Accumulation copper (Cu) in the *Halimione portulacoides* (L.) Aellen and *Suaeda prostrata* subsp. *prostrata* pall. taxa, spreading in Ayvalık saltern (Balıkesir-Turkey)

Güngör AY[1], Murat KILIÇ[2], Fatma KOÇBAŞ[1], Fatma MUNGAN KILIÇ[2,*]

[1] *Department of Biology, Faculty of Arts and Sciences, Celal Bayar University, Muradiye, Manisa, Turkey*
[2] *Department of Crops and Animal Production, Kızıltepe Vocational Training High School, Artuklu University, Kızıltepe, Mardin, Turkey*

Abstract

The aim of this present study was to compare the level of copper (Cu) in the soils and different organs (root, stem, leaves) of Halimione portulacoides (L.) Aellen and Suaeda prostrata subsp. prostrata Pall. taxa which are widespread distribution in every locality of from Ayvalık saltern in Balıkesir. Each month samples were collected from determined seven stations, from soil dam surrounding saltern. Analyses were done by using Perkin Elmer Analyst 700 Flame Atomic Absorption Spectrophotometer (FAAS) device. The level of Cu in H. portulacoides (0.003-0.925 ppm) and S. prostrata subsp. prostrata (0.004-0.896 ppm) was observed to be quite different. Levels of copper in the soils were below the limits of the Turkey soil pollution control regulations standard (TSP, 24609). The levels of Cu were found in the soil in H. portulacoides was 0.862-1.111 ppm, S. prostrata subsp. prostrata 0.858-1.111 ppm.

Güngör AY, gungoray@hotmail.com, https://orcid.org/0000-0002-3292-4932
Murat KILIÇ, muratkilic04@gmail.com, https://orcid.org/0000-0002-6408-9660
Fatma KOÇBAŞ, fatma.1970@hotmail.com, https://orcid.org/0000-0002-1053-3455
* Fatma MUNGAN KILIÇ, fatmamungankilic@artuklu.edu.tr, https://orcid.org/0000-0001-6858-3458

Ayvalık tuzlasında yayılış gösteren *Halimione portulacoides* (l.) ve *Suaeda prostrata* subsp. *prostrata* pall. taksonlarında bakır (Cu) birikimi (Balıkesir-Türkiye)

Öz

Çalışmamızın amacı Balıkesir ilinde bulunan Ayvalık tuzlasının her lokalitesinde yayılış gösteren ve yoğun olarak bulunan Halimione portulacoides (L.) Aellen ve Suaeda prostrata subsp. prostrata Pall. taksonlarının topraklarında ve farklı organlarında (kök, gövde ve yaprak) bakır (Cu) seviyesini karşılaştırmaktır. Her ay tuzlayı çevreleyen toprak set üzerinde belirlenen yedi istasyondan örnekler alınmıştır. Analizler Perkin Elmer Analyst 700 model alevli atomik absorbsiyon Spektrofotometresi (FAAS) cihazı ile yapıldı. Cu seviyesi H. portulacoides (0.003-0.925 ppm) ve S. prostrata subsp. prostrata (0.004-0.896 ppm) oldukça farklı olduğu gözlemlenmiştir. Topraktaki Cu değerleri; H. portulacoides'de 862-1.111 ppm, S. prostrata subsp. prostrata'da ise 0.858-1.111 ppm olarak bulunmuş olup, Türkiye toprak kirliliği kontrol yönetmeliği değerlerinin altında çıkmıştır.

Anahtar kelimeler: Ayvalık tuzlası, bakır, halofitik bitki, kirlilik.

1. Introduction

Heavy metals have a different significance to other chemical pollutants because they may be found in several different sources, they create a prevalent source of pollution, they are resistant to environmental conditions, they always affect biological systems and may accumulate in living beings in increasing concentrations by easily entering the food chain [1, 2]. Industrial activities exhaust gases of motorized vehicles, mineral deposits and establishments, volcanic activities, fertilizers and pesticides that are used in agriculture and urban waste are some of the factors that cause heavy metals to spread in the environment [3]. 50% of this pollution is created by motorized vehicles [4]. Trace elements such as Mn, Fe, Cu, Zn and Ni among the heavy metals are elements that are needed for plants [5]. The effect of heavy metal pollution on plants is dependent on the quantity of the pollutant, distance of the source of the pollutant, time of exposure and meteorological conditions [6]. As copper is a material that is used in various fields, there are multiple sources of pollution that is caused by this element. Using this element in processes or packaging may pollute the products and harm the environment [7]. While plants can collect heavy metals from their roots and store them in their other organs, they also take them into their bodies through their stomata [8]. When there is excessive accumulation of heavy metals in plant tissues, multiple processes such as mineral nutrient intake, photosynthesis, enzyme activity, chlorophyll biosynthesis and germination are affected negatively. These problems may be accompanied by physiological processes such as membrane damage, disruption of the hormonal balance and alterations in water status [9]. Additionally, regardless of whether or not they are micronutrient elements, excessive accumulation of heavy metals in the plant leads to physiological stress and reduction in growth and development [10]. *H. portulacoides*, which is a halophytic plant, is an edible, long-rooted, perennial, evergreen plant with succulent leaves whose height in saltern forms reach up to 40 cm. *S. prostrata* subsp.

prostrata is a long-rooted, annual halophytic plant with succulent leaves and erect stem whose height in saltern forms reach up to 20 cm. While several studies have been carried out on the *H. portulacoides* in the world, especially in Portugal [11-13], no study was found in Turkey regarding *H. portulacoides* or *S. prostrata* subsp. *prostrata* except for the study by [14] which investigated the heavy metal contents and the pollution of the Ayvalık Saltern.

The Ayvalık Saltern, which is located near the İzmir-Çanakkale highway in İzmir, is the second largest seawater saltern in Turkey. Here, an annual average of 20,000 tons of salt is produced. The salt that is produced here is used in the leather and intestine sector, snow control work and especially in the food industry. Due to the excessive concentration of salt in the soil barriers that connect the 17 evaporation and 5 crystallization pools found in the Ayvalık Saltern, almost no plants can grow there. There are halophytic plants such as *H. portulacoides*, *Salicornia europaea* L. and *S. prostrata* subsp. *prostrata* in the soil barrier that surrounds the saltern. This study aimed to determine the Cu levels of the soils in which the plants *S. prostrata* subsp. *prostrata* and *H. portulacoides* naturally grow in the Ayvalık Saltern, as well as the changes in these levels based on distance to the highway.

2. Materials and methods

The Ayvalık Saltern, which was established in 1980 on an area of 930,000 m², is located near the İzmir-Çanakkale highway at a distance of 11 km from the district of Ayvalık in Balıkesir, Turkey. The saltern is surrounded by a soil barrier of approximately 1 m of height and a water-filled drainage trench of 2-3 m of width that restricts it. The materials of our study consisted of the *H. portulacoides* and *S. prostrata* subsp. *prostrata* plants that are abundantly spread on the soil barrier and the soils they grew in. Sampling took place between the dates of June 2009 and May 2010 regularly in monthly intervals on 7 stations that were determined on the soil barrier surrounding the saltern (Figure 1). The plants were collected without using metal tools, brought to the laboratory and dried for 16 hours in a stove set at 105ºC. Each of the dried samples was turned into a powder in a porcelain mortar, 1 gr of the sample was collected by weighing on a precision scale and put in 250-ml beakers. HCl: HNO_3 (3: 1) added onto the samples. The samples were subjected to a burning process in a fume hood for 2 hours at 150-200ºC until approximately 1 ml of white-colored plant melt remained on the hot plate. After the melt was filtered through blue-grade filter paper, it was filled up to 50 ml with distilled water. The analyses were carried out by a Perkin Elmer Analyst 700 Model Flame Atomic Absorption Spectrophotometer (FAAS).

The soil samples were also collected without using metal tools from a depth of approximately 20 cm by extracting cores for 0.5-kg samples. The soils that were put into plastic bags were brought to the laboratory in iceboxes and stored at -21ºC until the analyses. The soils that were put in a certain amount on petri dishes before the analyses were dried for 16 hours in a stove set at 105 ºC. Each dried sample was turned into powder in a porcelain mortar, homogenized and filtered through a 160 μ sieve. The sieved soil samples were weighed as 0.5 gr on a precision scale, and HCl: HNO_3 (3: 1) was added. They were then subjected to a burning process under a fume hood for 2 hours at 150-200ºC until a white-colored melt remained. After the melt was filtered through blue-grade filter paper, the samples were completed to 25 ml with distilled

water [15]. The analyses were carried out by a Perkin Elmer Analyst 700 Model Flame Atomic Absorption Spectrophotometer (FAAS).
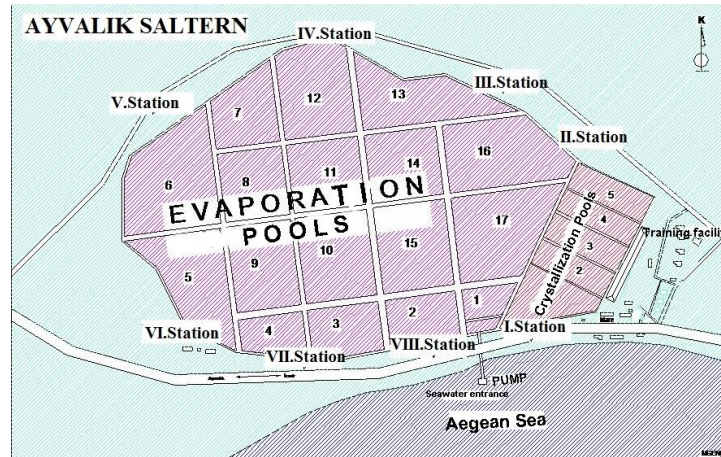

Figure 1. The Ayvalık saltern and sampling stations.

2.1. *The 2009-2010 meteorological (precipitation and wind) data for the district of ayvalık*

Table 1 shows the monthly average precipitation values and wind speeds based on the meteorological data obtained from the General Directorate of Meteorology, the Turkish Ministry of Forestry and Water Affairs.

Table 1. June 2009 - may 2010 monthly total precipitation (mm) average wind speed (m/sec) for the district of Ayvalık.

Month	Jun. 09	Jul. 09	Aug. 09	Sep. 09	Oct. 09	Nov. 09	Dec. 09	Jan. 10	Feb. 10	Mar. 10	Apr. 10	May. 10
Precipitation	21,6	0	0	15,6	11,0	91,8	162,9	96,5	270,1	21,7	30,9	26,0
Wind	2,1	2,7	3,4	2,4	2,1	1,4	2,0	2,4	1,9	1,9	2,3	1,6

3. Results and discussion

The *H. portulacoides* and *S. prostrata* subsp. *prostrata* samples that were collected from the Ayvalık Saltern were analyzed by analyzed by dividing them into root, stem and leaf parts. All analysis results (including soil) are shown in Table 2. According to these results, the Cu concentration of the *H. portulacoides* species was found to be in the range of 0.003-0.925 ppm. This rate was in the range of 0.862-1.111 ppm in the soil. According to the average values of 7 stations, the lowest monthly Cu concentration values were 0.927±0.01 ppm in the soil (March 2010), 0.039±0.03 ppm in the root (September 2009), 0.029±0.02 ppm in the stem (August 2009) and 0.042±0.03 ppm in the leaves (April 2010). The highest Cu concentration values were 0.968±0.07 ppm in the soil (February 2010), 0.845±0.04 ppm in the root (June 2009), 0.418±0.05 ppm in the stem and 0.482±0.05 ppm in the leaves (June 2009). According to the 12-month average values of each station, the lowest Cu concentrations were found as 0.924±0.03 ppm in the soil (station 3), 0.147±0.23 ppm in the root (station 4), 0.096±0.09 ppm in the stem (station 5) and 0.104±0.13 ppm in the leaves (station 4). Based on the same values, the highest Cu concentrations were found as 0.986±0.07 ppm in the soil (station 7), 0.238±0.29 ppm in the root (station 1), 0.116±0.12 ppm in the stem (station 4) and 0.135±0.13 ppm in the leaves (station 5) (Table 2, Figure 2).

Table 2. 12-month average Cu concentration values for all stations in *H. portulacoides*
and growth soil (dry weight).

Station Cu No ppm		Soil	Root	Stem	Leaf
1	Mean/SD	0.953±0.04	0.238±0.29	0.114±0.12	0.121±0.16
	Min-Max	0.890-1.057	0.005-0.925	0.021-0.376	0.012-0.500
2	Mean /SD	0.944±0.03	0.213±0.23	0.103±0.11	0.105±0.11
	Min-Max	0.862-0.995	0.046-0.892	0.011-0.435	0.022-0.450
3	Mean/SD	0.924±0.03	0.164±0.22	0.099±0.10	0.119±0.11
	Min-Max	0.872-0.982	0.015-0.845	0.013-0.419	0.029-0.440
4	Mean /SD	0.928±0.03	0.147±0.23	0.116±0.12	0.104±0.13
	Min-Max	0.872-0.984	0.003-0.882	0.023-0.456	0.008-0.500
5	Mean/SD	0.947±0.04	0.164±0.22	0.096±0.09	0.135±0.13
	Min-Max	0.898-1.055	0.011-0.832	0.023-0.354	0.006-0.502
6	Mean/SD	0.948±0.03	0.188±0.22	0.097±0.11	0.122±0.13
	Min-Max	0.913-1.022	0.005-0.846	0.012-0.435	0.031-0.525
7	Mean/SD	0.986±0.07	0.151±0.22	0.096±0.13	0.125±0.14
	Min-Max	0.900-1.111	0.006-0.861	0.013-0.498	0.022-0.556
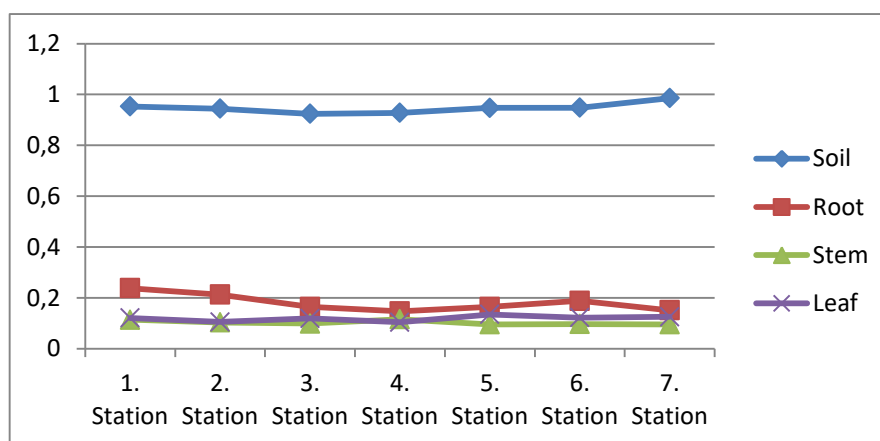
Figure 2. 12-month average Cu concentration values for all stations in *H. portulacoides*
and growth soil (dry weight).

The *S. prostrata* subsp. *prostrata* samples that were collected from the Ayvalık Saltern
were analyzed by dividing them into root, stem and leaf parts. All analysis results
(including soil) are shown in Table 3. According to these results, the Cu concentration
of the *Suaeda* species was found to be in the range of 0.004-0.896 ppm. This rate was
in the range of 0.858-1.111 ppm in the soil. According to the average values of 7
stations, the lowest monthly Cu concentration values were 0.919±0.03 ppm in the soil
(March 2010), 0.067±0.04 ppm in the root (August 2009), 0.034±0.01 ppm in the stem
and 0.064±0.03 ppm in the leaves (July 2009). The highest Cu concentrations were
0.966±0.06 ppm in the soil (June 2009), 0.860±0.01 ppm in the root (June 2009),
0.821±0.02 ppm in the stem (June 2009), and 0.861±0.01 ppm in the leaves (December
2009). According to the 12-month average values of each station, the lowest Cu
concentrations were found as 0.924±0.03 ppm in the soil (station 3), 0.140±0.22 ppm in
the root (station 7), 0.114±0.22 ppm in the stem (station 5) and 0.150±0.27 ppm in the
leaves (station 2). Based on the same values, the highest Cu concentrations were found

as 0.986±0.07 ppm in the soil (station 7), 0.186±0.22 ppm in the root (station 3), 0.139±0.22 ppm in the stem (station 6) and 0.209±0.26 ppm in the leaves (station 5) (Table 3, Figure 3).

Table 3. 12-month average Cu concentration values for all stations in *S. prostrata* subsp. *prostrata* and growth soil (dry weight).

| Station No	Cu ppm	Soil	Root	Stem	Leaf
1	Mean/SD	0.953±0.04	0.158±0.24	0.124±0.23	0.180±0.29
	Min-Max	0.890-1.057	0.011-0.854	0.004-0.872	0.034-0.896
2	Mean /SD	0.944±0.03	0.148±0.22	0.131±0.21	0.150±0.27
	Min-Max	0.862-0.995	0.023-0.861	0.025-0.801	0.030-0.836
3	Mean/SD	0.924±0.03	0.186±0.22	0.120±0.22	0.168±0.28
	Min-Max	0.872-0.982	0.052-0.872	0.009-0.812	0.020-0.856
4	Mean /SD	0.928±0.03	0.180±0.21	0.120±0.21	0.195±0.27
	Min-Max	0.872-0.984	0.050-0.863	0.012-0.798	0.047-0.876
5	Mean/SD	0.947±0.04	0.146±0.22	0.114±0.22	0.209±0.26
	Min-Max	0.898-1.055	0.007-0.850	0.009-0.803	0.021-0.856
6	Mean/SD	0.948±0.03	0.177±0.22	0.139±0.22	0.180±0.27
	Min-Max	0.913-1.022	0.057-0.882	0.015-0.824	0.024-0.846
7	Mean/SD	0.986±0.07	0.140±0.22	0.136±0.22	0.208±0.26
	Min-Max	0.900-1.111	0.022-0.852	0.009-0.828	0.056-0.862
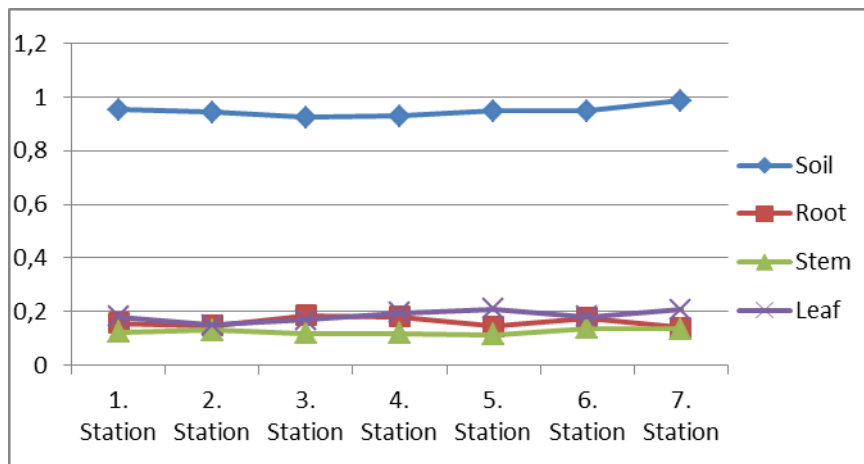
Figure 3. 12-month average Cu concentration values for all stations in *S. prostrata* subsp. *prostrata* and growth soil (dry weight).

The Ayvalık Saltern is located at a 11 km of distance from the district of Ayvalık, 9 km from the town of Altınova and 8 km from the town of Küçükköy where it resides within the borders of the town, and it is not surrounded by any industrial sites. There are a motel and a private hotel owned by the saltern establishment in the east of the saltern, 2 small-sized breeding farms in its north, an olive grove in its west and the İzmir-Çanakkale highway and the Aegean Sea next to it in its south. The İzmir-Çanakkale highway, that passes from the south of the saltern in adjacency, goes on towards the west by an approximately 3-4 km of distance and towards the north next. As a result of the analyses that were carried out on all plants and their growth soils, the Cu concentrations were found to be very low.

In Ref. [16] showed that the toxic limit of Cu in the dry matter of plants was a maximum of 40 ppm. In Ref. [17] reported that exceeding the concentrations of 100 ppm in the soil and 15-30 ppm in the plant dry matter would result in toxic effects. Considering the levels of heavy metals in plant parts in general, it is seen that the highest accumulation was in the leaves. Likewise, studies by ın Ref. [18-21] showed a significant relationship between distance to roads and levels of heavy metals in plant leaves. Separate analysis and interpretation of both plants and their growth soils revealed the ranges of 0.003-0.925 ppm in the plant and 0.862-1.111 ppm in the soil. The accumulation rates of Cu in plant parts was in the order of root>leaves>stem. Considering that In Ref. [22] reported Cu release into the atmosphere to be caused by abrasions and rusting in alloys on brake pads and engine parts, it was a similar finding that the highest Cu concentration was found at the 5th station which is the closest to the İzmir-Çanakkale highway and in the leaves of the plants. Looking at the 12-month average values of each of the stations shown in Table 2 and Figure 2, it may be seen that the highest Cu concentration in the leaves was 0.135±0.13 ppm (station 5), while the lowest value was 0.104±0.13 ppm (station 4).

The Cu concentrations in the *S. prostrata* subsp. *prostrata* plant and its growth soil were much lower than the limit values. These ranges were found to be 0.004-0.896 ppm in the plant and 0.858-1.111 ppm in the soil. The concentration values of Cu in plant parts was in the order of leaves>root>stem. Considering that Novotny and Chesters (1981) reported Cu release into the atmosphere to be caused by abrasions and rusting in alloys on brake pads and engine parts, it was a similar finding that the highest Cu concentration was found at the 5th station which is the closest to the İzmir-Çanakkale highway and in the leaves of the plants. Looking at the 12-month average values of each of the stations shown in Table 3and Figure 3, it may be seen that the highest Cu concentration in the leaves was 0.209±0.26 ppm (station 5), while the lowest value was 0.150±0.27 ppm (station 2). This was supported by the reports of the studies by In Ref. [23,24] that Cu concentrations will drop as the distance to roads increases.

The most important reason for the lower results of all the analyses that were conducted on the plants was that the predominant wind direction in the location of the Ayvalık Saltern is northwest (from the saltern to the highway). Furthermore, absence of any junctions or signalization on the highway that passes from the south of the saltern also prevented the pollution caused by vehicles from increasing. Moreover, important factors also include the fact that olive oil factories that may lead to polluting effects are closer to the district of Ayvalık in general, and there are no other effective industrial facilities that are close to the saltern. It was also found that there was no significant correlation between the soil and root, stem and leaf heavy metal levels and precipitation or wind.

In both plant species, the Cu accumulation increased in relation to the distance to the highway. The concentrations of Cu were higher in *H. portulacoides* than *S. prostrata* subsp. *prostrata* consequently, finding all heavy metal values to be lower than the limit values showed that the Ayvalık Saltern was built at a highly clean location. Considering the significant proportion of using the salt that is produced at the saltern in the food industry and those plants such as *H. portulacoides* that naturally grow here are consumed as food, it is clearly and crucially important to preserve the saltern's non-polluted status. This is why it is believed to be important to prohibit secondary developments and industrialization around the saltern and act very responsibly while

preparing the Environmental Impact Assessment Reports of facilities that are to be built in the area. This study will provide a significant contribution to the literature in terms of being the first heavy metal study that was conducted on the halophytic plants in the region.

Acknowledgements

References

[1]   Merlini, M., Some Considerations on Heavy Metals in the Marine Hydrosphere and Biosphere, **Thallasia Jugoslavica**, 16(2-4), 367-376, (1980).

[2]   Engel, D. W., Sunda, W., Fowler B. A., Factors affecting trace metal uptake and toxicity to estuarine organisms. I. Environmental Parameters, **Biological Monitoring of Marine Pollutants**, 127-144, (1981).

[3]   Zengin, F. K., Munzuroğlu, Ö., Fasulye Fidelerinin (*Phaseolus vulgaris* L. Strike) klorofil ve karotenoid miktarı üzerine bazı ağır metallerin (Ni+2, Co+2, Cr+3, Zn+2) etkileri, **Fırat Üniversitesi Fen ve Mühendislik Bilimleri Dergisi**, 17, 164-172, (2005).

[4]   Seaward, M. R. D., Richardson D. H. S., **Atmospheric sources of metal pollution an effects on vegatation**. In:SHAW A.J. editors. Heavy metaltolerance in plants. 75-92, (1989).

[5]   Nedelkoska, T. V., Doran, P. M., Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining, **Mineral Engineering**, 13, 549-561, (2000).

[6]   Öztürk, M., Türkan, İ., Heavy metal accumulation by plants alongside the motor roads. **Plants as biomonitors**, 640-650, (1993).

[7]   Nuhoğlu, Y., Malkoç, E., Gürses, A., Canpolat, N., Removal of Cu (II)   from aqueous solution by *Ulothrix zonata*. **Bioresource Technology**, 85, 3, 331-333, (2002).

[8]   Motto, H. L., Danies, R. P., Motto C. K., Lead in soils and plants; its relationship so traffic volume and proximity so highways, **Environmental Science & Technology**, 4, 231-237, (1970).

[9]   Zengin, F. K., Munzuroğlu, Ö., Fasulye Fidelerinin (*Phaseolus vulgaris* L.) Kök, Gövde ve Yaprak Büyümesi Üzerine Kurşun (Pb^{++}) ve Bakır (Cu^{++})'ın Etkileri. **Gazi Üniversitesi Fen Bilimleri Dergisi**, 17, 1-10, (2004).

[10]   Phalsson, A. M. B., Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. **Water, Air, & Soil Pollution**, 47, 287-319, (1989).

[11]   Reboreda, R., Caçador, I., Copper, zinc and lead speciation in salt marsh sediments colonised by *Halimione portulacoides* and *Spartina maritima*. **Science Direct, Chemosphere**. 69, 1655-1661, (2007).

[12]   Válega, M., Lillebø, A. I., Caçador, I., Pereira, M. E., Duarte, A. C., Pardal, M. A., Mercury in salt marshes ecosystems: *Halimione portulacoides* as biomonitor. **Science Direct, Chemosphere**, 72, 1224-1229, (2008).

[13]   Almedia, R. M. C., Dias, C. A., Mucha, P. A., Bordalo, A. A., Vasconcelos, S. D. T. M., Study of the influence of different organic pollutants on Cu

accumulation by *Halimione portulacoides*, **Estuarine, Coastal and Shelf Science**, 627-632, (2009).

[14] Kılıç, M., Ay, G., Koçbaş, F., Mungan, F., Karabaş, M., Dikicioğlu, R., Kardaş, T., Ayvalık Tuzlasında *Halimione portulacoides* (L.) Aellen Bitkisi Kullanılarak Nikel Düzeyinin Belirlenmesi, **Anadolu Doğa Bilimleri Dergisi**, 2, 2, 16-23, (2011).

[15] UNEP. Sampling of selected marine organisms and sample preparation for trace metal analysis, **Reference Methods for Marine Pollution Studies**, 7, 2, 15, (1984).

[16] Chang, C. C., Vander Mallie, R. J., Garvey, J. S., A radioimmunoassay for human metallothionein. **Toxicology and Applied Pharmacology**, 55, 94-102, (1980).

[17] Sossé, B. A., Genet, P., Dunand-Vinit, F., Toussaint, L. M., Epron, D., Badot, P.M., Effect of copper on growth in cucumber plants (*Cucumis sativus*) and its relationships with carbonhydrate accumulation and changes in ion contents, **Plant Science,** 166, 1213-1218, (2004).

[18] Tam, N. F. Y., Liu, W. K., Wong, M. H., Wong, Y. S., Heavy metal pollution in roadside urban parks and gardens in Hong Kong, **The Science of The Total Environment**, 59, 325-328, (1987).

[19] Caselles, J., Levels of lead and other metals in *Citrus alongside* a motor road. **Water, Air & Soil Pollution**, 105,(3-4), 593-602, (1998).

[20] Bu-Olayan, A. H., Thomas, B. V., Biomonitoring Studies on The Lead Levels in Mesquite (*Prosopis juliflora* (Sw.) DC.) in The Arid Ecosystem of Kuwait. **Kuwait Journal of Science & Engineering**, 29,1, 65-73, (2002).

[21] Çelik, A., Kartal, A. A., Akdoğan, A., Kaska, Y., Determining the heavy metal pollution in Denizli (Turkey) by using *Robinio pseudo-acacia* L. **Environmental International**, 31,1, 105-112, (2004).

[22] Novotny, V., Chesters, G., **Handbook of nonpoint pollution, sources and management,** Van Nostrand-Reinhold Publishers: New York, (1981).

[23] Ndiokwere, C. L., A study of heavy metal pollution from motor vehicle emissions and its effect on roadside soil, vegetation and crop in Nigeria, **Environmental Pollution Series B, Chemical and Physical**, 7, 1, 35-42, (1984).

[24] Ho, Y. B., Tai, K. M., Elevated Levels of Lead and Other Metals in RoadsideSoil and Grass and Their Use to Monitor Aerial Metal Depositions in Hong Kong, **Environmental Pollution**, 49, 1, 37-51, (1988).