ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ
C- YAŞAM BİLİMLERİ VE BİYOTEKNOLOJİ

Eskişehir Technical University Journal of Science and Technology C- Life Sciences and Biotechnology

2023, 12(2), pp. 64-72, DOI: 10.18036/estubtdc.1172036

EVALUATION OF HEAVY METALS CONCENTRATIONS OF *Verbascum diversifolium* AND *Alcea calvertii* PLANTS

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ABSTRACT

Depending on atmospheric and industrial pollution, heavy metals can accumulate in the soil and affect the ecosystem. Plants are important biological controllers of environmental pollution. Elemental analysis in plants is among the alternative effective methods used for ecological research. In this study, the usability of plant species such as *Verbascum diversifolium* and *Alcea calvertii*, whose heavy metal content was determined, as biomonitors was investigated. The mineral content measurements of the extracts prepared from the leaves and flowers of the examined plant species were made with an Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES). Heavy metal (Cd, Pb, Cr, As, Zn, Ni) concentrations in plant extracts were determined. In our study, it was determined that the heavy metal tolerance of these two plant species was high. It is thought that it will be important to determine plant species resistant to heavy metals, to clean nature and to prevent human-induced heavy metal pollution from becoming a major problem for all living things in the future.

Keywords: Heavy Metal, *Verbascum diversifolium*, *Alcea calvertii*

1. INTRODUCTION

The problem of urbanization due to rapid population growth in the world has caused heavy metal pollution and brought various problems over time [1-3]. In our age, environmental problems are one of the most important dangers that threaten the natural balance and living things. Heavy metals are a group of non-biodegradable environmental chemicals [4]. Heavy metals are metals with a density greater than 5 g/cm3. Heavy metals that are not absolutely necessary for living things in nature; cadmium (Cd), chromium (Cr), mercury (Hg) and lead (Pb). They have toxic effects even in trace amounts. Heavy metals such as copper (Cu), chromium (Cr^{+3}), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn) and nickel (Ni) are necessary for living things up to a certain level. These elements, which are intensely present in living things, can cause serious diseases and even death when they reach effective doses [5,6].

There are many factors that affect the accumulation of heavy metals in the plant structure. The interaction of many factors such as plant-related factors such as plant species and genetic structure, plant organ-related factors, heavy metal-related factors such as type of heavy metal, interaction with the plant, exposure time, etc. play a role in the entry and accumulation of heavy metals into the plant. [7,8].

However, the possible interactions of these chemicals with each other in the ecosystem are not fully known. For a long time, traditional methods and chemical analysis methods have been used to determine environmental pollution [9,10]. Plants contribute to the reduction of air pollution by accumulating heavy metals, and in this way, the use of plants is an important source of choice for reducing air pollution [11,12]. Recent studies have increased on “bioindicator” or “biomonitor” living things that accumulate toxic substances. However, these methods do not give much information about the effects of pollutants on living things in the environment. Thus, it is possible to have an idea about their concentrations in different ecosystems by analyzing toxic substances in various organs of living things with biomonitoring

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Received: 07.09.2022 Published:26.07.2023

properties [13]. Analyzing the contaminants accumulated in the tissues of living things, evaluating the effects of these substances that cause environmental pollution on the environment where living things live, and representing potential pollution are the most important reasons for using biomonitors. Especially in environmental pollution, pollution caused by radioactive materials is an important problem today. Plants are important biological controllers of environmental pollution [14]. The concept of metal accumulation by plants has aroused great interest as it can be used economically to clean metal-contaminated soils. Detoxifying metal-contaminated soils with plants may represent a low-cost eco-friendly technique [15].

Study material *Verbascum diversifolium* Hub. – Purple, is an endemic specie for our country. Verbascum L. is one of the largest genera of the Scrophulariaceae family. Verbascum genus, which is represented by more than 360 species in the world, usually grows in the temperate regions of the Northern Hemisphere [16]. In our country, the Malvaceae family is represented by 14 genera. One of these genera, Alcea, is represented by 20 species in our country and 2 of these species are endemic to our country. It mostly spreads in the whole of Europe except the north, in North America, in the north of Africa, in parts of the Caucasus and Southern Russia, and in the part from Anatolia to Afghanistan [17].

Heavy metal accumulation may vary according to plant species and plant organs. [18-20]. The most suitable plant species and organ should be determined separately for the monitoring of each heavy metal. In the study, heavy metal contents of medicinal plant species such as *Verbascum diversifolium* and *Alcea calvertii* were evaluated.

2. MATERIALS and METHODS

2.1. Collection of Plant Materials

Verbascum diversifolium Hochst., *Alcea calvertii* Boiss. species were collected from the roadside of the Gözeli plain during the flowering period of the plants. The region where it was collected is shown in Figure 1. The above-ground parts of the collected plant materials were dried in the shade. The aerial parts of the dried plant samples were extracted with a soxlet device using ethanol as a solvent [21]. Extraction was continued until the final extract was colorless. The ethanol content of the extracts obtained from the Soxlet device was removed in the evaporator and the dry extracts formed were stored at +4 °C.

Figure 1. Distribution areas of plants and the region where they are collected in Turkey

2.2. ICP-OES Heavy Metal Analysis

For solubilization process with microwave oven, 0.5 g of sample was transferred to Teflon solubilization vessel and 6 mL of 65% HNO_3 and 2 mL of 30% H_2O_2 were added and teflon bombs were placed in the microwave oven. The program of the microwave device was adjusted to go up to 200ºC for 15 minutes and stay at 200ºC for 15 minutes (Table 1). After the incineration process, the samples that were in solution were taken into balloons and made up to 50 ml with ultrapure water. The plasma of the ICP device is burned and ultrapure water is passed through the system for 15 minutes to stabilize it. According to the elements to be analyzed, mixture standard solutions were prepared and a calibration chart was created. After the calibration chart was created, the samples were given to the system and the reading process was performed. According to the results of the analysis that did not fall into the calibration graph, different calibration graphs were created at ppm or ppb level and re-reading was performed.

Table 1. Microwave program

Step	**Time**	**T1**	**T2**(1)	**Power**
1	00:15:00	200℃	110℃	Maxpower*
2	00:15:00	200℃	110℃	Maxpower*

(1)Optionalsensor, *MaxPower: 1500W forEthosand 1200W for Start units. Useupto 500 Wattforoperationswith 3 orlessvesselssimultaneously.

3. RESULTS AND DISCUSSION

The mineral content of the extracts prepared from the leaves and flowers of the studied endemic plants *Verbascum diversifolium*, *Alcea calvertii* were measured by Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES). Concentrations of toxic minerals (Cd, Pb, Cr, As, Al and Ni) in plant extracts were compared in Table 2.

Table 2. Heavy metal limit values accepted by FAO/WHO in plants [22,23].

Heavy Metal	**VDF** (mg kg^{-1})	**VDL** (mg kg^{-1})	**ACF**(mg kg^{-1})	**ACL**(mg kg^{-1})	**FAO/WHO** (mg kg^{-1})
Pb	31.864	3.633	2.441	5.134	10
As	6.165	6.989	5.573	11.346	3
Cd	0.4278	0.4604	0.261	0.4814	0.3
Cr	29.094	32.077	1.161	48.146	2.0
Ni	28.746	0.0027	16.824	45.774	1.63
Zn	13.390	17.907	29.605	55.643	27.4

Verbascum diversifolium flower (VDF), *Verbascum diversifolium* leaf (VDL), *Alcea calvertii* flower (ACF), *Alcea calvertii* leaf (ACL).

Pb, one of the toxic minerals, was found to be highest in the leaves of *A. calvertii* when all plants were evaluated. However, when all the data were compared with the FAO/WHO data, it was determined that it was not above the toxic limits, but below the consumable limits. When the As concentrations of the plants were examined, it was found that the As values in all plants were above the FAO/WHO limits, but the highest concentration was in the leaves of *A. calvertii*. Cd and Cr values in plants were found to be above the FAO/WHO accepted limits in all plants except the flowers of *A. calvertii*. Another Ni element was found to be above the specified limits only in the flowers of *V.diversifolium* and the leaves of *A. calvertii*. It was determined that Zn was below the limits in *V.diversifolium* and above the specified limits in the flowers and leaves of *A. calvertii*.

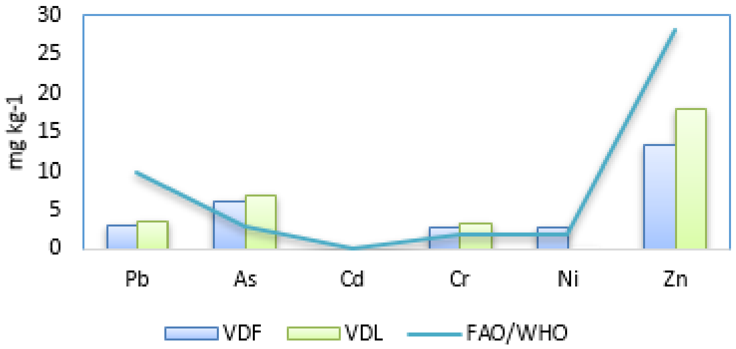

Figure 2. Comparison of VDL and VDF Heavy metal results with WHO values

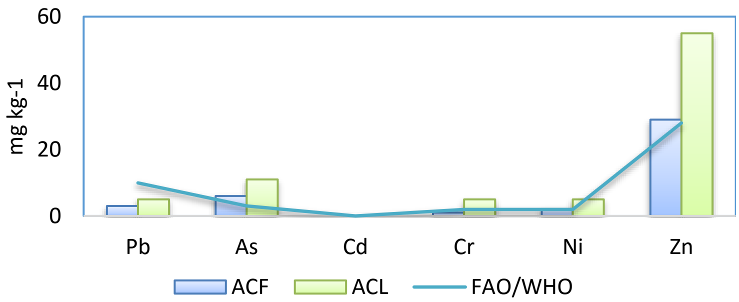

Figure 3. Comparison of ACL and ACF Heavy metal results with WHO values

In terms of usability as a biomonitor, the plant species we studied here, were not found in the literature research. However, findings obtained from different species in similar studies were compatible with the results of our study. Kaya and Gülser (2018) investigated the heavy metal (Fe, Mn, Cu, Zn, Cd, Cr, Ni and Pb) contamination potential in the leaves of the *Alcea rosea* (L.) plant grown in roadside soils. Significant differences were found in the heavy metal concentrations of the leaves. They found that the Fe, Cu, Cr, Ni, and Pb contents of the leaves decreased significantly ($P<0.05$). The highest average content of Fe, Mn, Cu, Zn, Cd, Cr, Ni, and Pb is 810.20 mg kg^{-1}, 63.01 mg kg^{-1}, 34.02 mg kg^{-1}, 29.12 mg kg^{-1}, 25.08 mg kg^{-1}, 14.47 mg kg^{-1}, the lowest average heavy metal content 7.42 mg kg^{-1}, and roadside sampling in leaves, respectively 7.00 mg kg^{-1} 157.75 mg kg^{-1}, 30.49 mg kg^{-1}, 8.20 mg kg^{-1}, 13.89 mg kg^{-1} , 0.01 mg kg^{-1}, 0.76 mg kg^{-1}, 0.57 mg kg^{-1} and 0.70 mg kg^{-1}. Fe, Cu, Zn, Mn, and Ni averages of *A. rosea* seeds were reported to be 24.38, 0.016, 0.179, 0.526, and 0.004 mg kg^{-1}, respectively [24]. In a study conducted by Yener (2007), as a result of the measurements carried out to determine whether *Alcea pallida* can be used as a biomonitor or not, it was stated that the highest value of Zn and Cd accumulation in *A. pallida* was found in the leaf and the lowest value in the flower [25]. When we compared this study with the results we obtained, Pb and Cd were found in the leaf extract of *Alcea calvertii*, and Zn was found to be high in the flower extracts of *Alcea calvertii*. The results obtained are consistent with our findings.

Idrees et al. (2018) analyzed the toxic metal contents of common medicinal plants. All selected metals were found to be higher than the WHO allowed limits in most plants. It was reported that the highest Cr (5.10 mg kg^{-1}), Ni (4.78 mg kg^{-1}) and Fe (129.04 mg kg^{-1}) concentrations were detected in *Verbascum thapsus*. It has also been reported that the Co concentration is found at 4.40 mg kg^{-1} in *Verbascum thapsus* [26]. When we compared the results of the *Verbascum diversifolium* plant that we used in our study, it was found that the Cr and Ni concentrations were close. In their study, Chaplygin et al. (2022) determined the levels of Mn, Cr, Ni, Cu, Zn, Pb, Cd for *Verbascum thapsus*. Zn, Pb, Cr and Cd and polyelement contamination were detected in *V. thapsus* plants. While the accumulation of Zn by plants mostly occurred in the root system, most of the other accepted heavy metals were found in the vegetative parts of the plants [27]. In our study, higher amounts of Pb Cr Ni Zn were detected for *Verbascum diversifolium*, especially in flower extracts. In a study by Tuncturk et al., (2018) in which they studied *Verbascum orientale* L., which grows naturally around Lake Van in the Eastern Anatolia Region, the heavy metal contents of Cr 0.05 mg kg^{-1}, Cd 0.14 mg kg^{-1}, Co 1.54 mg kg^{-1}, Zn was determined as 29.81 mg kg^{-1} and Pb as 0.04 mg kg^{-1}[28]. When compared with our study, it was determined that the Pb, Cr and Cd values were lower than the concentrations we obtained, and the Ni concentration was much higher than the data we obtained Eren and Mert (2017), in their study; According to the findings obtained from rhododendron (*Inula helenium*), lantern grass (*Physalis angulata*) and mullein (*Verbascum thapsus*) plants in soils contaminated with Ni, Cd and Cu, it was determined that the Ni, Cd and Cu concentrations accumulated in the tissues of the plants did not reach levels that would cause toxicity. The highest Ni content was obtained from 400 mg kg^{-1} application of lantern grass, the highest Cd content was obtained from 10 mg kg^{-1} application in lantern and mullein plants, and the highest Cu concentration was obtained from 200 mg kg^{-1} application of lantern plant As a result of the experiment, it was determined that lantern grass and mullein plants have the potential to be used in the cleaning of soils contaminated with heavy metals [29]. Although the species we used in our study were different from the species used in these studies, the results obtained as shown in Figure 2 were parallel to these results due to their structural features. Güleryüz et al. (2005) analyzed the element contents (Cu, Fe, Mn, Ni, Pb and Zn) to determine the index value of *Verbascum olympicum* Boiss. They also examined the element values in different organs of the plants. The maximum values of all the metals examined were determined at a very high level in the organs. Metal content in different organs of *V. olympicum* showed differences [30]. These results show that the metal content in the organs is reflected in the soils of the regions. Many factors such as differences in plant physiology, soil properties, agricultural management practices, ecological interaction can explain the different metal accumulation in plants. The accumulation capacities and biomonitoring properties of other Verbascum species were supported by our findings and previous studies on heavy metal. When we compare the data obtained as a result of our study with the heavy metal limit values accepted by FAO/WHO in plants, the acceptable limit values for As are reported as 1.63 mg kg^{-1} for 3 mg kg^{-1} Ni, 0.3 mg kg^{-1} for Cd, and 2.0 mg kg^{-1} for Cr [31]. According to Table 2, As; in all plant extracts studied; Cd and Cr in other plant extracts except flower extracts of *Alcea calvertii*; Ni was found above the reference value in other plant extracts except the leaf extracts of *Verbascum diversifolium.*

As a result of the study, it was determined that most of the elements in the leaf and flower extracts were at different levels. Different heavy metal concentrations have been reported in different organs in studies conducted to date [32, 33, 12]. Therefore, the structure and characteristics of the organ can significantly affect heavy metal uptake [34, 35].

As a result of the data obtained, as shown in Figure 3, it was found that the Pb, As, Cd, Cr, Ni, Zn results of the leaf extracts of *Alcea calvertii* were much higher than the reference values. As a result of the determination of the metals in the flower and leaf extracts of the plants, it was determined that some of them could be considered as metal accumulators. The collected plant samples were found to be acceptable as metal accumulators, although they had different deposition abilities for each metal. According to the results, the best accumulator was found to be *Alcea calvertii*. It was determined that the heavy metal accumulation of *Alcea calvertii* was higher when compared to the other studied species. Bioaccumulator plants are expressed as plant species that accumulate pollutants at higher concentrations than the living things in their environment [36]. Therefore, *Alcea calvertii* can be considered as a good bioaccumulator plant.

The use of some plant species commonly found in natural vegetation in the cleaning of ecosystems polluted with heavy metals is gaining increasing importance today. As a result of all the evaluations, the studied *Verbascum diversifolium, Alcea calvertii* species show that the plant species has high tolerance to heavy metals. Identifying plant species resistant to heavy metals will be important in cleaning nature and preventing heavy metal pollution caused by humans from becoming a major problem for all living things in the future. Measures are needed to prevent contamination of agricultural lands. One of the methods that can be applied in such problematic or potentially problematic areas is the use of tolerant plant species and varieties.

4. CONCLUSION

The results obtained, the heavy metal content of the plants we studied can be evaluated as a biomonitor. It is important to find plant species resistant to heavy metals, which is one of the factors that pollute the nature. The results of the analysis on the examined plant species show that they can be used as an important source for further studies.

FUNDING

Finance of this research has been provided by Firat University Scientific Research Project Council of Turkey (Project number: SYO.18.06).

CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article.

AUTHORSHIP CONTRIBUTIONS

Tuba TÜRKOĞLU: Writing - original draft, Visualization. **Semra TÜRKOĞLU:** Formal analysis, Investigation, Conceptualization, Supervision, Visualization, Conceptualization.

ACKNOWLEDGMENT

In this study, we would like to thank Prof. Dr. İsmail TÜRKOĞLU for his contributions to the collection and identification of plants.

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