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Heavy metal (Cr, Cu, Ni, Pb, and Zn) contents of endemic *Salvia halophila* plants around Lake Tuz

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Abstract

Heavy metals occur naturally in ecosystems at varying concentrations. However, heavy metal sources that have emerged in present-day mainly due to human influence, i.e. industrial activities, agricultural waste, pesticides, use of fossil fuels and traffic, have included a part of heavy metals in the ecosystem. Lake Tuz, together with the entire lake surroundings, water beds and important steppe areas, was declared Turkey's Specially Protected Area (SPA) in 2001. Our aim in this investigation was to determine the levels of heavy metals such as Chrome (Cr), Copper (Cu), Nickel (Ni), Lead (Pb) and Zinc (Zn) in endemic *Salvia halophila* grown in different areas of Lake Tuz. The results of the heavy metal contents analyzed at the plant were compared with the international standard levels of heavy metals. The consequences displayed that differing extents of heavy metals are accumulated in *S. halophila*. The results obtained differed in accordance with the collection time and localities. When the outcomes are appraised, it is achievable to say that Pb is higher than the standard values. The findings of this investigation are the first reported results for this endemic *S. halophila* species that grows naturally at Lake Tuz and are important as they are newly discovered results.

Introduction

The problem of environmental pollution, which arises with the rise of heavy metals above the natural level, has increased significantly from the beginning of the industrial revolution to the present and continues. Heavy metals either essential or non-essential are naturally present in the ecosystem at trace concentrations. While heavy metals are found in harmless amounts and forms in nature, their amounts have reached harmful concentrations due to increasing industrialization, fossil fuels, road traffic, wrong agricultural techniques and pesticides in the last decades. The presence of heavy metals in the ecosystem is ecologically critical as they can transport to the biosphere in soil and water ecosystems and become toxic to all living organisms at certain concentrations. Also, the responses of plants to high levels of heavy metals in their growth media vary in a species-specific manner. Some plant species can be adversely affected and damaged by relatively smaller concentrations of heavy metals while others can tolerate extremely high concentrations (Srivastava et al., 2017; Srivastava et al., 2020; Nedjimi, 2021).

Heavy metals, which are found in excess in our environment, are an important source of problems for all living environments due to their permanence properties and pose a serious threat to human health (Capparos et al., 2022). As a result of many studies, it has been emphasized that the heavy metal concentration in the soil where the plants used for various purposes (such as food, medicinal and herbal tea) grow, will adversely affect human health (Barthwal et al., 2008; Chary et al., 2008; Capparos et al., 2022).

It was reported that many medicinal plants used in China can uptake harmful metals from the soil and accumulate them in their shoots and/or roots (Meng et al., 2022). The concentrations of Cu, Cd, Hg and Pb in the plants were above the permissible limits; for this reason, it was stated that it would be more accurate to use these plants to be used for medicinal purposes by producing them under controlled conditions instead of collecting them from nature (Meng et al., 2022).

Heavy metal (Pb, Cu, Zn, and Cd) accumulations in different organs of *Mentha piperita* L. *Salvia officinalis* L. and *Salvia sclarea* L. species collected at different growth periods from the industrial area near Plovdiv (Bulgaria) were investigated. The results revealed that the amounts of Pb, Zn, and Cd in the leaves were above the permissible limits. It has also been emphasized that these plants, which have the potential to be used as spice and herbal tea, can pose a great danger to human health if they are collected and used from the region (Angelova et al., 2006). In a different study conducted by the same researcher group, the potential to use *Salvia sclarea* L. collected from this region for the phytoremediation of the soils, where heavy metal pollution is observed, was investigated. According to the results, it has been reported that *S. sclarea* plant is a hyperaccumulator for Pb and an accumulator for Cd and Zn. It was emphasized that this plant has the potential to be used for phytoremediation of these soils, where heavy metal pollution problems are seen (Angelova et al., 2016).

It is known that halophyte plants, which represent 1% of the global plant wealth, can activate more than one tolerance mechanism against salinity. It is also reported that halophytes are better adapted than glycophytes to avoid the harmful effects of heavy metal accumulation. With these features, it has been emphasized by many researchers that halophytes have an important potential for the improvement of problem areas (Flowers & Colmer, 2008; Manousaki & Kalogerakis, 2011a; Manousaki & Kalogerakis, 2011b; Caparros et al., 2022; Peng et al., 2022). Li et al. (2019) reported that halophytes could be used for phytoremediation in both salt-affected and heavy metal-contaminated soils. According to the results of their study with halophyte *Halogeton glomeratus*, it was emphasized that this plant was a promising plant for soils with such problems.

Turkey's Authority for Specially Protected Areas (ASPA) conducts various studies so as to reduce the number of pollutants that may affect special areas for managing and protecting their different natural characteristics. In 2001, Lake Tuz was declared one of the Specially Protected Areas (SPA) of Turkey, including all of the lake surface, surrounding water beds and some of the important neighboring steppic areas. The lake is surrounded by salt marshes are the richest areas in terms of endemism and plant communities with halophyte characteristics (Baysal- Furtana et al., 2013). Although, it is such an important area, it has been facing a pollution problem, especially in the last decades. The main sources of pollution are pesticides, heavy metals, detergents, oil, and waste water, which are carried by drainage systems (Tuğ & Duman, 2010; Demir et al., 2021).

The *Lamiaceae* family is the third-largest family in Turkey. Also, Turkey has about 10% of all *Lamiaceae* members in the World. Salvia is one of the largest genera (945 species) in *Lamiaceae* and has a high endemism ratio (Celep & Dirmenci, 2017). *S. halophila*, a species belonging to this genus, is an endemic perennial herb in the Irano-Turanian phytogeographic region and is spread on the inland salt steppes of central and western Anatolia, Turkey. The essential oils in sage are of great medicinal importance (Gruenwald et al., 2004). For this reason, it is used in the pharmaceutical industry in many parts of the world. The antiseptic and antispasmodic properties of the essential oils increase the medicinal value of the plant. It is used in traditional medicine for colds, wound healing, stomach complaints, rheumatic pains, and liver diseases (Baytop, 1984; Sezik & Yeşilada, 1999). Due to its medicinal importance, its unconscious collection from nature causes great problems for the continuation of generations. *Salvia halophila*, which is distributed in a limited area in our country, is one of the endemic species under threat and is in the endangered (EN) (species with a very high risk of extinction in the wild) category (Kuşaksız, 2019). For this reason, it is very important to protect the biodiversity of endemic and economically important plants such as *S. halophila*.

In this study, we aimed to determine the heavy metals (Chrome [Cr], Copper [Cu], Nickel [Ni], Lead [Pb], and Zinc [Zn]) levels of *S. halophila*, an endemic plant that grows naturally in various parts of Lake Tuz. For this purpose, heavy metal contents in plant samples collected at different times in their natural areas were analyzed and the results were compared with international standard heavy metal levels. It is crucial that the findings of this investigation are the first reported results for this endemic species of Salvia located at Lake Tuz, Turkey.

Materials and Methods

Lake Tuz, which is a tectonic lake, is located in the vicinity of Ankara-Konya-Aksaray provinces of the Central Anatolia Region. It is the second-largest lake in Turkey, situated 905 m above the sea level. Although it has a large area (1500 km^2), it is a very shallow (0.5 to 1 m deep) lake, especially in the dry summer months when water evaporates in large quantities, its depth drops to 30 cm and a thick salt crust forms on its surface. The highest temperatures are recorded in June and September when the rain is minimal. The arid season begins at the end of June, continues for 3–4 months. The area is under the influence of a semiarid cold Mediterranean climate. Seventy-eight per cent of the soils in the basin are characterized as saline and alkaline. Consequently, they behave as suitable habitats for halophytic plants and a few glycophytic ecotypes (Baysal-Furtana et al., 2013).

Figure 1. Localities of *Salvia halophila* marked in Google Earth. The coordinates were determined by Global Positioning System (GPS)

The study areas are located in Eskil district of Aksaray province and Cihanbeyli district of Konya province, which are the natural habitats of *S. halophila* (Figure 2). Plant materials were collected from these areas at month intervals between June-September 2016 in accordance with the vegetation period. The aerial parts (shoots, leaves, flowers) of the three plant samples that best represent the population were regularly collected from designated localities (Eskil (N 38°24´-29´ E 33°26´-30´) and Cihanbeyli (N 38°32´-34´ E 38°06´-08´) at 4 different times (Figure 1). Fresh plant samples were kept in ice-box and immediately transferred in to laboratory. The contaminants such as dust and soil on the plant material were removed by sequential washing with dilute acid, tap water and distilled water. The samples were oven dried at 65°C and grinded to have homogenous alicots.

Then, 1 g of powdered samples were digested with 10 mL of concentrated HNO_3 (Merck, 64%) by means of a the microwave oven (EPASW-846 3051) and passed through a 0.45 µm (Minisart® NML Syringe Filters) filter and the volume was made up to 15 ml by adding distilled water. Finally, the heavy metal concentrations (Cr, Cu, Ni, Pb, Zn) of the digests were determined by Atomic Absorption Spectrophotometer (Perkin Elmer, AAnalyst 800 Atomic Absorption Spectrometer). The AAS were calibrated by a High Purity Standards brand QCS-27 series and an internal standard (10 ppb 209Bi) was used to increase the precision of the measurement that produced a calibration curve with a determination coefficient above 0.99.

Figure 2. General view of Salvia halophila

Taking into account the density and abundance ratios from the saline steppe vegetation, which is the habitat of *S. halophila*, from the areas where the vegetation is homogeneous, 3 representative individuals determined as the representative of the population were collected for analysis. Plant samples for analysis were collected from locations with at least 10 individuals in an area of 25 m^2, paying attention to the distribution, abundance and overlap of the population of the species, so that collection could be made from the same location at all sampling times. It is very difficult to collect numerical data from natural populations, but it may be possible to obtain results representative of the population by studies that can be planned under laboratory conditions with plant materials collected in limited amounts from natural areas. Plant samples were collected from localities at 4 different times. The experiments were set up in a factorial randomized block design. All analyses were carried out with 3 replications. The findings were evaluated with SPSS 23, ANOVA statistical methods. Analysis of variance (ANOVA) was performed to determine significant differences. Means were seperated using Duncan Multiple Range Test at $p < 0.05$.

Results and Discussion

Halophytes, which can easily live and reproduce in saline soils, can be ideal alternative phytormediators for saline soils exposed to heavy metal pollution (Flowers & Colmer, 2008; Manousaki & Kalogerakis, 2011a, 2011b; Wang et al., 2013). Salinity in the soil can also increase heavy metal mobility (Acosta et al., 2011; Manousaki et al., 2008). The resistance of halophytes to heavy metals may be related to the salt tolerance properties of these plants (Wang et al., 2013; Liang et al., 2017) because they use almost the same tolerance mechanisms for the heavy metals (Nedjimi, 2021).

In this study, the heavy metal (Cr, Cu, Ni, Pb and Zn) levels of *S. halophila*, which is an endemic halophytic plant that grows naturally in various parts of Lake Tuz, were determined. The results reveal that heavy metals such as Cr, Cu, Ni, Pb and Zn accumulated in varying amounts in *S. halophila* plants (Table 1). The table also presents recommended optimum levels according to FAO/WHO standards (EC, 2001; Srivastava et al., 2017). When the Table 1 showing the heavy metal concentration of the plant is examined, it can be seen that the accumulated heavy metal amounts varied in accordance with the collection time (as plant's growth period, season) and/or the locality.

According to the observed results, *S. halophila* was seen to accumulate Pb above the permissible limit at both locations. When the plant analyzes collected from the Cihanbeyli are evaluated; it is seen that the investigated metals in the plant are lower than the samples collected from Eskil. The highest concentration of Pb was recorded as 1.440 $\mu g.g^{-1}$ (±0.510) in the sample taken from Eskil in June (Table 1). According to the results, it is achievable to conclude that the amount of Pb determined in the samples collected from Eskil is above the standard values. The metal content of plant changes according to time and vegetation period. (Angelova et al., 2006; Naser et al., 2011; Mensah et al.,2008) accounted for that the concentration of Pb increased consistently during the growth period in lettuce plants. Nevertheless in our study, the highest Pb concentration was recorded in June, and it was found to be below this value in other investigated months. It has been reported by Naser et al. (2011) that the time-dependent changes in metal concentrations in plants differ according to the plant, its species and the type of metal. It was determined that there were significant differences in Pb concentrations of the examined plant depending on time ($p<0.05$) and locality ($p<0.01$) (Table 2).

Diacu et al. (2011) analyzed the cadmium and lead content of Sage- *Salvia officinalis* samples from the Prahova Valley region–Romania. They reported that the potential *S. officinalis* to be used as a detoxifier cannot be ignored due to its heavy metal accumulating capacity. Lead is unstable and can readily decompose into oxides, carbonates, and sulfates, often penetrating into soil through airborne deposition on roadsides (Wuana & Okieimen, 2011). The cause of Pb accumulation was thought to be due to the air pollution, the traffic emissions and domestic waste and energy. Also, Pb accumulation can be attributed to a high concentration in the soil where the plants grow.

The highest mean concentration of Ni was recorded as 0.583 $\mu g.g^{-1}$ (±0.161) in the sample taken from Cihanbeyli in September (Table 1). It has been observed that the accumulated Ni levels varied in accordance with the collection time and the locality. The time-dependent changes in the amount of the Ni in the plant samples collected from Eskil were not significant. However, time-dependent changes in the amount of the Ni in the plant samples collected from Cihanbeyli were significant ($p<0.05$) (Table 2). It has been reported that some plant groups are called "Ni hyperaccumulators" because they have the capacity to tolerate and store more than 1 $\mu g.g^{-1}$ Ni in their bodies (Baker et al., 1994), whereas the permissible limit set by FAO/WHO (Srivastava et al., 2017) in plants was 1.5 $\mu g.g^{-1}$. When the Ni concentrations given in Table 1 are examined, none of the samples examined in our study had a Ni value above 1.5 $\mu g.g^{-1}$.

In various studies conducted around Lake Tuz, it has been reported that especially the Pb and Ni ratios are quite high (Tuğ & Duman, 2010; Demir et al., 2021). In a similar study by Kılıç (2019), the heavy metal accumulation (Ni, Fe, Co, Mn) and the usability of *Calepina irregularis* species that naturally grown in Amasya, as a biomonitor was investigated. The heavy metal content in the roots, stems and leaves of the plant samples was evaluated. The samples were collected from 4 different areas: the city center, close to the highway, suburban and traffic-free (control) areas. According to the data obtained, it has been stated that the accumulation of heavy metals is higher in the leaves and root parts of the plant samples grown around the highway, and in the stems of the plants grown in the suburbs. Kılıç (2019) also stated that Ni and Mn may be found higher in plants, which are close to the highways due to air pollution because of traffic.

The highest mean concentration of Cr was recorded as 0.487 $\mu g.g^{-1}$ (±0.075) in the sample taken from Eskil in August (Table 1). When the results of the

Table 1. The concentrations (µg g^{-1}) of heavy metals (Cu, Cr, Ni, Pb and Zn) analyzed in *Salvia halophila*

Location	Date		Pb	Ni	Cr	Cu	Zn
Eskil	June	Range	0.930-1.440	0.160-0.300	0.214-0.380	0.420-0.453	0.560-1.550
		Mean	1.440	0.230	0.297	0.437	1.055
		Std. Dev.	0.510	0.070	0.083	0.017	0.495
	July	Range	0.480-0.870	0.220-0.600	0.105-0.230	0.230-0.430	0.660-1.220
		Mean	0.623	0.420	0.143	0.300	0.980
		Std. Dev.	0.215	0.121	0.075	0.113	0.288
	August	Range	0.880-0.940	0.105-0.660	0.210-0.770	0.330-1.100	1.060-1.600
		Mean	0.913	0.420	0.487	0.697	1.320
		Std. Dev.	0.215	0.191	0.075	0.113	0.288
	September	Range	0.740-1.500	0.100-0.520	0.250-0.660	0.120-1.200	1.030-2.020
		Mean	1.180	0.340	0.413	0.550	1.370
		Std. Dev.	0.215	0.191	0.075	0.113	0.288
Cihanbeyli	June	Range	0.105-0.700	0.105-0.140	0.311-0.455	0.370-0.530	0.140-0.950
		Mean	0.403	0.123	0.359	0.450	0.545
		Std. Dev.	0.198	0.018	0.083	0.080	0.205
	July	Range	0.130-0.162	0.150-0.340	0.110-0.270	0.228-0.700	0.490-0.950
		Mean	0.144	0.230	0.210	0.433	0.696
		Std. Dev.	0.016	0.098	0.087	0.142	0.194
	August	Range	0.370-0.660	0.200-0.300	0.120-0.350	0.330-0.880	0.660-0.980
		Mean	0.523	0.260	0.200	0.543	0.803
		Std. Dev.	0.146	0.053	0.095	0.195	0.103
	September	Range	0.200-0.320	0.400-0.700	0.100-0.550	0.150-0.410	0.940-1.060
		Mean	0.253	0.583	0.260	0.257	0.980
		Std. Dev.	0.061	0.161	0.152	0.096	0.069
	Optimum Value		0.3	1.5	1.5	10	50

*Optimum reference value declared by FAO/WHO (EC, 2001; Srivastava et al., 2017)

Table 2. ANOVA for variation of concentrations (µg g^{-1}) of heavy metals (Cu, Cr, Ni, Pb and Zn) analyzed in *Salvia halophila* by locality and/or time

Source of variance	df	Pb	Ni	Cr	Cu	Zn
Replication	2	ns	ns	ns	ns	ns
Locality (L)	1	**	ns	ns	ns	**
Time (T)	3	*	*	ns	ns	ns
L x T	3	ns	ns	ns	ns	ns
Error	12	0.082	0.023	0.028	0.085	0.130

ns: nonsignificant, ** and *, significant in 1% and 5% area (n=3) analysis were evaluated, it was determined that there were differences in the amount of Cr according to the collection time and the locality (Table 1). While time-dependent changes in the content of the Cr in the plant samples collected from Cihanbeyli were not significant, the differences between the samples collected from Eskil in July and those collected in August and September were significant. According to Allen (1989), the chromium level, which is toxic to plants, is 0.5 $\mu g.g^{-1}$, while according to Markert (1994), this limit has been reported as 1.5 $\mu g.g^{-1}$. In our study, the mean Cr concentration was generally below the limit level.

The highest mean concentration of Cu was recorded as 0.697 $\mu g.g^{-1}$ (±0.113) in the sample taken from Eskil in August (Table 1). Although the amount of accumulated Cu varied according to the time (as plant's growth period, season) and location that the plants were collected, these differences were not significant (Table 2). The permissible limit set by FAO/WHO (Srivastava et al., 2017) in plants was 10 $\mu g.g^{-1}$. According to the results of the analysis we performed of the samples we collected, it was determined that the amount of Cu was below the limit values.

The highest mean concentration of Zn was recorded as 1.370 $\mu g.g^{-1}$ (±0.288) in the sample taken from Eskil in September (Table 1). It has been observed that the accumulated Zn levels varied in accordance with the collection time and the locality (Table 1). It was determined that the difference depending on the locality was significant ($p<0.01$).

Due to a study conducted with *Celtis australis* L. in 40 different localities of Istanbul, it was determined that there was a direct correlation between the accumulation of investigated heavy metals (Pb, Cd, Cu and Zn), the traffic density and proximity to the roadside (Ozturk et al., 2017).

According to the study results of Demir et al. (2021), the main sources of heavy metal pollution observed around Lake Tuz are domestic and industrial wastes, sewage and septic wastes, volcanic dust and gases, agricultural activities, pesticide use, use of artificial fertilizers and traffic. They reported that Ni and Pb pollution from heavy metals examined in soil samples analyzed from Eskil and Cihanbeyli was higher than the others. Researchers attribute the reason for this pollution in the region to the fact that it is close to the main drainage channel carrying the industrial waste coming from Konya, that the surrounding districts are garbage collection areas, and that there is an open and irregular sewer system in parallel. Along with all these situations, it is thought that the location of both regions in the volcanic area is effective in heavy metal accumulation (Demir et al., 2021). It has been reported that the volcanic activity, dust particles blown by the wind and desert dust reaching the area are also important heavy metal sources (Nagajyoti et al., 2010; Srivastava et al., 2017).

In accordance with the results of the analysis, it was observed that the heaviest Ni, Cr, Cu and Zn accumulations were in August and September. This situation may be due to the high temperature and evaporation of the research area during these months. In the areas around Lake Tuz, it has been reported by Tuğ & Duman (2010) that the heavy metals and minerals that have become mobile rebound to the upper layers from deeper levels of the soil by evaporation with increasing temperature in summer. For this reason, an increase in heavy metal concentrations in the soil is observed during the summer.

Conclusion

The data we obtained from this study are critical as they are the first results presented to the scientific world for this endemic *Salvia halophila* species that grows naturally in Lake Tuz. The fact that our results can be used in similar studies in the future will both increase the economic and ecological value of the plant and determine its potential for use phytoremediation. Additionally, it is vital because it can provide the use of plants determined to have phytoremediation properties with such studies as a biomonitor that will allow monitoring of short-term changes in environmental pollution in the near future.

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Author Contributions

GBF: carried out the field study, collected the plant samples and prepared them for analysis, wrote manuscript (review and editing); **AD**: carried out conceptualization and statistical analysis; **MT**; carried out the field study, collected the plant samples; **AR:** carried out the analysis of the investigated heavy metals; **RT**: carried out conceptualization.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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