Anatolian Journal of Botany
3(2): 44-50 (2019)
Research article *doi:10.30616/ajb.583148*

Investigation of heavy metal accumulation and biomonitoring of *Calepina irregularis* species growing in Amasya (Turkey) province

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Received : 27.06.2019
Accepted : 28.07.2019
Online : 01.08.2019

Amasya'da yetişen *Calepina irregularis* türünde ağır metal birikimi ve biyomonitör olarak kullanılabilirliğinin araştırılması

Abstract: In this study, heavy metal accumulation (Ni, Fe, Co, Mn) in *Calepina irregularis* (Asso) Thell. (Brassicaceae), growing naturally in Amasya province, and the usability of it as abiomonitor was investigated. The amount of heavy metals in the root, stem and leaves of plants which were collected from the city center, near the highway, suburban and non-traffic (control) localities, were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) and the obtained data were evaluated. The Ni, Fe, Co and Mn values in compete plants, growing in traffic areas, were found between the ranges 14.32-35.66 $mgkg^{-1}$, 827.61- 2716.72 $mgkg^{-1}$, 12.52-16.51 $mgkg^{-1}$ and 175.93-826.75 $mgkg^{-1}$ respectively. The amount of element accumulation in the plant was listed as Fe>Mn>Ni>Co. Ni and Mn were found to be higher in plants growing near the highway while Fe and Co were higher in plants collected from city centre. Heavy metal accumulation was higher in leaves and roots of the plants growing around the highways while it was higher in stems of the plants growing in suburban areas. According to the correlation with plant and soil samples taken from the localities, the relationship between soil and plant, Fe and Mn contents was found significant at P<0.01 level. This shows that the plant receives Ni and Co elements due to air pollution, and that Fe and Mn are mostly taken from the soil through its roots. According to the results of the study, *C.irregularis* can be used as a biomonitor since it can monitor the short term changes in environmental pollution in urban areas due to its wide distribution area and it has several individuals in its habitat and its conformity with standard analysis methods.

Key words: *Calepina irregularis*, heavy metal, biomonitor, Amasya, Turkey

Özet: Bu çalışmada, Amasya ilinde doğal olarak yetişen *Calepina irregularis* (Asso) Thell. (Brassicaceae) türünde ağır metal birikimi (Ni, Fe, Co, Mn) ve biyomonitör olarak kullanılabilirliği araştırılmıştır. Şehiriçi, otoyol kenarı, kenar semt ve trafiğin olmadığı alanlardan toplanan bitki örneklerinin; kök, gövde ve yapraklarında ağır metal miktarları İndüktif Eşleşmiş Plazma-Optik Emisyon Spektrometresi (ICP-OES) ile belirlenmiş ve elde edilen veriler değerlendirilmiştir. Trafik bulunan alanlarda yetişen bitkilerin toplam kütlesindeki Ni, Fe, Co ve Mn değerleri sırasıyla 14.32-35.66 $mgkg^{-1}$, 827.61-2716.72 $mgkg^{-1}$, 12.52-16.51 $mgkg^{-1}$ and 175.93-826.75 $mgkg^{-1}$ aralığında bulunmuştur. Bitkide element biriktirme miktarı Fe>Mn>Ni>Co şeklinde sıralanmıştır. Ni ve Mn elementi yol kenarında yetişen bireylerde, Fe ve Co ise şehir içinde toplanan örneklerde yüksek değerde tespit edildi. Yol kenarında yetişen bitki örneklerinde yaprak ve kökte ağır metal birikimi daha fazla olurken, kenar semtte yetişen bitkilerde ise gövde de birikim daha fazla bulunmuştur. Lokalitelerden alınan bitki ve toprak örnekleri ile yapılan korelasyona göre toprak ve bitki Fe ve Mn içerikleri arasındaki ilişki P<0.01 düzeyinde anlamlı bulunmuştur. Bu da bitkinin Ni ve Co elementlerini hava kirliliği kaynaklı aldığını, Fe ve Mn'yi daha çok kökleri yoluyla topraktan aldığını ortaya koymaktadır. Çalışmanın sonuçlarına göre *C. irregularis* türünün yayılış alanının geniş olması ve habitatında birey sayısı fazla olması, standart analiz metotlarına uygun olması nedeni ile kentsel alanlarda çevresel kirlilikteki kısa vadeli değişiklikleri izleyebildiği için biyomonitör olarak kullanılabilir.

Anahtar Kelimeler: *Calepina irregularis*, ağır metal, biyomonitör, Amasya, Türkiye

1. Introduction

Increasing industrial and traffic intensity in recent years has led to an increase in heavy metal pollution in ecosystems. The increase in the concentration of heavy metals in the atmosphere, water and soil above a certain level causes serious problems for all living things and leads to deterioration of soil quality, reduction of biological production and harm to the health of living things (Blaylock and Huang, 2000). Heavy metals are classified as necessary and unnecessary for life according to their degree of impact on biological processes. Those required for life must be present in the organism at a certain rate, even they are toxic at high concentrations (Kahvecioğlu et al., 2003; Hamutoğlu et al., 2012).

The main sources of air and soil pollution are the fuels used for heating in residantial, industrial activities and especially transportation vehicles (Beckett et al., 1998; Yeşilyurt and Akcan, 2001). Urban areas are considered to be the main sources of pollutants due to the presence of high concentrations of pollutant spreading activities (Wiseman et al., 2001; Markert et al., 2003; Galal and Shehata, 2015). Vehicle traffic emissions are of concern, because they are made up of gaseous pollutants (Laschober et al., 2004) that may remain in the air for a while, most are deposited on roadside soils and plant materials near the road. For this reason, the toxicity and tolerance of metal in plants has been an issue for more than thirty years (Das et al., 1997; Clemens, 2001; Mertens et al., 2005). Increasing environmental pollution has led to the development of several methods for the determination of pollution and taking measures. One of them is the use of biomonitoring organisms that do not harm the environment and are cheaper than other physical and chemical methods (Marinho et al., 2018; Sevik et al., 2019).

The organisms used to obtain certain characteristics and information of the biosphere are called "bioindicator" or "biomonitor" (Markert, 1993). In order to use a species as a biomonitor, it is necessary for it to be represented in large numbers in the collection area, to have a wide distribution, to be collected from the same area throughout the year, and it should be easy to exemplify and should not have an identity problem (Aksoy et al., 1999; Conti and Cecchetti, 2001). First noticeable things in the detection of heavy metal pollution are lichens, fungi, trees and tree shells (Lopes et al., 2019; Aricak et al., 2019).

In later studies, it is used as a biomonitor to detect instant changes in herbaceous plants. For example, *Taraxacum officinalis* is a common herbaceous weed that is frequently used as a bio-monitor of environmental pollution and used in different countries (Balasooriya et al., 2009). *Calepina irregularis*, which grows in large populations in intensive traffic areas in Amasya province, was chosen as research material. Rapid growth capability without being harmed by the traffic originated pollutants, widespread distribution, and the development of root and green parts at sufficient amounts, were the major consideration criteria while species selection.

2. Materials and Method

Sampling area: Amasya province is located in Black Sea region of Turkey, between 41°04′54″ - 40°16′16″ northern latitudes and 34°57′06″ - 36°31′53″ eastern longitudes. Major causes of air pollution in the province of Amasya can be listed as fuels used in heating, exhaust gas emissions from motor vehicles and industrial emissions. Insufficient air currents due to the mountainous structrure and the heavy traffic in city roads because of the absence of a ring road or a freeway also play role in pollution in Amasya province.

For plant sampling, 20 different stations were determined in 4 different localities (5 at each stations) (Fig. 1). The coordinates of each point were determined and recorded by GPS (Global Positioning System). Plant samples were collected in August 2015. The mean values were used for the data obtained from the samples for each station. Daily vehicle densities of the selected localities are as follows: City center (16201), near the highway (11291), suburban (3-5 vehicles per minute), control (non-traffic) (Fig. 1). The distribution areas of the species are the parks, gardens and vacant areas in the city, the field edges near the highway and the suburb and the glades in the control group.

Morphological characteristics of *Calepina irregularis* (Asso) Thell.: The species to be used as a biomonitor should have some properties. It should have a widespread distribution and large individual density, be easy to grow and able to accumulate heavy metals, be resistant to herbal medicines and diseases, complies with the standard methods of analysis and should have very low genetic variations (Aksoy and Ozturk, 1996, 1997).

Calepina irregularis (Brassicaceae) provides many of these criteria. This plant is widespread in the fields, at streamsides, slopes and especially in heavy traffic areas. *Calepina irregularis* is an annual plant with a length of 15-70 cm. It blooms from May to June. Flowers are small, 2-4 mm, white, thin, calyx are upright and 4 pieces. The fruit is in the form of a small cluster and is full of seeds.

Collection and analysis of plant samples : Plants were collected by plastic gloves and some of them were prepared as herbarium samples. Plant samples were washed twice with tap water and then with distilled water. As a result of the washing process, the plant samples were divided into three parts as root, stem and leaf and kept in the oven at 70 °C for 24 hours. After drying, the samples were ground. Samples for analysis were added with 10 ml of concentrated nitric acid (HNO_3) and 2 ml of hydrogen peroxide (H_2O_2) and burned. The amounts of heavy metal accumulated in soil and plant organs were determined as three replicates (ICP-OES) and the obtained data were evaluated (Çayır and Coşkun, 2007). All data were analyzed using SPSS (18.00) statistical package program.

Figure 1. Sampling areas on the map

3. Results and Discussions

Heavy metal values for *C. irregularis* in traffic areas were measured as 14.32-35.66 $mgkg^{-1}$ for Ni, 827.61- 2716.72 $mgkg^{-1}$ for Fe, 12.52-16.51 $mgkg^{-1}$ for Co, and 175.93-826.75 $mgkg^{-1}$ for Mn were found (Fig. 2). The amount of element accumulation in the plant is ordered as Fe>Mn>Ni>Co. Ni and Mn were found to be high in the plants growing around the highway and Fe and Co were found to be high in the samples collected from the city centre.

In this study; nickel (Ni^{+2}) values were determined as 2.902-3.12 $mgkg^{-1}$ in the soil and 14.32-35.66 $mgkg^{-1}$ in the total plant (Table 1).

While permissible boundary nickel values in the soil are 35 $mgkg^{-1}$ according to the WHO standard, nickel values in the plant are 10 $mgkg^{-1}$ (FAO/WHO, 2003). In our study, the Ni value in the soil was found below the limit values in all localities. No differences were found in the soil between the localities in terms of Ni ($p \geq 0.05$) (Fig. 2). It was found that Ni accumulation in the leaves of the plants collected from city centre is more than other localities. It was determined that there was a decrease in Ni values as we go farther from traffic density and there

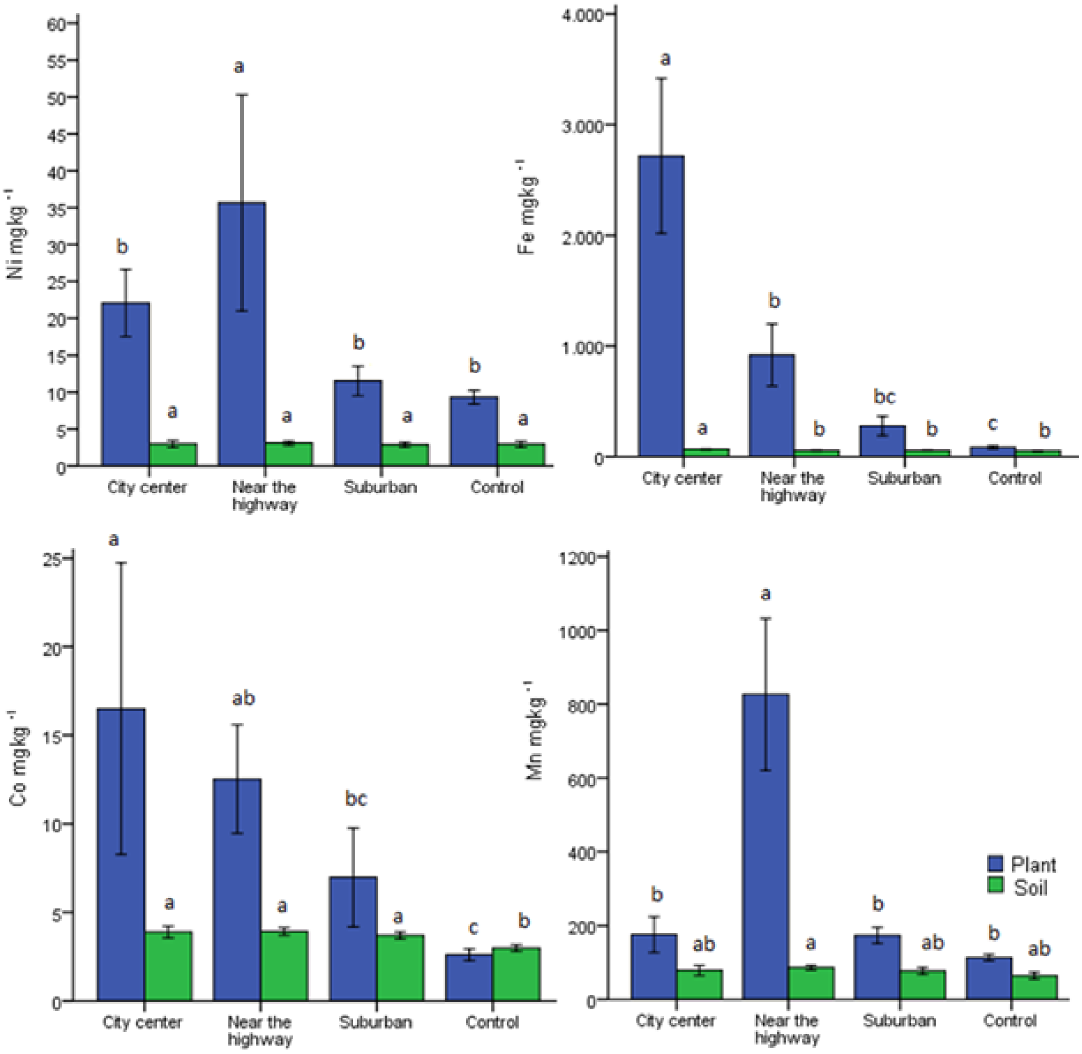

Figure 2. Mean heavy metal contents of *C. irregularis* and soil and Tukey's HSD test (Means followed by the same letter are not significantly different at the 0.05 level)

was a significant relationship between the data and localities at the level of $p \leq 0.05$. As the traffic density increased, the proportion of Ni accumulation also increased and values above the WHO limits were found. Ni contamination is mostly applied to the soil with waste water from the metal processing and coating industry. As a result of a study carried out in Muğla province, with *Pyracantha coccinea* Roem, Ni accumulation was found to be highest in the industrial zone (14.34 ± 1.59 μgg^{-1}) while least in the urban area (4.05 ± 0.51 μgg^{-1}) (Akgüç et al., 2010). Osma et al. (2012) found the Ni amount as 3.06-13.65 μgg^{-1} in *Brassica oleracea* L. var. *acephala*. These studies also support the high accumulation possibility of Ni in industrial zones. Actually Ni is a component of the urease enzyme responsible for the hydrolysis of urea nitrogen (Gerendás et al., 1999; Barcelos et al., 2018). A large part of the nickel nutrient taken from the Ni element is excreted with feces without being absorbed by intestines, some of them accumulate in tissues such as the lung, intestine and skin, and nickel has been reported to have carcinogenic effects especially in children (Qing et al., 2015; Vural, 1993).

Iron is an essential elements for all cells and is an important component of hemoglobin molecule. In plants, it plays an important role in respiratory and photosynthetic reactions in the form of Fe2+, Fe3+. Iron activates enzymes such as catalase, peroxidase and cytochrome oxidase in plants, and catalyzes many biochemical reactions. Though chlorophyll molecules don't contain iron, chlorophyll production decreases in the case of iron deficiency (Bolat and Kara, 2017). Fe pollution is caused by factors such as flue gases and heavy traffic. The normal limits for Fe concentration in plants are reported to be 2-250 μgg^{-1} (Kabata-Pendias, 2000). In our study, Fe value was found to be 54.62-67.89 $mgkg^{-1}$ in the soil and 827.61-2716.72 $mgkg^{-1}$ in plant samples (Table 1). The limit value is 30 $mgkg^{-1}$ according to the FAO/WHO (2003) standards, The reported permissible iron (Fe^{+2}) limit values for soil is 50 $mgkg^{-1}$ and 50-150 $mgkg^{-1}$ for plants (Yücel, 2010; Fergusson, 1990). Our Fe findings in soil and plant samples are above the limit values. As the traffic density increases, Fe pollution also increases, and a statistically significant relation at the level of $p \leq 0.05$, was determined between the data obtained from the localities, plant organs and the soil. Yıldırım et al. (2012) found the Fe values for *Elaeagnus angustifolia* L. as 26.37 ± 2.89 μgg^{-1} and for *Pinus brutia* Ten. as 67.22 ± 11.34 μgg^{-1} in Amasya. In this study, Fe was found within normal limits. In a study performed with *Tradescantia pallida* (Rose) Hunt., Fe was determined as 39.3 $mgkg^{-1}$ and Mn was determined as 16.27 $mgkg^{-1}$ (Santos et al., 2015).

Table 1. Heavy metal averages in plant organs and soil in studied localities (mgkg^{-1})

Locality	Plant Part	N	Ni Mean	Fe Mean	Co Mean	Mn Mean
City Center	Leaf	15	3.68±0.18*	686.00±6.23	4.31±0.34	45.81±0.40
	Stem	15	13.01±0.93	1073.20±4.03	5.85±0.87	68.25±0.29
	Root	15	5.40±0.23	872.12±8.71	6.34±0.51	61.87±0.53
	Total Plant	15	22.09±0.82	2716.72±13.18	16.51±1.49	2.97±0.74
	Soil	15	3.003±0.85	67.89±9.13	3.89±0.60	78.57±24.44
Near the Highway	Leaf	15	13.88±1.56	501.41±18.44	3.65±0.38	379.66±2.22
	Stem	15	3.18±0.11	497.20±0.00	0.93±0.08	47.46±0.32
	Root	15	18.60±2.28	605.42±46.17	7.94±0.43	399.63±3.59
	Total Plant	15	35.66±2.64	1603.63±50.33	12.52±0.55	826.75±3.72
	Soil	15	3.12±0.55	54.62±5.24	3.92±0.39	86.14±11.28
Suburban	Leaf	15	4.83±0.35	119.75±5.23	2.59±0.35	73.70±0.28
	Stem	15	5.99±0.42	478.71±40.65	8.01±0.52	137.39±1.29
	Root	15	3.50±0.20	229.15±10.21	3.46±0.31	52.61±0.13
	Total Plant	15	14.32±0.36	827.61±16.19	14.06±0.50	263.7±0.39
	Soil	15	2.90±0.59	55.19±5.37	3.69±0.33	77.3±16.024
Control	Leaf	15	6.22±0.13	22.83±1.63	1.61±0.03	73.18±0.10
	Stem	15	2.16±0.16	51.68±5.46	0.52±0.04	27.94±0.18
	Root	15	0.94±0.06	10.99±2.41	0.48±0.04	12.22±0.05
	Total Plant	15	9.32±0.17	85.50±27.21	2.61±0.06	113.35±0.16
	Soil	15	175.93±0.87	50.972±4.02	2.99±0.34	64.43±15.99

* SD : Standart deviation

Heavy metal accumulation was found to be higher in the stems and roots of the plants collected from the city centre. But it was found to be higher in the leaves and roots of the plant samples collected around the highway. On the other hand it was higher in the stems of plants collected from the suburbs (Table 1). The reason for this difference may be the difference in heavy metal accumulation capacity of species.

In a study carried out by Hüseyinova et al. (2009) in Ordu province, the Fe value for the herbaceous species were reported as 188.9-519.9 mgkg^{-1}. Çubukçu and Tüysüz (2007) determined the Fe values above the normal range in the studies carried out in on soils from KBİ Izabe, Tügsaş and Organized Industrial Zones of Samsun and in a group of plants, including cabbage (*Brassica oleracea* L.).

Cobalt, which is classified as a micro element in plants, is one of the most common elements on earth. Cobalt is used in industry, especially in paint and glass industry. Cobalt is a metal component of coenzyme cobalamin with vitamin B12, which has an important function in nitrogen fixation by legume plants (Kaçar, 1995). In the case of cobalt deficiency, decreasing in nitrogen binding, decreasing in leaves, decreasing in chlorophyll content and decreasing in seed weight are observed. As a result of inhalation of cobalt in the air and contact of skin with cobalt salts, cobalt poisoning occurs and there is a risk of being carcinogenic material although there is no research on effects of cobalt on cancer yet (Kahvecioğlu et al., 2003). According to Carrigan and Erwin (1951), the total Co content of soils is 1-40 mgkg^{-1}, and the extractable Co content is between 0.03 and 0.09 mgkg^{-1}. According to the researchers, the toxicity limit value of extractable Co was determined as 0.09 mgkg^{-1} in agricultural soils. In accordance with the relevant regulation, the limit values of Co for soils in our country could be 40 mgkg^{-1} (Tok, 1997). In this study, cobalt was determined as 3.69-3.96 mgkg^{-1} in soil and 16.51-14.06 mgkg^{-1} in the plant. The values obtained in the soil and the plant were below the limit value (Table 1).

Plants can take Mn^{+2} ion by their roots and leaves. It has been reported that the soils mostly contained Mn at the levels of 200 and 300 mgkg^{-1} (Kacar, 1995). Mn pollution is caused by factors such as industrial activities, fossil fuels and pesticides. The limit values of Mn in the plant and soil were determined as 100 mgkg^{-1} (Alvarenga et al., 2006). Toxic values were reported to be between 300-500 µgg^{-1}. In our study, Mn amonut in plant samples were 263.70-826.75 mgkg^{-1} and were above the toxic limit while it was 77.33-86.14 mgkg^{-1} in soil and were below the toxic limit. In a study performed with *Tradescantia pallida*, Mn was found to be 16.27 mgkg^{-1} (Santos et al., 2015).

According to correlation with plant and soil samples taken from localities, the relationship between soil and plant Fe and Mn contents was found to be significant at $P<0.01$ level. This shows that Ni and Co intake of plants depends on air pollution while Fe and Mn mostly were taken from soil through their roots (Fig. 3). Plants accumulate heavy metal by their roots. This is because most heavy metals exist in the soil system and are mostly absorbed by plants through the root system. Besides the roots, plants can also absorb heavy metals from leaves, fruits and flowers

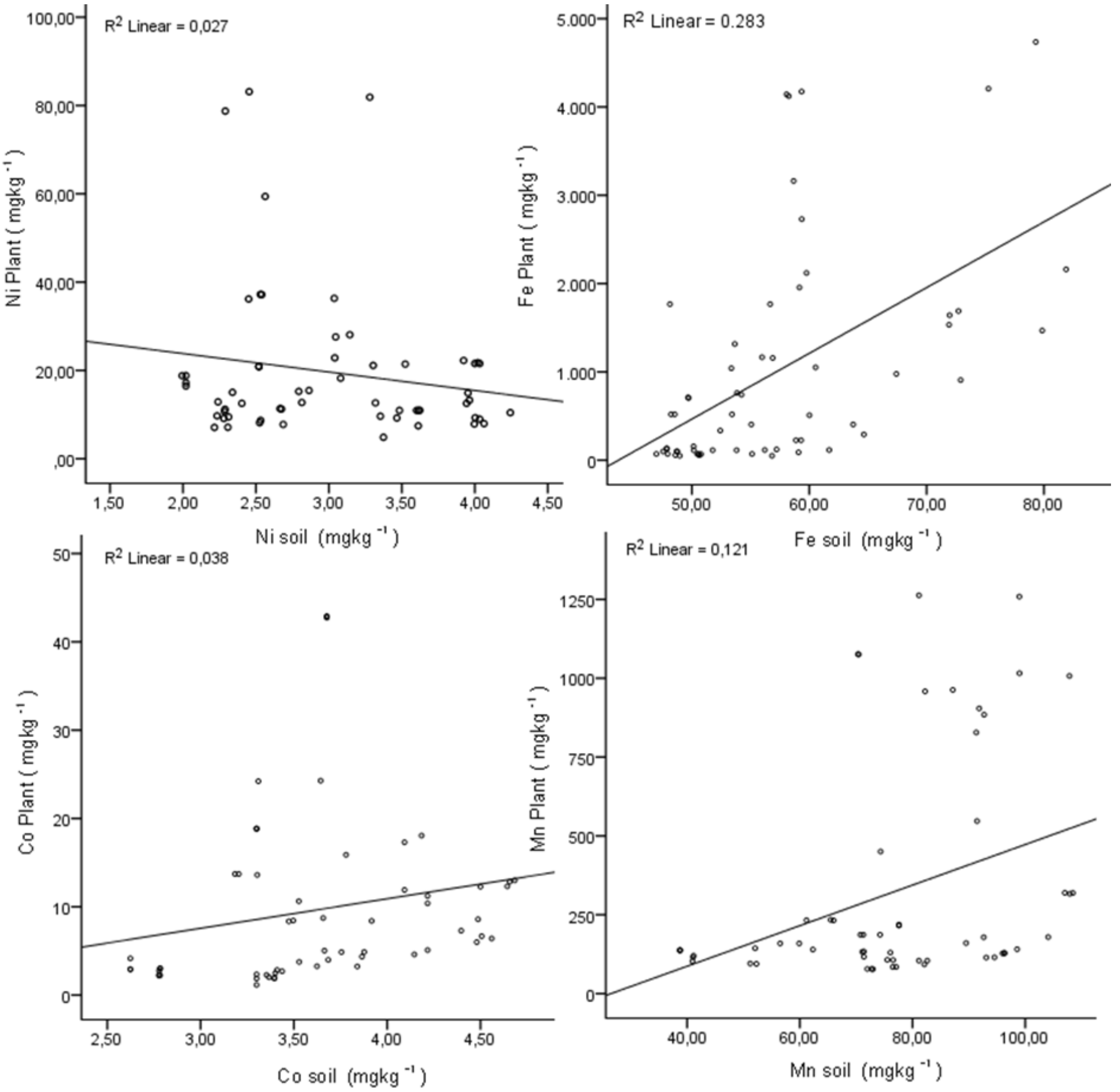

Figure 3. Correlations between the metal levels in the plants and soils of *C. irregularis*

(Bondada et al., 2004; Sevik et al., 2019). Heavy metal accumulation was found to be different depending on the organs. In this study, different amount of accumulation in different plant organs were also changed depending on the locality and the pollution level.

For example, Ni accumulation was determined to be high in stem while it was low in stem and leaves. On the other hand determined Co value was higher in the roots of the plant samples collected from the city centre and near the highway.

Heavy metal accumulation was found to be high in roots and leaves of the plant samples spreading along the highway while it was high in stems of the plants collected from suburban arears (Table 1). Reported literature data indicate that heavy metal accumulation may also vary depending on plant organelles (Emamverdian et al., 2015; Dimitrijević et al., 2016; Tošić et al., 2016; Shahid et al., 2017; Sevik et al., 2019).

Heavy metal contents vary considerably depending on plant species, plant organs and sampling locality. Except soil Ni content, the relations between these parameters were found to be significant (Table 2). According to the results Ni, Fe, Co and Mn concentrations in the plant increased depending on traffic density. It is known that, industry and traffic density are the main sources of heavy metal pollution (Uzu et al., 2011; Martley et al., 2004) and heavy metal concentration in plants varies depending on traffic density (Galal and Shehata, 2015).

Table 2. MANOVA results in heavy metal contents in soil, plant organs and localities (**p<0,01,* p<0,05)

	Plant	F (Plant)	F (Soil)
Locality	Ni	19,72**	0,665ns
	Fe	84,75**	57,023**
	Co	17,30**	35,107**
	Mn	75,45**	9,470**
Plant	Ni	23,78**	1,343ns
	Fe	23,31**	27,514**
	Co	18,10**	10,254**
	Mn	32,35**	7,011**
Locality * Plant	Ni	5,44**	1,885ns
	Fe	12,93**	10,066**
	Co	4,91**	3,871**
	Mn	14,57**	2,245**

4. Conclusions

Nickel accumulation in *C. irregularis* was found to be the highest in traffic areas. Heavy metal concentration changed significantly depending on traffic density. Contrary to expectations, Ni, Fe and Mn contents were determined in higher amounts. It was also found that Ni and Co accumulation in plants depended on air pollution while Fe and Mn were taken from the soil through its roots.

According to the results of the study, *C. irregularis* can be used as a biomonitor since it can monitor the short term changes in environmental pollution in urban areas due to its widespread distribution and its density in each habitat and its conformity with standard analysis methods.

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Cite this article: Kılıç DD (2019). Investigation of heavy metal accumulation and biomonitoring of *Calepina irregularis* species growing in Amasya (Turkey) province. Anatolian Journal of Botany 3(2): 44-50.