged widely. Unlike other elements, B shows a wide range of concentrations in soil (2). The B concentrations in soils from site III (polluted) were higher than those in samples from site II, but lower than those in soil samples from site I. Therefore, the mean value of B at unpolluted sites was compared with the B content at the polluted site. The B levels in leaf samples were lower at polluted sites than at the unpolluted site. There is competition between W and Mo, as reported earlier (15), and a positive correlation between B and Mo has also been reported (10). Thus, there is some competition or completion for transport between B and W and between B and Mo, respectively. As a result, low B and Mo levels in leaves were associated with higher W levels. This is consistent with the results shown in Table 1; that is, leaf samples with higher W levels had lower Mo and B levels. There also appeared to be some competition between B and Mo in soil samples; that is, soil samples with higher levels of B had lower levels of Mo, and vice versa. Thus, B and Mo also showed complementary behaviors in leaves of this species, as outlined by Liu *et al.* (10). This may indicate that they are interchangeable in some physiological functions of the plant. The association between B and Mo may have some significance in terms of the adaptation of this plant to the polluted area.

When W levels were present at excessive levels in soils at the polluted site, this appeared to interfere with the functions of B and Mo as they became competitive elements. Thus, any mutual effects of elements also depends on the other elements present, their concentrations, and how they interfere with other elements in the soil. As shown in Table 1, all of the other studied elements, especially W, were present at higher concentrations in soil at the polluted site than in soil at the unpolluted sites. However, the transportation ratios (element concentration in leaves divided by element concentration in soil) of B, Fe, Zn, and Mn were lower at the polluted site than at the unpolluted site. These three elements (Fe, Zn, and Mn) may be associated with B. The only element present at lower concentrations at the polluted site than at the unpolluted site was Mo.

Apart from other micro-elements, macro-elements and their quantity in the area, together with soil pH, are important factors that can enhance or inhibit the uptake of the studied elements from soil into plants. Phosphate was one of the main macro-elements, since water removal pools are known to be rich in phosphate (13). Thus, the high levels of the selected elements in leaf samples from the polluted (waste removal pools) site may be due to water-soluble phosphate complexes that are readily taken up by plants, depending on the pH (16).

Although it might be expected that large amounts of an element in the soil will lead to high transportation, and thus accumulation in plants, the opposite effect was observed for B. This may have been due to direct interference from W and Mo with the indirect interference from Mo to B. Higher W levels in leaves were associated with lower Mo and B levels. However, when the soil B levels were high, the B levels in leaves were low. This illustrates the importance of transportation in determining which elements will be present at high levels in leaves. Different transportation behaviors were observed for Mo. Therefore, interactions among elements may also inhibit B transport in plants.

As shown in Table 1, the other effected elements with their transportation ratios were Zn and Cd. Table 2 summarizes correlations between pairs of elements in plant parts. There was a significant correlation (r^2 =0.849) between B content and Mo content in leaf tissues of *V. olympicum,* confirming the complementary effects between these elements for translocation. This led to the conclusion that B and Mo function similarly in this species. There was no significant correlation between B and other elements, even W. Thus the adaptation capability of *V. olympicum* depends on the complementary effects of B and Mo directly, and also B and W indirectly depending on the W–Mo interaction. There was no significant correlation between leaf and soil B levels in this species (r^2 = 0.011, P = 0.789, regression equation = 21.706 + 0.06233 x *Soil B*). This result highlighted that high B levels in leaves were not associated with high B levels in soils, but depended on competition with other elements for uptake in the natural adaptation processes of this plant.

Table 2. Simple correlation coefficients (r^2), significance levels (P) and linear regression equations (Y = a + bx) between contents of various elements and boron in *Verbascum olympicum* (n = 9, P < 0.05 considered as significant correlation).

	r^2	P	Y= a + bx
Leaf-B	0.294	0.131	Leaf-B = 30.922 – 0.2747 x *Leaf-W*
Leaf-B	0.262	0.159	Leaf-B = 17.402 + 0.30398 x *Leaf-Fe*
Leaf-B	0.849	**0.000**	Leaf-B= 8.0080 + 41.361 x *Leaf-Mo*
Leaf-B	0.143	0.315	Leaf-B = 32.319 – 0.0990 x *Leaf-Zn*
Leaf-B	0.048	0.571	Leaf-B = 31.339 – 0.3669 x *Leaf-Cu*
Leaf-B	0.319	0.113	Leaf-B = 33.179 – 68.47 x *Leaf-Cd*
Leaf-B	0.187	0.245	Leaf-B = 39.696 – 13.93 x *Leaf-Pb*
Leaf-B	0.101	0.406	Leaf-B = 33.979 – 0.0071 x *Leaf- Mn*

CONCLUSION

Determining soil composition through elemental analyses is important for evaluating the synergism and antagonism between elements in terms of uptake by plants. Apart from environmental factors, the competition between B and other elements for uptake by *V. olympicum* plants affected its final concentration in plant tissues. This information provides a realistic picture about the uptake of elements under natural conditions. These results will be useful for optimizing the formulation of fertilizers containing B, especially those intended for use in areas with high concentrations of particular elements. As excess uptake of certain elements from fertilizers can lead to stress responses in plants, it is important to understand the synergistic or antagonistic relationships among elements. These data will also be useful for designing management strategies for the area around the abandoned mine. Although the data suggested that there is a relationship between B and Mo, this may not be the case in other plant species or under different environmental conditions. For this reason, many elements should be taken into account in environmental risk assessments. There is still much to learn about the roles of various micro-elements, including B, in the synthesis and function of molecular and macromolecular structures. This research on the relationships among micro-elements in plants growing under stress conditions can provide a useful baseline for further laboratory-based research on the role of B in higher plants. Further research at the organelle and molecular levels is necessary to clarify the role of B. The knowledge gained from such studies will be useful for producing environmentally friendly materials.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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