# Element Content (Cu, Fe, Mn, Ni, Pb, and Zn) of the Ruderal Plant *Verbascum olympicum* Boiss. from East Mediterranean

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In this study, heavy metal content (Cu, Fe, Mn, Ni, Pb, Zn) was determined in soils and different organs of *Verbascum olympicum* Boiss. This species is endemic to Uludağ and spreads on destroyed areas such as: roadsides, developed building areas, ski lift stations and sheep folds. Soils and different organs (roots, stems, leaves and flowers) of plant samples were analyzed using an atomic absorption spectrophotometer for determining the element content. Heavy metal contents in soils and different organs in this species were highly correlated ($P < 0.05$). However, the contribution of plant organs to the accumulation capacity varied according to the metal. These results suggest that this species may be useful as a bioindicator for heavy metals.

*Key words:* Heavy Metal, Biomonitoring, *Verbascum olympicum*

## Introduction

The purpose of monitoring hazardous waste sites is to characterize soil and groundwater pollution in sufficient detail to facilitate site remediation (Rifai *et al.*, 2000). Reports on plants growing in polluted areas without being seriously harmed indicate that it should be possible to detoxify contaminants using agricultural and technological approaches (Schwitzguebel, 2001). Although soil and water monitoring is the main subject, plants can be used as bio-indicators for toxicity assessment in aquatic and terrestrial ecosystems (Pugh *et al.*, 2002). Zurayk *et al.* (2001) pointed out that detecting environmental pollution using biological material as indicators is a cheap, reliable and simple alternative to the conventional sampling methods. The use of higher plants as biomonitors of heavy metal pollution in the environment has increased in the past few decades (Piczak *et al.*, 2003; Swaileh *et al.*, 2004).

*Verbascum olympicum* Boiss. is one of the main species of natural plant covers on destroyed areas of Uludağ National Park, Bursa, Turkey. It became dominant in many areas such as roadsides, developed building areas, sheep folds, rubbers and mining areas after destruction. In most plant community types, soil disturbance creates openings for establishment frequently of weedy or ruderal species. Some plant species become dominant in these areas and they are the pioneer species of ruderal plant communities (Ellenberg, 1988). Ruderal plant communities are the first plant communities growing on destroyed areas and they begin to restore these areas after soil disruption. These plants appear to have some ability to restore the harmful effects of these activities. Rehder *et al.* (1994) reported that this species was the pioneer species of ruderal plant communities in this area. It was reported that this species was useful in restoration of destroyed areas such as roadsides, developed building areas, rubber and mining areas, due to high organic matter production (Güleryüz and Arslan, 2001).

In this study, *Verbascum olympicum* Boiss., collected from four populations and their corresponding soils were analyzed for element contents (Cu, Fe, Mn, Ni, Pb and Zn) in order to ascertain the indicator value of *V. olympicum*. Element values in different organs of the plant were also studied in order to gain information about their distributions.

## Experimental

### *Species*

*Verbascum* is the second largest genus in the Turkish flora, including numerous other endemic species. The endemism ratio of this genus is 70.4%

 

(Akman, 1993). *V. olympicum* Boiss. is a biannual species, local endemic, and present only in Uludağ (Güleryüz and Malyer, 1998). It belongs to the Scrophulariaceae family. This species is a hemicryptophyte with broad basal leaves. Stems are robust and angular with numerous branches, which can reach up to 100–200 cm. Flowers with congested clusters of 3–11 are on numerous thick branches forming a broad ovate-pyramidal panicle. Roots are robust and staked. Flowering time is from June through August.

*Sample sites*

Sample sites were selected from four different places in sub-alpine belts of Uludağ Mountain (1600–2000 m altitude). These sites are around Etibank Mine, Mandra, Sarıalan and Kirazlıyayla. The geological structure of the investigation areas is composed of granites substratum.

*Mine Work:* This site is near the Etibank Wolfram Mine at an altitude of 1900–2000 m and is explained in detail in our earlier work (Güleryüz *et al.*, 2002). The main vegetation of this area is composed of hard cushion (*Festuca punctoria*-community and *Festuca cyllenica*-community) and dwarf shrub (*Juniperus communis*-community) types (Rehder *et al.*, 1994). Ruderal vegetation, represented by the *Verbascum olympicum*-community, is dominant on rubbishes, waste removal pools, canals and around the buildings in this area.

*Mandra:* There is a ski lift station on this site and the *Nardus stricta* meadow-community is dominant in this area. But *Verbascum olympicum* also forms vegetation patches in this area.

*Sarıalan:* Sampling on this site was taken from Sarıalan roadsides. *Abies bornmuelleriana*-community is the dominant vegetation type in this area. No formation of a ruderal plant community was observed. *V. olympicum* grows on a line along the roadsides and under forests in this area.

*Kirazlıyayla:* This sample site is used for recreational activities, *i.e.*, picnicking and ball playing, especially in summer. Meadow vegetation, represented by the *Nardus stricta*-community, is dominant at this site. But *V. olympicum* also dominates some areas of Kirazlıyayla, and samples were taken from this site.

*Sampling*

Soils and plant samples were taken from five separate locations at each sampling site measuring approx. 200 $m^2$ in size each. Soils were taken from a 0–5 cm layer, sifted with a standard 2-mm sieve, and then air-dried. Sampling of all plants was performed in the flowering phase. Plant samples were harvested together with above- and below-ground parts and divided into compartments (*i.e.* roots, stems, leaves and flowers) with stainless steel knives. All samples were transported to the laboratory in plastic bags. Plant parts were washed with tap water to clean away other dried plant parts and soil particles from the source samples. In addition, they were washed again carefully with distilled and de-ionized water (Demir *et al.*, 1990). Plant materials were then put into paper bags and dried in an oven (80 °C) until their weights became constant. Dried material was homogenized for heavy metal analyses.

*Chemical and statistical analyses*

Aqua regia (1 mol $HNO_3$ : 3 mol HCl) was used for the analysis of total cation contents in soils. 1 g of homogenized plant samples was soaked for 1 d in a $HNO_3/H_2O_2$ solution and digested. Digested samples were diluted to 50 ml of de-ionized water. All soil and plant material solutions were analyzed for Cu, Fe, Mn, Ni, Pb and Zn using an atomic absorption spectrophotometer (Massmann *et al.*, 1976). Interference, caused by plant matrices, was corrected by the standard addition method (Demir *et al.*, 1990). All chemicals were analytical reagent grade. Detection limits of Cu, Fe, Mn, Ni, Pb, Zn are 5 mg/kg, 24 mg/kg, 15 mg/kg, 15 mg/kg, 1.5 mg/kg and 7.5 mg/kg, respectively.

Simple correlation between heavy metal contents of the soils and different plant organs were tested on a significance level of 0.05 with STATISTICA Ver 6.0 (StatSoft Inc. 1984–1995).

## Results and Discussion

*Heavy metal levels in the soils*

Min/max values of heavy metal contents (mg/kg dry weight, DW) in the soils are given in Table I. These values (Cu, Fe, Mn, Pb, Ni, Zn) in soils were higher than those of different uncontaminated soils (Temmerman *et al.*, 1984). An increase in these elements was clearer in the soils of the Etibank Wolfram Mine and Mandra sampling sites. For instance, Temmerman *et al.* (1984) reported that the Cu content of different uncontaminated soils varied by 15–25 mg/kg DW. But Cu content was between 394 and 1718 mg/kg DW in the soils of the Etibank Mine site, and 632 and 1606 mg/kg

Table I. Min/max values of elements determined in soil of the investigation area.

| | Sampling sites | | | | |
|---|---|---|---|---|---|
| Element [mg/kg DW] | Mine Work | Mandra | Sarıalan | Kirazlıyayla | All plots |
| Cu | 394– 1718 | 632–1606 | 130– 687 | 125– 558 | 125– 1718 |
| Fe | 3496–10177 | 2127–6103 | <24* | 88– 940 | <24*–10177 |
| Mn | 455– 4575 | 1274–2879 | 467–2114 | 46– 636 | 46– 4575 |
| Ni | 88– 1650 | 33–1254 | 284–1444 | <15*– 620 | <15*– 1650 |
| Pb | 208– 655 | 656–1164 | <15*– 670 | <15*– 126 | <1.5*– 1164 |
| Zn | 557– 2952 | <7.5*– 948 | 33– 168 | <7.5*–1405 | <7.5*– 2952 |

* Under detection limits.

DW in the soils of the Mandra site. Min/max Fe contents were found in the soils of both the Etibank Mine (3496–10177 mg/kg DW) and Mandra (2127–6103 mg/kg DW) sites. While Fe was found under detection limits (24 mg/kg DW), the highest Ni values were determined in the soils of the Sarıalan site. Heavy metal levels show that there is contamination in the soils of Etibank Mine and Mandra for all metals, but in the soils of the Sarıalan site only Ni was discovered (Table I). This all-metal contamination can be attributed to the mining activities around the site (Güleryüz *et al.*, 2002), and to the construction of buildings and ski lifts on Mandra.

### *Heavy metal levels in plant organs*

Min/max and mean values of heavy metal contents in different organs of *V. olympicum* are shown in Table II. Maximum values of all examined metals were at considerably high levels in organs. These results indicate that the metal contents in organs are reflected in the soils of sites.

Since this species shows a tissue content reflecting external Cu concentration, it can be concluded that *V. olympicum* is an indicator for external Cu concentration. The copper content in organs of this species is up to 594 mg/kg DW, and this value is higher than the copper content in normal plants (4–15 mg/kg DW) (Shaw *et al.*, 2004). The increase in tissue Cu content was quite high in plant samples collected from the Etibank Mine and Mandra sample sites (Table II). The highest mean tissue content was found in the leaves of plant samples collected at all samples sites (Table II). For this reason, it can be said that leaves are Cu accumulating organs. The accumulation of Cu in the leaves is related to the function of these organs in basic metabolic activities. The biochemical function of copper is mainly to be a co-factor in enzymes, *e.g.* plastocyanin, superoxide dismutase and amine oxidases (Hagemeyer, 2004). On the other hand, the Cu content of flowers and fruits was higher than that of roots and stems. Therefore, it can be concluded that these organs have Cu assimilating capacity.

The mean iron content of plants was highest in samples collected from mine work (Table II) and this value was also found in the related soils (Table I). The lowest iron content of this species was found in plant samples collected from Kirazlıyayla, which has the lowest iron content in its soils (Table I). Except for plant samples collected from the Sarıalan and Kirazlıyayla sample sites, the mean Fe content of organs was found to be higher than the normal Fe content of plants (140 mg/kg DW) (Shaw *et al.*, 2004). Also, Fe content of whole organs collected from the Etibank Mine and Mandra sites exceeds the phytotoxic levels reported by different researchers. According to Levy *et al.* (1999), Fe contents which are higher than 500 mg/kg DW are poisonous. Romheld and Marschner (1991) suggested that Fe phytotoxicity is 400–1000 mg/kg DW. However, the iron contents of soil and plant samples from Sarıalan were under the detection limit (24 mg/kg DW). Whereas roots seem to be the main organs in the distribution of Fe among plant organs, Fe can be translocated to above-ground organs, especially to leaves. This was expected, due the role of iron in the biosynthesis of heme co-enzymes and chlorophyll (Hagemeyer, 2004).

Similar to the Fe content in organs of this species, manganese was highest in samples collected from Mine Work and Mandra (Table II) and this values were also found in the related soils (Table I). Although Mn accumulated in all organs,

Table II. Min/max and mean (± standard deviation, SD) values of Cu, Fe, Mn, Ni, Pb and Zn determined in organs and whole plant (mg/kg DW) of *V. olympicum* collected from different sites.

| | | Sampling sites | | | |
|---|---|---|---|---|---|
| | Organ | Mine Work | Mandra | Sarıalan | Kirazlıyayla |
| Cu | Flowers | 93–461 (267 ± 137) | 153–424 (288 ± 103) | 49–169 (109 ± 43) | 64–125 (98 ± 26) |
| | Leaves | 87–594 (278 ± 194) | 216–411 (312 ± 76) | 39–163 (109 ± 47) | 39–281 (106 ± 104) |
| | Stems | 106–340 (218 ± 102) | 96–368 (245 ± 109) | 28–207 (113 ± 73) | <5*–60 (17 ± 25) |
| | Roots | 96–373 (234 ± 125) | 72–403 (234 ± 127) | 14–148 (81 ± 55) | <5*–123 (40 ± 53) |
| Fe | Flowers | 417–1839 (1269 ± 610) | 70–1199 (833 ± 462) | <24* | <24*–328 (98 ± 135) |
| | Leaves | 1291–2802 (2018 ± 589) | 674–1181 (977 ± 208) | <24* | <24*–256 (71 ± 112) |
| | Stems | 252–1724 (947 ± 554) | 452–1280 (850 ± 372) | <24* | <24*–428 (136 ± 186) |
| | Roots | 1113–4734 (2521 ± 1474) | 577–2519 (1411 ± 748) | <24* | <24*–339 (113 ± 138) |
| Mn | Flowers | 120–1258 (590 ± 475) | 58–632 (365 ± 243) | 28–471 (255 ± 204) | <15*–18 (91 ± 86) |
| | Leaves | 218–1555 (859 ± 577) | 444–1197 (760 ± 276) | 265–723 (466 ± 195) | 46–487 (193 ± 180) |
| | Stems | 24–1324 (637 ± 538) | 223–809 (491 ± 250) | 55–568 (299 ± 221) | <15*–219 (77 ± 90) |
| | Roots | 94–1264 (680 ± 542) | 83–816 (427 ± 283) | 98–518 (340 ± 176) | <15*–263 (106 ± 110) |
| Ni | Flowers | 68–530 (253 ± 176) | 33–348 (221 ± 123) | 97–391 (247 ± 114) | <15*–184 (921 ± 83) |
| | Leaves | 21–418 (204 ± 150) | <15*–324 (166 ± 119) | 81–379 (237 ± 124) | <15*–176 (71 ± 73) |
| | Stems | <15*–372 (165 ± 141) | <15*–307 (179 ± 130) | 57–352 (215 ± 124) | <15*–146 (65 ± 60) |
| | Roots | <15*–331 (136 ± 124) | <15*–274 (157 ± 116) | 50–322 (200 ± 117) | <15*–115 (46 ± 47) |
| Pb | Flowers | 26–175 (115 ± 65) | 171–318 (240 ± 59) | <1.5*–302 (147 ± 143) | <1.5*–38 (17 ± 17) |
| | Leaves | 30–161 (95 ± 53) | 193–288 (241 ± 37) | <1.5*–258 (114 ± 116) | <1.5*–42 (27 ± 16) |
| | Stems | 43–205 (120 ± 61) | 142–286 (223 ± 56) | <1.5*–112 (57 ± 53) | <1.5*–44 (26 ± 16) |
| | Roots | 41–188 (109 ± 60) | 102–272 (208 ± 70) | <1.5*–67 (30 ± 33) | <1.5*–32 (23 ± 13) |
| Zn | Flowers | 45–1116 (403 ± 480) | <7.5*–349 (184 ± 135) | 26–56 (38 ± 12) | <7.5*–476 (109 ± 207) |
| | Leaves | <7.5*–578 (321 ± 210) | 145–255 (181 ± 44) | <7.5* | <7.5*–437 (104 ± 187) |
| | Stems | <7.5*–593 (180 ± 249) | <7.5*–243 (139 ± 99) | <7.5*–81 (16 ± 36) | <7.5*–492 (115 ± 211) |
| | Roots | 48–666 (236 ± 250) | <7.5*–306 (117 ± 127) | <7.5*–56 (23 ± 24) | <7.5* |

* Under detection limits.

leaves were preferred as Mn accumulation organs (Table II). Accumulation of Mn in leaves depends on its function in photosynthesis. The most important role of Mn in green plants was in the Hill reaction, the $H_2O$ splitting and $O_2$ releasing process (Hagemeyer, 2004).

According to Allen (1989), normal Ni contents in plants range from 0.5 to 5 mg/kg and the values over these limits are poisonous. The min and max range in organs of our plant species varied from under detection limit (15 mg/kg DW) to 1650 mg/kg DW (Table II). These results indicate that *V. olympicum* may have a special Ni detoxifying capacity. Ni content of *V. olympicum* reflects the soil Ni content of its environment (Tables I and II). The distribution of this element among organs appears to be different from other metals. Ni content of flowers and fruits was higher than that of other organs (Table II).

The capability of *V. olympicum* for taking Fe, Mn, Ni, Cu linearly from soil was also observed for Pb and Zn. Pb contents in organs of this species (Table II) varied from under detection limit (1.5 mg/kg DW) to 318 mg/kg DW (Table II). However, maximum value in plants was found at Mandra site (Table II). This value is also much higher than that of normal plants (1–13 mg/kg DW) (Shaw *et al.*, 2004).

The highest Zn content of tissues was found in flowers and fruits in plants collected from all sample sites (Table II). For this reason, it can be said that Zn was localized mainly in flowers and fruits. It is possible that these organs have a special mechanism for detoxifying Zn in plant tissues. The Zn content in all organs of *V. olympicum* varied from under detection limit (7.5 mg/kg DW) to 1116 mg/kg DW (Table II). Except for plant samples taken from Sarıalan, max Zn content of plant

Table III. Simple correlation coefficients between element content of soils and different organs (mg/kg DW) of *V. olympicum* Boiss. ($n$ = 20, $P$ < 0.05 significant correlation).

| | $r^2$ | $P$ | $Y = a + bx$ |
|---|---|---|---|
| Soil-Cu | | | |
| Root-Cu | 0.909 | 0.000 | Root-Cu = −22.4421 + 0.246837 × *Soil-Cu* |
| Stem-Cu | 0.926 | 0.000 | Stem-Cu = −15.4164 + 0.23797 × *Soil-Cu* |
| Leaf-Cu | 0.853 | 0.000 | Leaf-Cu = 12.29275 + 0.27527 × *Soil-Cu* |
| Flower-Cu | 0.932 | 0.000 | Flower-Cu = 25.52459 + 0.23997 × *Soil-Cu* |
| Soil-Fe | | | |
| Root-Fe | 0.906 | 0.000 | Root-Fe = −83.9122 + 0.389596 × *Soil-Fe* |
| Stem-Fe | 0.850 | 0.000 | Stem-Fe = 48.40739 + 0.154707 × *Soil-Fe* |
| Leaf-Fe | 0.863 | 0.000 | Leaf-Fe = 37.9909 + 0.259136 × *Soil-Fe* |
| Flower-Fe | 0.940 | 0.000 | Flower-Fe = −2.46946 + 0.196568 × *Soil-Fe* |
| Soil-Mn | | | |
| Root-Mn | 0.923 | 0.000 | Root-Mn = −59.7162 + 0.270053 × *Soil-Mn* |
| Stem-Mn | 0.924 | 0.000 | Stem-Mn = −72.9981 + 0.270613 × *Soil-Mn* |
| Leaf-Mn | 0.867 | 0.000 | Leaf-Mn = 72.91508 + 0.299328 × *Soil-Mn* |
| Flower-Mn | 0.408 | 0.002 | Flower-Mn = 59.86859 + 0.159981 × *Soil-Mn* |
| Soil-Ni | | | |
| Root-Ni | 0.973 | 0.000 | Root-Ni = −16.6234 + 0.228183 × *Soil-Ni* |
| Stem-Ni | 0.983 | 0.000 | Stem-Ni = −7.73679 + 0.247037 × *Soil-Ni* |
| Leaf-Ni | 0.968 | 0.000 | Leaf-Ni = 0.509478 + 0.254471 × *Soil-Ni* |
| Flower-Ni | 0.963 | 0.000 | Flower-Ni = 23.8409 + 0.270305 × *Soil-Ni* |
| Soil-Pb | | | |
| Root-Pb | 0.769 | 0.000 | Root-Pb = −4.79508 + 0.216963 × *Soil-Pb* |
| Stem-Pb | 0.880 | 0.000 | Stem-Pb = 1.603746 + 0.234395 × *Soil-Pb* |
| Leaf-Pb | 0.888 | 0.000 | Leaf-Pb = 1.324758 + 0.263404 × *Soil-Pb* |
| Flower-Pb | 0.835 | 0.000 | Flower-Pb = 1.854604 + 0.285217 × *Soil-Pb* |
| Soil-Zn | | | |
| Root-Zn | 0.734 | 0.000 | Root-Zn = −10.6972 + 0.196556 × *Soil-Zn* |
| Stem-Zn | 0.802 | 0.000 | Stem-Zn = −2.75495 + 0.216422 × *Soil-Zn* |
| Leaf-Zn | 0.691 | 0.000 | Leaf-Zn = 39.21343 + 0.210732 × *Soil-Zn* |
| Flower-Zn | 0.809 | 0.000 | Flower-Zn = −11.2365 + 0.365948 × *Soil-Zn* |

samples, collected from other sites, was much higher than that of normal plants (8–100 mg/kg) (Shaw *et al.*, 2004).

Significantly high positive correlations were found between all heavy metal contents of soils and all organs of this species ($P < 0.05$) (Table III). This indicates that the contribution of plant organs in monitoring heavy metal content of the environment by this species is similar to each other.

Our findings suggest that *V. olympicum* may be considered a bio-indicator for Cu, Fe, Mn, Ni, Pb, Zn, and may be a useful tool for monitoring the changes in the contents of these metals in the environment. The reason for using this species for monitoring the changes in heavy metal contents of the environment is that it can contribute to the stabilization of heavy metals on polluted areas in Uludağ National Park, due to the accumulation of these metals in its organs, and, since it becomes dominant on destroyed areas, distribution of this species in Uludağ National Park supports this consideration.

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