# RESPONSES OF *VERBASCUM OLYMPICUM* BOISS. TO EXCESS MANGANESE

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## ABSTRACT

*Verbascum olympicum* Boiss. (Scrophulariaceae) is one of the main species of natural plant on destroyed areas of Uludag National Park, Bursa, Turkey. This is the pioneer species of ruderal plant communities on these areas and it has restoration capability of destroyed areas by different ecological properties such as heavy metal accumulation and high organic matter production. The variations in elemental compositions (Mn, Fe, Zn, Cu, Mo, B) of *V. olympicum* was evaluated in the presence of excess manganese under laboratory conditions. Seedlings (10 weeks old) were grown in Hoagland's solution containing manganese (0, 50, 200 µM $MnSO_4$) for 7 days. Acid digestion procedure was applied for leaves and roots of the samples and the concentrations of six elements in roots and leaves were determined by inductively coupled plasma-mass spectrometry (ICP-MS). The accuracy of the method was confirmed by the analysis of the certified reference material (GBW07605 tea leaves). The increased Mn concentrations were observed in Mn content of both roots and leaves. It also seen that there were influence on nutritional balance of this species.

**Keywords**:Manganese stress, element composition, *Verbascum olympicum*, inductively coupled plasma-mass spectrometry

## 1. Introduction

Many anthropogenic activities lead to elevated release of heavy metals into the environment. Numerous heavy metals included the manganese (Mn) are essential for plant growth and productivity. But they may be toxic at higher concentrations (Kováčik *et al.,* 2014). Manganese is an essential micronutrient participating the photosynthesis, enzyme activation, carbohydrate metabolism and phosphorylation. It has also important role in electron transfer (oxidation-reduction) reactions and electron transport systems (Marschner, 1995). Excess Mn may retard plant growth and development together with toxicity symptoms such as stunted growth, chlorosis and crinkled leaves (Foy, 1984). Also it is reported that excess Mn interferes with the absorbtion, translocation and utilization of some mineral such as Ca, Mg, Fe and P (Clark,1982; Lee *et al*., 2011).

Plants which are capable of their survival under high metal concentration are classified in two types as metal-tolerant and accumulator plants (Juárez-Santillán *et al.,* 2010). While metal tolerant plant utilize different mechanisms such as complexation, metal accumulator plants can take up large amounts of metal and translocate it to the above ground parts. These plants have capacity to detoxify heavy metals via binding them to cell wall and organic acids or proteins and compartmentalize in vacuoles. Due to the their detoxifying capacities, metal accumulator plants may serve as biological tools for cleaning up heavy metals in contaminated soils. This process is called as phytoremediation and accepted as a novel environmental clean-up technique (Moreira *et al*., 2011).

*Verbascum olympicum* Boiss. (Scrophulariaceae) is an endemic species to Uludağ Mountain (Bursa-Turkey) and has important role ecosystem process in this mount such as secondary succession (Rehder *et al*. 1994). In our early studies, we determined the potential for this species to restore degraded ecosystems (Güleryüz *et al*., 2006) and its tolerance to Cd and Ni under laboratory conditions some in previous studies (Arslan *et al*., 2014; Akpınar *et al*., 2015).

The aim of this study is to investigate the metal mobilities (manganese, Mn; iron, Fe; zinc, Zn; copper, Cu; molybdenum, Mo; magnesium, Mg; boron, B; cobalt, Co; bismuth, Bi) of *V. olympicum* under Mn stress.In order to reach this goal we analyzed the heavy metals in plant organs of *V. olympicum* seedlings.

## 2. Material and method

### 2.1. Plant Culture and Experimental Design

Seeds of *V. olympicum* collected from the alpine belt of Uludağ Mountain (1850-1900 m asl) in August 2011 were surface sterilized with 5% sodium hypochlorite for; 5 min and then rinsed with double-distilled water. Then, they were placed on Petri dishes on filter paper moistened with distilled water for germination under dark conditions (20 °C). The experiments were conducted with seedlings cultivated in Hoagland's nutrient medium (Hoagland and Arnon, 1950). At 10 days after germination, the seedlings were transferred to small beakers (20×15 ×10 cm) filled with one-tenth (v/v)-strength Hoagland nutrient medium (600 mL). The seedlings were transferred to a growth chamber (Heraeus Vötsch HPS500, Balingen, Germany) with a 15 °C/25 °C day/night temperature regime and a 16-h light/8-h dark photoperiod. Plants were irradiated by cool white fluorescent 36-W tubes that provided an irradiance (400–700 nm) of ca. 80 μmol $m^{-2}$ $s^{-1}$. The nutrient medium (pH 6.0) was renewed every other day, and its concentration was increased by 10% once a week. Eight-week-old seedlings (with 8 leaves) were selected and exposed to different concentrations of Mn (control, 50, 100, 200 μM) in 80% Hoagland's nutrient medium, with Mn supplied as $MnSO_4$ (Merck, 1020270100, Darmstadt, Germany). Four plants were harvested on days 1, 3, and 7 of the experiment. After the plant samples were washed thoroughly with de-ionized water; the roots and leaves were separated and their fresh weights were determined. The plant samples were oven-dried at 105°C for 24 h, homogenized, and then weighed.

### 2.2. Trace Element Analyses

Elan 9000 inductively coupled plasma–mass spectrometry (ICP-MS) (PerkinElmer SCIEX, Shelton, CT, USA) was used to determine the contents of Mn, Fe, Zn, Cu, Mo, Mg; B, Co, Bi in the plant tissues (roots and leaves) separately. Perkin-Elmer Ryton cross-flow nebulizer, a Scott-type double-pass spray chamber, a standard glass torch, nickel sampler and skimmer cones (i.d.:1.1 mm and 0.9 mm, respectively) were the components of ICP-MS equipment. Additionally, the optimum instrument conditions 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. The classical open wet digestion procedure was applied to the samples (10-20 mg) with 3 mL $HNO_3$ and 1mL $H_2O_2$ in a borosilicate glass vessel. A multi-element standard solution of 30 elements (Merck 110580) were used to prepare working solutions for external calibration. Calibration curves were constructed with eight points (0.1–30 μg $L^{-1}$ for Mn).

### 2.3. Statistical Analysis

The experiments had a completely randomized design and the heavy metal analyses included four replicates ($n$=4).The differences among the mean values for heavy metal with respect to different Mn concentrations and Mn-exposure periods were analyzed by two-way ANOVA. All statistical tests were performed at the significance level of 0.05 using SPSS 16.0 for Windows (SPSS Inc. 2007). Translocation factor (TF) was calculated by dividing the element content in the shoots to the content in the roots for each trace element (Brooks, 1998)

## 3. Results and discussion

The mean contents and TFs metals were outline in Table 1. In general, increased Mn concentrations were resulted in increased Mn content of both roots and leaves (Table 1; $P<0.05$). But the accumulation of Mn was performed in roots at all treatments and durations and reached up to 5681 ± 3320 mg $kg^{-1}$ DW in 200 μM Mn-treated plants on the 7th day. This value which is above the average range of Mn in plant tissues (1-700 mg $kg^{-1}$ DW) (Visioli and Marmiroli, 2013)

is attributed to the Mn accumulation capacity of roots. It was reported that Mn could be taken up via an active transport system in epidermal root cells and transported as divalent cation $Mn^{2+}$ into the plants (Pittman, 2005) and $Mn^{2+}$ appears to be adsorbed by the negatively charged cell wall constituents of the root cell apoplastic spaces (Humphries *et al*., 2007). But the translocation of Mn to above ground parts is restricted. Similar responses were observed for Fe, Zn, Mo, Co and Cu. Contrary, B decreased in roots but increased in leaves.

**Table 1:** Trace element content (mg $kg^{-1}$DW) in plant parts and TF values of these elements in *V. olympicum* seedlings exposed to Mn treatment series and exposure periods. (Mean ± SD; n = 4, α; 0.05)

| Treatments | Days | Mn | | | |
|---|---|---|---|---|---|
| | | **Roots** | **Leaves** | **Whole Plant** | **TF** |
| **Control** | **1** | 506 ± 172 | 76 ± 18 | 581 ± 181 | 0.16 ± 0.05 |
| | **3** | 1498 ± 385 | 65 ± 15 | 1564 ± 370 | 0.05 ± 0.03 |
| | **7** | 681 ± 180 | 86 ± 14 | 767 ± 168 | 0.14 ± 0.06 |
| **50 µM** | **1** | 1375 ± 383 | 79 ± 15 | 1455 ± 373 | 0.06 ± 0.02 |
| | **3** | 761 ± 324 | 76 ± 5 | 837 ± 324 | 0.10 ± 0.08 |
| | **7** | 1375 ± 585 | 74 ± 15 | 1448 ± 598 | 0.06 ± 0.02 |
| **200 µM** | **1** | 1109 ± 324 | 92 ± 20 | 1201 ± 332 | 0.09 ± 0.02 |
| | **3** | 3044 ± 1107 | 133 ± 28 | 3177 ± 1096 | 0.05 ± 0.02 |
| | **7** | 5681 ± 3320 | 153 ± 13 | 5834 ± 3332 | 0.04 ± 0.02 |
| | | **Fe** | | | |
| **Control** | **1** | 4787 ± 1997 | 187 ± 31 | 4974 ± 1993 | 0.05 ± 0.02 |
| | **3** | 5507 ± 2068 | 215 ± 68 | 5722 ± 2046 | 0.04 ± 0.02 |
| | **7** | 1467 ± 424 | 199 ± 26 | 1665 ± 426 | 0.14 ± 0.04 |
| **50 µM** | **1** | 9494 ± 5716 | 305 ± 109 | 9799 ± 5746 | 0.05 ± 0.04 |
| | **3** | 9813 ± 4681 | 148 ± 18 | 9961 ± 4695 | 0.02 ± 0.01 |
| | **7** | 6291 ± 1317 | 168 ± 35 | 6459 ± 1323 | 0.03 ± 0.01 |
| **200 µM** | **1** | 8398 ± 5724 | 186 ± 55 | 8585 ± 5692 | 0.10 ± 0.17 |
| | **3** | 7255 ± 5197 | 168 ± 22 | 7423 ± 5179 | 0.09 ± 0.14 |
| | **7** | 5238 ± 3655 | 96 ± 21 | 5434 ± 3658 | 0.07 ± 0.07 |
| | | **Zn** | | | |
| **Control** | **1** | 115 ± 5 | 47 ± 21 | 163 ± 20 | 0.41 ± 0.19 |
| | **3** | 138 ± 4 | 49 ± 18 | 187 ± 16 | 0.36 ± 0.13 |
| | **7** | 53 ± 18 | 42 ± 10 | 95 ± 26 | 0.81 ± 0.15 |
| **50 µM** | **1** | 165 ± 47 | 34 ± 12 | 199 ± 42 | 0.23 ± 0.12 |
| | **3** | 156 ± 61 | 42 ± 5 | 198 ± 58 | 0.32 ± 0.17 |
| | **7** | 137 ± 29 | 53 ± 10 | 190 ± 29 | 0.40 ± 0.11 |
| **200 µM** | **1** | 119 ± 42 | 46 ± 13 | 164 ± 32 | 0.45 ± 0.25 |
| | **3** | 148 ± 33 | 33 ± 3 | 181 ± 35 | 0.23 ± 0.04 |
| | **7** | 139 ± 49 | 49 ± 14 | 188 ± 57 | 0.38 ± 0.12 |

**Root Mn Content**, $F_{Concentration\ (2,69)}$ =13.95, p=0.000, $F_{Duration\ (2,69)}$ = 5.14, p=0.013, $F_{Concentration\ x\ Duration\ (4,69)}$ =5.19, p=0.003. **Root Fe Content,** $F_{Concentration\ (2,69)}$ =4.31, p=0.024, $F_{Duration\ (2,69)}$ = 2.69, p=0.086, $F_{Concentration\ x\ Duration\ (4,69)}$ =0.09, p=0.985. **Root Zn Content**, $F_{Concentration\ (2,69)}$ =5.62, p=0.009, $F_{Duration\ (2,69)}$ = 3.15, p=0.059, $F_{Concentration\ x\ Duration\ (4,69)}$ =1.85, p=0.149. **Leaf Mn Content**, $F_{Concentration\ (2,69)}$ =35.80, p=0.000, $F_{Duration\ (2,69)}$ = 5.28, p=0.012, $F_{Concentration\ x\ Duration\ (4,69)}$ =5.17, p=0.003. **Leaf Fe Content**, $F_{Concentration\ (2,69)}$ =0.65, p=0.529, $F_{Duration\ (2,69)}$ =3.06, p=0.064, $F_{Concentration\ x\ Duration\ (4,69)}$ =4.34, p=0.008. **Leaf Zn Content**, $F_{Concentration\ (2,69)}$ =0.23, p=0.796, $F_{Duration\ (2,69)}$ = 0.94, p=0.403, $F_{Concentration\ x\ Duration\ (4,69)}$ =1.69, p=0.182. **Whole plant Mn Content**, $F_{Concentration\ (2,69)}$ =14.51, p=0.000, $F_{Duration\ (2,69)}$ = 5.26, p=0.012, $F_{Concentration\ x\ Duration\ (4,69)}$ =5.28, p=0.003. **Whole plant Fe Content**, $F_{Concentration\ (2,69)}$ =4.32, p=0.024, $F_{Duration\ (2,69)}$ = 2.72, p=0.084, $F_{Concentration\ x\ Duration\ (4,69)}$ =0.09,

p=0.984. **Whole plant Zn Content**, $F_{Concentration\ (2,69)}$ =4.79, p=0.017, $F_{Duration\ (2,69)}$ = 2.03, p=0.151, $F_{Concentration\ x\ Duration\ (4,69)}$ =2.44, p=0.071.

Table 1 (continued)

| Treatments | Days | Roots | Leaves | Whole Plant | TF |
|---|---|---|---|---|---|
| | | **Cu** | | | |
| **Control** | **1** | 20 ± 5 | 10 ± 2 | 30 ± 5 | 0.50 ± 0.13 |
| | **3** | 22 ± 11 | 13 ± 4 | 35 ± 14 | 0.65 ± 0.12 |
| | **7** | 8 ± 2 | 10 ± 3 | 18 ± 4 | 1.17 ± 0.37 |
| **50 µM** | **1** | 22 ± 10 | 12 ± 4 | 34 ± 13 | 0.57 ± 0.17 |
| | **3** | 25 ± 5 | 9 ± 1 | 34 ± 5 | 0.39 ± 0.15 |
| | **7** | 25 ± 6 | 9 ± 3 | 34 ± 7 | 0.38 ± 0.17 |
| **200 µM** | **1** | 32 ± 11 | 10 ± 2 | 42 ± 11 | 0.34 ± 0.14 |
| | **3** | 37 ± 17 | 9 ± 1 | 46 ± 18 | 0.27± 0.12 |
| | **7** | 13 ± 4 | 15 ± 4 | 28 ± 5 | 1.24 ± 0.51 |
| | | **Mo** | | | |
| **Control** | **1** | 10 ± 3 | 3 ± 0 | 13 ± 3 | 0.27 ± 0.09 |
| | **3** | 16 ± 3 | 2 ± 1 | 18 ± 3 | 0.14 ± 0.05 |
| | **7** | 13 ± 1 | 3 ± 1 | 16 ± 2 | 0.22 ± 0.06 |
| **50 µM** | **1** | 24 ± 1 | 2 ± 0 | 26 ± 1 | 0.09 ± 0.01 |
| | **3** | 25 ± 19 | 2 ± 0 | 27 ± 19 | 0.10 ± 0.06 |
| | **7** | 16 ± 3 | 2 ± 0 | 18 ± 3 | 0.11 ± 0.01 |
| **200 µM** | **1** | 15 ± 2 | 3 ± 1 | 18 ± 2 | 0.21 ± 0.06 |
| | **3** | 19 ± 5 | 3 ± 0 | 22 ± 5 | 0.14 ± 0.03 |
| | **7** | 16 ± 8 | 3 ± 0 | 19 ± 8 | 0.21 ± 0.10 |
| | | **B** | | | |
| **Control** | **1** | 28 ± 3 | 73 ± 7 | 100 ± 8 | 2.66 ± 0.39 |
| | **3** | 21 ± 7 | 73 ± 25 | 94 ± 29 | 3.50 ± 1.08 |
| | **7** | 34 ± 15 | 84 ± 16 | 118 ± 25 | 2.82 ± 1.13 |
| **50 µM** | **1** | 55 ± 15 | 84 ± 22 | 139 ± 31 | 1.58 ± 0.42 |
| | **3** | 29 ± 17 | 58 ± 11 | 87 ± 15 | 2.72 ± 1.71 |
| | **7** | 24 ± 10 | 57 ± 14 | 81 ± 17 | 2.75 ± 1.49 |
| **200 µM** | **1** | 26 ± 10 | 82 ± 11 | 108 ± 13 | 3.53 ± 1.38 |
| | **3** | 28 ± 6 | 79 ± 24 | 107 ± 26 | 2.89 ± 0.88 |
| | **7** | 17 ± 10 | 84 ± 13 | 101 ± 17 | 5.80 ± 2.63 |

**Root Cu Content**, $F_{Concentration\ (2,69)}$ =4.43, p=0.022, $F_{Duration\ (2,69)}$ = 6.19, p=0.006, $F_{Concentration\ x\ Duration\ (4,69)}$ =2.22, p=0.094. **Root Mo Content,** $F_{Concentration\ (2,69)}$ =4.42, p=0.022, $F_{Duration\ (2,69)}$ =1.45, p=0.252, $F_{Concentration\ x\ Duration\ (4,69)}$=0.721, p=0.585. **Root B Content**, $F_{Concentration\ (2,69)}$ =3.64, p=0.040, $F_{Duration\ (2,69)}$ = 3.65, p=0.040, $F_{Concentration\ x\ Duration\ (4,69)}$ =3.71, p=0.016 **Leaf Cu Content**, $F_{Concentration\ (2,69)}$ =0.77, p=0.474, $F_{Duration\ (2,69)}$ = 0.477, p=0.626, $F_{Concentration\ x\ Duration\ (4,69)}$ =4.23, p=0.009. **Leaf Mo Content**, $F_{Concentration\ (2,69)}$ =11.37, p=0.000, $F_{Duration\ (2,69)}$ =3.26, p=0.054, $F_{Concentration\ x\ Duration\ (4,69)}$ =1.07, p=0.391. **Leaf B Content**, $F_{Concentration\ (2,69)}$ =2.66, p=0.088, $F_{Duration\ (2,69)}$ = 1.01, p=0.377, $F_{Concentration\ x\ Duration\ (4,69)}$ =1.53, p=0.222. **Whole plant Cu Content**, $F_{Concentration\ (2,69)}$ =3.56, p=0.042, $F_{Duration\ (2,69)}$ = 3.86, p=0.034, $F_{Concentration\ x\ Duration\ (4,69)}$ =0.96, p=0.448. **Whole plant Mo Content**, $F_{Concentration\ (2,69)}$ =3.75, p=0.036, $F_{Duration\ (2,69)}$ = 1.20, p=0.317, $F_{Concentration\ x\ Duration\ (4,69)}$ =0.817, p=0.526. **Whole plant B Content**, $F_{Concentration\ (2,69)}$ =0.08, p=0.923, $F_{Duration\ (2,69)}$ = 2.92, p=0.071, $F_{Concentration\ x\ Duration\ (4,69)}$ =3.73, p=0.015.

## 4. Conclusions

We conclude that *V. olympicum* is capable of accumulating high concentrations of Mn in its organs, especially its roots, under high-Mn conditions in the laboratory. Many other heavy metals

which studied were also accumulated in the organs of Mn-treated plants. The translocation properties of some trace elements were not affected by Mn except B. Further studies should be conducted in contaminated soil environments to test the suitability of this species for phytoremediation.

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