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Original Article

Determination of Heavy Metal Levels in *Echium italicum* L. Plants

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Abstract

Plants and their components have maintained importance in human life as medicinal and aromatic ingredients that have been used by people for many centuries. Nature is the chief source of these valuable resources and humans use these plants directly after collection from their habitat. Therefore, the places where such plants grow have become highly important for understanding the role and behaviour of the accumulation of various elements, especially heavy metals. The aim of the present study was to evaluate the levels of heavy metals in *Echium italicum* L. plants collected from seven different locations in the province of Sivas in Northern Turkey. Heavy metal pollution was determined in the analysis of the plants. A significant difference was observed in the amounts of heavy metals between plants growing in areas with high vehicle traffic and in the plants growing in low traffic areas. This is of great importance for medical and aromatic plants, which are usually collected from areas of natural growth. Therefore, when these plants are collected from the wild, low traffic areas should be selected.

Keywords: *Echium italicum*L; heavy metal; Pb-Cd-Hg; pollution

Introduction

Echium italicum L. is a perennial plant commonly known as Italian bugloss, which belongs to the Boraginaceae family. As a medicinal plant, *E. italicum* L. has attracted great interest as the red pigmented roots have been widely used to treat burns and wounds. Similarly, the essential oil is very popular because of high antimicrobial activity (Morteza-Semnani *et al*., 2009). *E. italicum* L. is found throughout several continents, whereas Australia, Europe, Mediterranean and Asian countries contain a huge diversity of this plant (Rechinger, 1967).

Turkey is considered a diversity hot spot and centre of origin for various crops, mainly due to differences in climatic, geographic and geomorphologic conditions (Karakoy *et al*., 2012, 2014; Baloch *et al*., 2014). A total of 9 *Echium* species have been reported in Turkey (*E. italicum* L., *E. angustifolium* Miller, *E. plantagineum* L., *E. orientale* L., *E. russicum* J.F.Gmel., *E. arenarium* Guss, *E. glomeratum* Poir., *E. vulgare* L., *E. parviflorum* Moench). Of these 9 species, *E. italicum* L. and *E. vulgare* L. are the most widely distributed in Turkey and most commonly used for ethnopharmacological purposes.

Echium mainly grows in Central and South Europe and South-West Asia, while is scattered throughout Turkey (Edmondson, 1978). This plant grows rapidly as a weed in forests and on agricultural land (Yıldırım and Ekin, 2003). At the same time, *Echium* species are cultivated in Europe and England (Berti *et al*., 2007) and the flowers are used as medicine in France. It is used for infectious diseases in traditional medicine of Iran (Mohsen Abolhassani, 2004). It has also been reported to be a urine enhancer, an emollient, an antidepressant and to have positive effects on wound healing and the relief of rheumatism pain (Tabata e*t al*., 1994; Pardo *et al*., 2000; Fujita *et al*., 2013).

The main chemical constituents of the plant are shikonin derivatives, flavonoids, phenolic acids, pyrrolizidine alkaloids and fatty acids (Eruygur, 2012). Fatty acids such as omega-3 and omega-6, which are present in the plant in large quantities, are beneficial for certain types of cancer, heart and skin disorders (Coupland, 2008).

Micronutrients such as Iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), nickel (Ni), molybdenum (Mo) and cobalt (Co) are essential for plant life, whereas heavy metals such as chromium (Cr), cadmium (Cd), mercury (Hg) and lead (Pb) are of no benefit for plants (White, 2012). These heavy metals, even in small concentrations, may result in

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toxic effects on living organisms; pollute the environment by producing exhaust gases, industrial waste, agricultural pesticides and chemical fertilizers. Environmental waste has become an important concern. With the passage of time, these heavy metals accumulate in the soil and have drastic effects on soil fertility, microbial activity, and biodiversity and even on human beings by creating toxic effects on human and animals through the food chain (Robinson, 1997; Tong *et al.*, 2017; Wu *et al.*, 2018; Rodríguez-Bocanegra *et al.*, 2018).

The main objective of the present study was to investigate the levels of various heavy metals in *Echium italicum* L. plants collected from different regions of Turkey. It was also aimed to investigate the effect of the area of plant growth on the heavy metal levels in the plants.

Materials and Methods

The obtaining of plant materials

The study was conducted in Cumhuriyet University Sivas Vocational School and West Mediterranean Agricultural Research Laboratories (Antalya) in 2016.

Plant and soil samples were collected from seven different locations around Sivas (Turkey) (Table 1, Fig. 1). Samples were collected of both the below-ground and above-ground sections of the plant parts.

Concentrations of various heavy metals, primarily cadmium and lead, were then determined.

Soil samples were also taken at a depth of 30 cm from the soil where the plants were growing and the heavy metal levels in these samples were determined.

Table 1. The locations of the plant and soil samples

Locations	Elevation (m)	Longitude	Latitude
L1 (Leblebici)	1,340	38°55'10" K	35°47'35" D
L2 (Gemerek)	1,300	39°13'34" K	36°7'8" D
L3 (Şarkışla 1. Location)	1,200	39°20'41" K	36°23'24" D
L4 (Şarkışla 2. Location)	1,320	39°36'47" K	37°1'25" D
L5 (İşhanı)	1,245	39°40'20" K	37°1'30" D
L6 (CU Campus)	1,270	39°42'35" K	37°2'4" D
L7 (CU Campus-N)	1,250	39°43'25" K	37°2'35" D

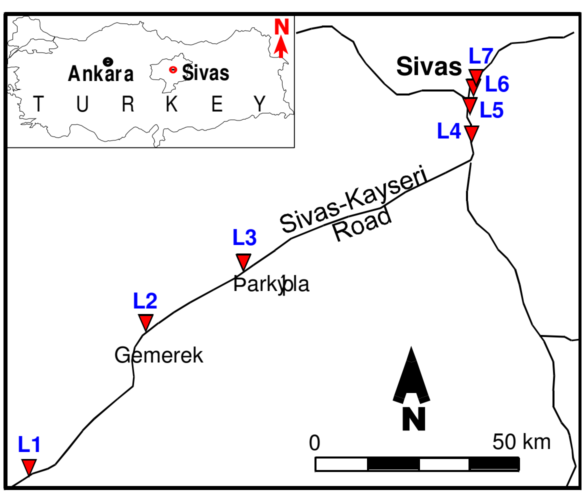

Fig. 1. Locations map

Determination of heavy metal levels in biological samples

For mineral analysis, samples were taken from the mature or fully expanded leaves of the plants. Leaf samples were washed with distilled water three times and then dried in a forced-air oven at 65 °C to a constant weight. Each leaf sample was separately ground in a stainless steel mill and was then passed through a 20-mesh screen and the analytical process was performed as stated by Kaçar and İnal (2008). Using wet-digested samples via ICP (17), concentrations of various elements, including Cd, Co, Ni, Zn, Mn, Pb and Cr in the same solution were determined (Kaçar and İnal 2008).

Results and Discussion

The results of the analysis of various heavy metals in the soil samples collected from 7 different regions of Sivas are shown in Table 2. The maximum and minimum concentrations of these elements, with some relevant properties are given in Table 3. The range of total heavy metal and micronutrients of Cd, Co, Cr, Ni, Pb, Cu and Zn in the soil samples were determined as 0.30-2.50, 14.50-26.50, 122.30-181.30, 149.70-306.90, 12.70-35.40, 20.40-57.60 and 54.60-88.20 ppm respectively (Table 3).

The total concentration of Cd, Pb, Cu and Zn in the soil taken from the *E. italicum* L. growing areas was lower compared to the values reported in the official report of soil pollution (Anonymous, 2005) (Table 2, Table 4). Kloke (1980) recommended a maximum cadmium limit in the soil of 3 ppm, while Alloway (1968) reported an average of 0.06 ppm cadmium with a range of 0.01 - 7 ppm. Therefore, it can be assumed that *E. italicum* L. plays an important role in lowering Cd concentrations and there are no Cd pollution problems where *E. italicum* L. plants are grown. According to Alloway (1990), 54-58% of soil Cd contamination is due to the application of phosphorus fertilizers, 39-47% is from the atmosphere and 2-5% is due to sewage waste. Moen *et al.* (1986) reported that Cd is present in the range of 0.01 to 1 ppm in uncontaminated soil. The limit values of Cd vary according to the countries, with levels of 0.5 in Denmark, Finland and Sweden, 2 in France, 1.5 in Germany, 1 in Spain, 3 in the UK and 20 ppm in the USA. Haktanir *et al.* (1995) reported that the concentration of Pb is higher near traffic areas and lower in the soil away from traffic areas, with reported levels of 120 ppm Pb in soil near to traffic and 25 ppm Pb in soil away from traffic and in uncontaminated areas. Horvath (1995) reported that soil Pb content can reach up to 1000 ppm in areas under intense pollution from industrial, urban and traffic-related activity. Bergmann (1993) and Alloway (1990) stated that the Pb content of uncontaminated soil was in the range of 1-20 ppm and 2-300 ppm respectively in normal conditions. El-Bassam and Tğetjen (1977) and Kloke (1980) emphasized that these values are 100 ppm. Taking the findings of earlier reports into consideration, it can be said that the soil where *E. italicum* L. is grown is not exposed to Pb pollution. The main reasons for Cu contamination are industrial activities or agricultural spraying; Schwertmann and Huit (1975) and Alloway (1990) reported that the total Cu content of the soil can range from 2 to 250 ppm. Other reports (El-Bassam and Tğetjen, 1977; Kloke, 1980; Kabata-Pendias and Pendias, 1992) have emphasised that a Zn level of 300 ppm is toxic for plants. In comparison with these ranges for both elements, no pollution or toxicity of these elements was determined in the studied soils due to the presence of *E. italicum* L.

Table 2. Results of heavy metal analysis of soil samples (ppm)

No	Cd	Co	Cr	Ni	Pb	Cu	Zn	Mn
L1	0.3	22.3	134.3	264.7	12.7	20.4	54.6	844.1
L2	1.3	26.5	122.3	191.0	24.9	40.4	81.1	1037.3
L3	0.6	25.3	144.5	255.5	21.3	39.9	73.7	1050.1
L4	0.8	25.3	163.2	254.8	20.2	39.8	74.1	1073.8
L5	0.7	22.6	163.0	265.1	16.4	32,2	58.4	1045.3
L6	2.5	14.5	142.9	149.7	35.4	30.5	83.1	665.7
L7	0.8	26.1	181.3	306.9	25.1	57.6	88.2	967.3

Table 3. The descriptive statistics of heavy metal content of soils (n = 7)

Parameters	Cd	Co	Cr	Ni	Pb	Cu	Zn	Mn
Minimum	0.30	14.50	122.30	149.70	12.70	20.40	54.60	665.70
Maximum	2.50	26.50	181.30	306.90	35.40	57.60	88.20	1073.80
Average	1.00	23.23	150.21	241.10	22.29	37.26	73.31	954.80
Stickiness	3.60	3.79	-0.61	0.34	1.11	1.42	-1,06	1.62
Distortion	1.80	-1.88	0.23	-0.91	0.72	0.50	-0.60	-1.51
Hydrangea	0.80	25.30	144.50	255.50	21.30	39.80	74.10	1037.30
StdS	0.73	4.18	20.07	52.79	7.29	11.49	12.59	149.57
Variance	0.53	17.50	402.84	2786.94	53.10	132.09	158.56	22371.52
DK	72.57	18.01	13.36	21.90	32.70	30.85	17.18	15.67

Table 4. Heavy metal limit values in the ground (Moen *et al.*, 1986)

Heavy Metal (Total)	pH 5 - 6 (ppm)	pH > 6 (ppm)
Pb	50	300
Cd	1	3
Cr	100	100
Cu	50	140
Ni	30	75
Zn	150	300
Co	-	20

The total concentrations of Co, Cr and Ni in all of the surveyed soils (except one sample for Co) were above the limit value officially reported (Anonymous, 2005) (Table 4). A possible reason for the excess of these elements could be the soil parental material. Smith (1990) identified Co concentrations in the range of 40-200 ppm, 10-20 ppm and 1-3 ppm in Scottish soils from serpentine, andesite and granite deposits, respectively. Bourrelier and Bertelin (1998) stated that the Cr contents of soils that formed on serpentine generally vary between 0 and 200 ppm. Dixon (1989) concluded that soil originated from serpentine parental rock contains higher concentrations of Cr and Ni.

Mengel (1991) reported soil Co concentrations in a range of 0.2-31 ppm, while Alloway (1990) reported the total Cr limit in soil in the range of 5-1500 ppm and Kabata-Pendias (1992) stated that the average Ni content of the earth's soil is 2.2 ppm. Different investigators have accepted 100 ppm as the limit for total Cr concentration in the soil (El-Bassam ve Tğetjen, 1977; Kloke, 1980). The limit value of total Ni for toxicity in soil has been reported as 100 ppm by El-Bassam ve Tğetjen (1977) and Linzon (1978) and 35 ppm by Goncharuk and Sidorenka (1986).

Heavy metal ratio of Echium italicum L.plants

Analysis of various heavy metals in soil samples collected from various soils where *E. italicum* L. was found are presented in Table 5.

Normally, heavy metals accumulating in soil are present in stable form, adsorbing on clay minerals, or forming organo-mineral compounds with organic mineral in the soil or near-surface depths (Tok, 1997). Cd (cadmium), which is toxic to plants, animals and humans even at a very low concentration, is one of the most important heavy metals to be noted. Cd accumulation causes the inhibition of photosynthesis, lowering the transpiration rate through the stomatal closure, restriction of chlorophyll synthesis and degradation of total chlorophyll in plants (Bergmann, 1992; Zincircioğlu, 2013). During the present study, Cd was determined in a range of 1.9 to 2.1 ppm. However, a minimum concentration of Cd content was found in plants collected from L5 (İşhanı) and there was little difference in the Cd content of the different plant parts. Cutler and Rains (1974) reported that the amount of cadmium decreased from the root to the top, while Soon (1998) found Cd in the range of 20-100 µg/kg in plant tissues.

Pb (lead) is one of the heavy metals that cause environmental pollution and which is quite harmful to human health. Tetra ethyl lead is the main source of Pb which is produced from the gasoline used in motor vehicles. Normally lead accumulates in the blood or soft tissues, resulting in toxicity in the human body. Lead toxicity or poisoning in humans may result in brain damage or even death. Infants and children are very sensitive to pollution and chronic poisoning of Cd may lead to mental retardation, learning disorders and hyperactivity, blood pressure elevation and chronic anemia (Vural, 1993; Çağlarırmak ve Hepçimen, 2010). Yoon *et al*. (2006) found 2-1183 mg/kg Cd concentrations in the plants growing in an area of maximum heavy metal pollution. Başar and Aydinalp (2005) reported that the concentrations of lead determined in the peach leaf and fruit differed.

In the hereby study, the lowest lead content was determined in the plant roots (93.7 ppm) collected from L4 (Şarkışla 2. location), while the maximum concentration of lead was obtained from the roots of plants collected from L7 (CU Campus-N) (525 ppm). There was also a difference between the leaves and roots of the plants in respect of lead concentrations.

Metals such as Cu (copper) and Zn (zinc), which are essential for both plants and humans, are naturally present in nature, although higher concentrations result in harmful effects. In plants, Zn regulates metabolic events and plays a role in enzyme activity (Allan, 1997; Okcu *et al*., 2009).

Kaçar and Katkat (2006) showed that zinc levels in tealeaves varied between 24-60 mg/kg during three different periods of shoot growth. Different plants have different abilities of obtaining Zn from the soil. According to different researchers, the plant root structure is the main factor in Zn availability in plants. Zinc accumulates in different proportions in various parts of the plant, and in the current study, the amount of zinc in the roots and leaves was different, with the highest zinc value determined at the L1 (Leblebici) gateway (80.6 ppm).

Co varied among the different organs of the plants and was determined in the range of 2.3-8. The lowest amount of Co was also determined within the plants in the campus area (2.3 ppm) where the vehicle traffic was low. The highest concentration (8 ppm) of Co was detected in the plants collected off campus where there was heavy vehicle traffic.

When the plant samples collected from the different locations were analysed for Cr concentration, the lowest Cr level (5.6 ppm) was found in the first region of the Şarkışla district and the highest amount (25.4 ppm) was present in the region of the L1 gateway. When the Cr content of the root and leaves of the plant were compared, it was seen that there was a difference between these values. The highest content of Ni was detected in the samples collected off campus (36.3 ppm), while the lowest Ni content (5.6 ppm) was found in the samples from the first region of the Şarkışla district. It has been reported that in plants, Co is generally present in a range of 0.1 to 10 ppm (Bakkaus *et al*., 2005), while Cr exists in trace amounts varying between 3 and 100 µg /mg (Welch and Cary, 1975).

Medicinal and aromatic plants have many important essential and beneficial elements. However, as plants may be contaminated with heavy metals from absorption, serious health problems may arise in people who consume these plants. Therefore, the determination of mineral nutrient and heavy metal accumulation is significant (Ozyigit *et al*., 2017).

According to the results of the analyses in the present survey, while the minimum concentration of Cd content was in plants collected from L5 (İşhanı) (1.9 ppm), there was little difference in the Cd content of the different plant parts. The lowest Pb content was determined in the plant roots (93.7 ppm) collected from L4, while the maximum concentration of lead was obtained from the roots of plants collected from L7 (525 ppm). It was determined that the areas where the plants were growing had an impact in terms of the elements in the plant content.

Table 5. Heavy metal ratios in the leaves and roots of *Echium italicum* plant (ppm)

Location No	Plant parts	Cd	Co	Cr	Ni	Pb	Zn	Mn
L1 (Leblebici)	Leaf	2.1±0.03	3.7±0.02	19.3±0.03	22.3±0.05	179±1.2	37.4±0.03	44.8±0.3
	Root	2.1±0.03	6.5±0.02	25.4±0.09	31.27±0.06	449±0.6	80.6±0,03	114±0.5
L2 (Gemerek)	Leaf	2.1±0.02	3±0.02	16.9±0.03	21.4±0.1	145±3.5	35.2±0.07	70.7±2.5
	Root	2.0±0.02	4.8±0.02	10.9±0.02	13.6±0.07	95±3	22.4±0.05	24.4±0.5
L3 (Şarkışla 1. Location)	Leaf	2±0.02	4.4±0.04	11.7±0.03	13.4±0.03	77.3±2.5	27.6±0.1	44.1±0.1
	Root	2±0.02	3.9±0.03	5.6±0.006	5.6±0.01	112.3±2.5	12.4±0.08	11.3±0.06
L4 (Şarkışla 2. Location)	Leaf	1.93±0.03	2.9±0.02	12±0.02	23.1±0.04	147±1.2	28.8±0.1	28.5±0.5
	Root	2.03±0.04	3,7±0.02	7.5±0.04	14.3±0.02	93.7±0.6	11.8±0.03	3.2±0.1
L5 (İşhanı)	Leaf	1.9±0.01	4.41±0.03	2.3±0.03	6.6±0.05	61±1	17.2±0.03	26.3±0.3
	Root	1.9±0.02	2.3±0.02	8.5±0.02	14.04±0.05	182±2	16.4±0.03	68.3±1.1
L6 (CU Campus)	Leaf	2.1±0.02	3.9±0.02	4.5±0.005	10.3±0.04	30,±0.6	22.4±0.05	20.1±0.1
	Root	2±0.006	6.9±0.04	16.1±0.1	23.9±0.03	455±0.6	34±0.06	70.6±0.3
L7 (CU Campus-N)	Leaf	2.05±0.03	5.8±0.02	8.3±0.03	11.4±0.01	169±1	16.7±0.1	26.3±0.3
	Root	2.03±0.01	8±0.02	23±0.02	36.3±0.2	525±0.6	31.5±5.9	73.9±0.4

Conclusions

The present study was conducted within the borders of the province of Sivas, along a Southwest-Northeast line. Samples of *Echium italicum* L. plants were collected from 7 separate locations defined in this area and laboratory analyses were applied to determine the concentrations of the elements of Cd, Co, Cr, Ni, Pb, Cu and Zn. Heavy metal pollution was determined in the analyses and higher values of these elements were obtained in samples from areas where there was heavy traffic. These locations were seen to be L4, L5 and L6 which were closer to the city centre. Therefore, it can be clearly understood that medicinal and aromatic plants should be collected from natural areas with little or no motor vehicle traffic for there to be no risk of heavy metal pollution.

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