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Heavy metal, soil pollution, dill, basil,  
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### Effects of Some Heavy Metals (Cd, Cu, Pb, and Zn) Concentration on Qualitative Traits of Dill (*Anethum graveolens* L.) and Basil (*Ocimum basilicum* L.)

#### Abstract

Soil pollution is a global concern from past to recent. Toxic and high resistance nature of chemical pollutants, such as heavy metals show their important as environmental risk and a serious problem for human health, because they can introduce to the food chain from contaminated environments. Experiments were conducted to evaluate the effect of Cd, Cu, Pb, and Zn on qualitative parameters of dill (*Anethum graveolens* L.), and basil (*Ocimum basilicum* L.) grown in polluted soil environment. Heavy metals concentration, total phenol and flavonoid, essential oil percentage, DPPH radical scavenging activity, super and nitric oxide radical scavenging activity in leave and stem parts of the plants were analysed in this study according to standard methods. According to results, Cu was the dominant heavy metal in leave and stem of both studied plants, followed by Pb, Zn, and Cd. The concentration of various heavy metals in dill was more than two times higher than basil. Total phenol and flavonoids showed significant differences in leave and stem in both plants. DPPH, super oxide and nitric oxide radical scavenging activity showed significant higher values in leave than stem in both plants.

## INTRODUCTION

Heavy metal contamination of agricultural soils is a major environmental problem that can reduce both the productivity of plants and the safety of plant products as foods and feeds (Valenzuela-Cota et al., 2019). Cleansing soil of heavy metals using traditional technologies such as excavation and chemical leaching of metals is expensive with estimated projected costs of US\$ 7–35.4 billion in the US (Roba et al., 2016), some US\$ 250,000 per acre (Barouchas et al., 2019). Phytoextraction of metals using hyper accumulator plants is promising emerging technology, but with many challenges yet to be resolved (Salama et al., 2015). Earlier studies have suggested that some common aromatic and medicinal crops might be capable of accumulating heavy metals from contaminated soil (Zahedifar et al., 2019), suggesting the possibility that such plants could be used in the phytoremediation of contaminated soils. Cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) are some of the most widespread heavy metal contaminants of agricultural soils (Wasli et al., 2018), and known to exert toxic effects in animals and plants at elevated concentrations (Meena et al., 2019). However, the effect of these contaminants

on medicinal and aromatic plants is not well known. Dill and basil have been traditionally grown as cash crops in Europe, US, Iran and recently in Canada (Samavatipour et al., 2019). Dry leaves or shoots are used in herbal teas, while fresh herbage is used as culinary herb or as minor adjuncts to salads. Essential oils and extracts from these crops have been shown to contain significant amount of antioxidants (Caunii et al., 2015) and to possess antimicrobial activity (Zohrehvand and Takdastan, 2017). So the aim of this study was to assess the effect of some heavy metals pollution (Cd, Cu, Pb, and Zn) on qualitative parameters and metal accumulation property of dill (*Anethum graveolens* L.), and basil (*Ocimum basilicum* L.) under open field condition.

## MATERIAL and METHODS

### *Experimental condition*

The trial was done at the experimental fields of Hamadan University, Iran during 2017-2018. The experimental land was plowed at the optimum moisture level (field capacity) and leveled. Sowing were done in an open field at the Department of Agronomy, Faculty of Agriculture, Hamedan University. The seeds were sowed in plastic pots filled with soil, sand, and peat moss substrate as a material to

germination. After sowing was irrigated regularly depending on field conditions and development stage of plants. Seedlings were harvested and planted in the experimental field.

### ***Soil and plant analysis***

Five soil samples were collected randomly from various sites. Sampling in each site was done in 0-30 cm depth in autumn before application of fertilizers, using stainless-steel auger. Soil samples were mixed together to form a combined sample, and collected using polyethylene bags and pre-treatment by being air-dried at room temperature ( $25 \pm 1^\circ\text{C}$ ), ground and sieved through 2- mm for physiochemical analysis. Soil analysis was performed based on standard methods (Rowell, 1994). Soil pH was measured using 1:5 soil to water ratio suspension with a glass electrode pH meter (model inolab pH 7110). Soil electrical conductivity (EC) was measured using a glass electrode (model 712 conductometer) after mixing the soil with water (1:5 w/v). Organic matter was determined according to the walky-black method, which is based on the oxidation of organic matter with  $\text{K}_2\text{Cr}_2\text{O}_7$  and  $\text{H}_2\text{SO}_4$  and titration with  $\text{FeSO}_4$ . Soil available P (Olsen-P) is the official factor for assessing soil plant available (Lu, 1999). Briefly, 1 g

of aired-dried soil sample and 20 ml of  $\text{NaHCO}_3$  ( $0.5 \text{ mol L}^{-1}$ , pH 8.5) were placed into a 50 ml extraction bottle; and the bottles were shaken mechanically for 30 min at room temperature. The suspension was filtered through a Whatman No. 42 free filter paper. The P concentrations in the filtrates were measured by the colorimetric method using ascorbic acid at 820 nm by spectrophotometer (model Varian Cary 100). Available potassium (K) concentration was determined using 1 M Ammonium Acetate. Particle-size distribution was determined by the hydrometer method (Kruma et al., 2008; Suanarunsawat et al., 2014). Total content of heavy metals (Cd, Cu, Pb, and Zn) were analysed by Atomic Adsorption Spectroscopy (AAS, model Varian Spectra, 220) according to standard method (Rowel, 1994). The leaves and stem of plants were harvested at the flowering stage and chemical analysis were performed after drying and digestion with  $\text{HNO}_3$  1N according to Rowel, 1994. The harvested leave and stem parts of the plants were dried at room temperature until they reached a constant weight. 1.0 g of any air-dried powder sample was mixed in 80% methanol (20 mL) for 24 hours at room temperature.

The extracts were filtered and kept in vials at  $-70^{\circ}\text{C}$  until their corresponding analysis. Essential oil extraction was performed using Clevenger apparatus (distilled water). 10 g of dried leaves were poured into a 1000 ml balloon, and about 100 ml of distilled water was added and extraction was performed. The extraction time was about 3 hours. During this time, the volatile compounds were extracted with water

vapor and after cooling, a distinct layer on the surface of the water was visible in the graduated tube of the Clevenger apparatus. The content of heavy metals in leave and stem parts of the plants were measured according to standard method, by Atomic Adsorption Spectroscopy (AAS, model Varian Spectra, 220) according to standard method (Rowel, 1994).

**Table 1.** Climatic data of the experiment city

Climatic factors	Months											
	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug
Rainfall (mm)	0	73.0	51.7	32.5	20.6	32.6	20.8	50.3	38.1	16.3	7.9	0
Temperature ( $^{\circ}\text{C}$ )	25.3	3.2	11.2	1.3	-2.8	1.7	6.2	11.3	19.0	21.9	30.1	34.7

### **Total phenolic content (TPC)**

Measuring of the total phenolic compounds in flowers was performed by Folin- Ciocalteu method adapted from Singleton et al. (1999). in details, 10  $\mu\text{L}$  of methanolic extracts and 1600  $\mu\text{L}$  of distilled water was mixed together then 200  $\mu\text{L}$  of Folin-Ciocalteu reagent (10% V/V prepared in distilled water) were added and left at  $25^{\circ}\text{C}$  for 5 min, then 200  $\mu\text{L}$  of sodium carbonate (7.5%) was added and kept for 30 min (at  $25^{\circ}\text{C}$  in dark place). The absorbance of the solution was determined at 760 nm using a spectrophotometer (DB-20/DB-20S UV/Visible Spectrophotometer,

USA) for quantitative analysis of TPC, the gallic acid was used as an external standard, and TPC was expressed as mg gallic acid  $\text{g}^{-1}$  DW.

### **Total flavonoid content (TFC)**

The analysis of total flavonoid content in flower extracts was carried out by aluminum chloride colorimetric method. Briefly, 30  $\mu\text{L}$  of the extract was mixed with 150  $\mu\text{L}$  of sodium nitrate (5% w/v) and was allowed to stand for 5 min, followed by the addition 3 mL of Aluminum chloride hexahydrate (10% w/v) and incubated for 5 min, Then, 1 mL of NaOH (1.0 M) was added and the mixture was diluted to the

mark with distilled water. After incubation at 25°C in dark place for 30 min, the absorbance of the solution was measured at 510 nm by spectrophotometer. For the quantification of TFC, the Quercetin (QE) was used as an external standard, and TFC was expressed as mg QE g<sup>-1</sup> DW (Tvrda et al., 2019).

### ***Radical scavenging activity***

The radical scavenging activity of extracts was evaluated using the colorimetric method described by Brand-Williams et al (1995). Briefly, 15 µL of methanolic extract was mixed with 2.0 mL of the DPPH solution and the mixture was incubated in dark place at 20°C for 30 min. Then the absorbance of the solution was measured at 517 nm. The following equation was used to calculation of DPPH inhibition (Szyrka and Słowik-Borowiec, 2019):

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad \text{Eq (1)}$$

Where A control and A sample are the absorbance of the control and the sample respectively.

### ***Super oxide radical scavenging activity***

Super oxide radical scavenging activity of samples was determined according to Jing and Zhao (1995). Briefly, 1 ml of extract was added to 9 ml of 5 mM Tris-HCl

buffer (pH 8.2). Then, 40 µL of 4.5 mM pyrogallol was added to the mixture. The mixture was shaken for 3 min and the absorbance of the solution was measured at 420 nm by spectrophotometer (Similar concentration extract was used as the blank to eliminate interference). Super oxide radical scavenging activity was expressed by the oxidation degree of a test group in comparison to that of the control. The percentage of scavenging effect was calculated using the following equation (Baghaie and Fereydoni, 2019):

$$\text{Super oxide radical scavenging (\%)} = [(A_0 - A_1 / A_0)] \times 100 \quad \text{Eq. (2)}$$

Where A<sub>0</sub> is the absorbance of the Tris-HCl buffer with pyrogallol, A<sub>1</sub> is the absorbance of the extract addition.

### ***Nitric oxide radical scavenging activity***

Nitric oxide radical inhibition can be estimated by the use of Griess Illosvoy reaction (Garrat, 1964). In this assay, Griess Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). 3 mL of the solution containing sodium nitroprusside (10 mM, 2 mL), phosphate buffer saline (0.5 mL) and Artemisia vulgaris extract (25 to 125 mg mL<sup>-1</sup>) or standard solution (rutin, 0.5 mL) was incubated at 25°C for 150 min. After

incubation, 0.5 mL of the solution mixed with 1 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and left for 5 min for completing diazotization. Then, 1 mL of naphthyl ethylene diamine dihydrochloride was added, mixed and left for 30 min at 25°C. A pink coloured chromophore is formed in diffused light. The absorbance of these solutions were measured at 540 nm against the corresponding blank solutions using spectrophotometer. The following equation was used to calculation of nitric oxide radical inhibition (Salama et al., 2015):

$$\text{Nitric oxide radical inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Eq. (3)

Where A control is absorbance of control sample and A sample absorbance in the presence of the samples of extracts or standards. All experimental sections were performed in triplicate, results were expressed as mean value. The statistical T test was performed between leave and stem for each plant using SAS (version 9.1.3)

software. Differences at  $p \leq 0.05$  were considered to be significant.

## RESULTS and DISCUSSION

Selected chemical and physical properties of the five studied soils are shown in Table 2. According to the obtained pH, soil classified as neutral (ranging from 6.5 to 7.5) but it is not alkaline soil due to the low EC (less than 2 dS m<sup>-1</sup>). As well as, relatively high organic carbon (1.14 %) and loamy texture of studies soil samples relevant the appropriate conditions was performed for growing of basil and dill based on soil nutrients analysis, no fertilization was necessary in studied soils (P and K concentrations more than 15 mg kg<sup>-1</sup> and 60 mg kg<sup>-1</sup> respectively). The use of organic manures and chemical fertilizers in Iranian agricultural farms is more than the needs of plants and soil and water research institute recommendations, which leads to the accumulation of organic and inorganic compounds in soil and their decomposition over time (Pingale et al., 2012).

**Table 2.** Mean physiochemical properties of studied soil

pH	EC (dS m <sup>-1</sup> )	OC (%)	Olsen-P (mg kg <sup>-1</sup> )	Available- K (mg kg <sup>-1</sup> )	CaCO <sub>3</sub> (%)	Sand (%)	Silt (%)	Clay (%)
7.33	0.066	1.14	37.60	166	9.0	44	33	24

**Table 3.** Mean total content of some heavy metals in studied soil sample

	Cd (mg kg <sup>-1</sup> )	Cu (mg kg <sup>-1</sup> )	Pb (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )
Soil Sample	1.2	53.69	46.25	72.13

According to soil heavy metals analysis, the soil was polluted in respect with Cd, Cu and Pb because all results are more than USPE (2005) standard concentrations. Concentration of heavy metals in medicinal plants beyond permissible limit is a matter of great concern to public safety all over the world. The problem is rather more serious in Iran, because medicinal plants which form the raw materials for the finished products are neither controlled nor properly regulated by quality assurance parameters (Barouchas et al., 2019). Lead (Pb) and cadmium are non-essential trace elements having functions neither in human body nor in plants. They induce various toxic effects in humans at low doses. The typical symptoms of lead poisoning are colic, anemia, headache, convulsions and chronic nephritis of the kidneys, brain damage and central nervous system disorders. Cadmium (Cd) accumulates inhuman body and damages mainly the kidneys and liver. WHO (1998) prescribed limit for Pb contents in herbal medicine is 10 ppm while the dietary intake limit for Pb is 3 mg/week. The lowest level of Cd which can cause

yield reduction is 5-30 ppm, while the maximum acceptable concentration for food stuff is around 1 ppm (Fattahi et al., 2019). Although Cu is an essential enzymatic element for normal plant growth and development but can be toxic at excessive levels. Phytotoxicity can occur if its concentration in plants is higher than 20-100 ppm DW (dry weight). Zinc is an essential trace element for plant growth and also plays an important role in various cell processes including normal growth, brain development, behavioral response, bone formation and wound healing. Zinc deficient diabetics fail to improve their power of perception and also causes loss of sense of touch and smell (Majdoub et al., 2017). The dietary limit of Zn is 100 ppm (Esmielpour et al., 2016). Heavy metals content in variable parts are shown in Table 4. According to results, the significant differences in different heavy metals concentration were observed in leave and stem. Copper had the highest concentration in both studied plants followed by Pb, Zn, and Cd respectively.

**Table 4.** Heavy metals concentration (mg kg<sup>-1</sup>) in leave and stem of the plants

Plant	Parts	Cd	Cu	Pb	Zn
Dill	Leave	0.31 <sup>a</sup>	32.55 <sup>a</sup>	23.10 <sup>a</sup>	12.39 <sup>a</sup>
	Stem	0.12 <sup>b</sup>	11.16 <sup>b</sup>	10.23 <sup>b</sup>	4.36 <sup>b</sup>
Basil	Leave	0.15 <sup>a</sup>	11.50 <sup>a</sup>	11.59 <sup>a</sup>	10.56 <sup>a</sup>
	Stem	0.06 <sup>b</sup>	9.69 <sup>b</sup>	0.98 <sup>b</sup>	3.49 <sup>b</sup>

Different letters indicate significant differences at  $p \leq 0.05$

Some metallic elements, the micronutrients Cu and Zn are constitutive elements with specific functions in the plant metabolism. They are also essential elements in the human or animal organism. Other hand, estimations of an input of 12.3 g Pb and 1.6 g Cd per hectare and year through fertilization and deposition from the atmosphere in agrarian ecosystems suggest a slow increase in the contamination (Caunii et al., 2015). The Cd availability to plants is also governed by parameters such as soil pH and the content on organic substances. In this context, some soils, may also present good Cd availability. The 90th percentile data reported by Majdoub et al (2017) are in all cases higher than the mean values reported in this study. The issue of how to deal with Cd-enriching species encompasses different aspects: the Cd uptake capacity may vary greatly with the plant variety considered. This is the case in poppy (Esmielpour et al., 2016), sunflower (Samavatipour et al., 2019), linseed (Okpashi et al., 2019).

Another aspect which has to be considered is the plant part or product derived from the plant. The final product used must be free from or low in toxic heavy metals. For instance, during extraction, about 28–32% of Cd and up to 32% of Pb from the medicinal plant could be transferred to the extract (Manzoor et al., 2018). In Eastern Europe, some regions are affected by heavy industrial pollution where the soils are contaminated with hazardous elements (Espanany and Fallah, 2016). Although the heavy metal contents in the plants grown there were high, the distilled essential oils were not contaminated. The conclusion was that cultivation on such soils is suitable for essential oil production. Various precautions may be suitable for minimization of the uptake of heavy metals by plants. Cd transfer from soil to plant may also be influenced by cutting time. In various herb species, the Cd content of later cuttings was higher than that of earlier ones (Mahdi, 2016).



In the case of chamomile, sowing in spring yielded higher Cd contents in the flower heads than when the sowing was done in autumn (Pisoschi et al., 2016). In trials with various wheat cultivars, (Seyedalikhani et al., 2019) observed that Cd availability for the plants was governed by Cd in the soil and was modified by sorption to organic matter and the concentration of Ca and Cl in the soil solution. To avoid Cd accumulation in

plants, they proposed a careful choice of the variety and adjusting the soil chemical conditions (Roba et al., 2016). Some phytochemical analysis are shown in Table 5. Based on obtained results, significant differences were observed among leave and steam in phytochemical analysis in both studied plants. More phytochemical analysis were observed in leave than steam in both studied plants.

**Table 5.** Some phytochemical analysis of studied plants

Plant	Parts	TPC (mg GAE g <sup>-1</sup> DW)	TFC (mg QE g <sup>-1</sup> DW)	DPPH radical scavenging (%)	Super oxide radical scavenging (%)	Nitric oxide radical scavenging (%)	Essential oil (%)
Dill	Leave	23.12 <sup>a</sup>	4.56 <sup>a</sup>	52.25 <sup>a</sup>	22.52 <sup>a</sup>	14.65 <sup>a</sup>	1.01 <sup>a</sup>
	Steam	8.56 <sup>b</sup>	4.13 <sup>a</sup>	34.72 <sup>b</sup>	9.36 <sup>b</sup>	8.56 <sup>b</sup>	0.56 <sup>b</sup>
Basil	Leave	15.62 <sup>a</sup>	9.65 <sup>a</sup>	43.56 <sup>a</sup>	18.65 <sup>a</sup>	15.63 <sup>a</sup>	1.23 <sup>a</sup>
	Steam	11.16 <sup>b</sup>	5.32 <sup>b</sup>	28.10 <sup>b</sup>	16.25 <sup>b</sup>	10.13 <sup>b</sup>	0.36 <sup>b</sup>

Different letters indicate significant differences at  $p \leq 0.05$ .

Phenolic compounds are a main diverse group of plant secondary metabolites that have been linked to numerous ecological functions. The differences among the various species of a genus for TPC were also found in other medicinal plants (Lajayer et al., 2019). Comparing of our results with other studies showed two times higher amounts of TPC in Iranian basil species than Turkish species (Meena et al., 2019). Environmental factors (such as soil composition, temperature, rainfall, and

ultraviolet radiation) are the most effective factors on the phenolic content (Miranzadeh et al., 2019). The low temperatures, high radiation, pathogen infection, herbivores, and nutrient deficiency can increase producing free radicals and reactive oxygen species (ROS) and as a result lead to increased accumulation of antioxidants such as phenolic compounds in plants (Padash et al., 2019). In recent years, free radicals have been proven to be the most important food oxidizing agents so that in

addition to their adverse organoleptic effects, they eliminate toxins and nutrients by eliminating essential vitamins and fatty acids (Carrubba, 2015). It is well known that phenylalanine ammonialyase (PAL) is an important marker for environmental stresses in different plant species also it plays a key role in the phenylpropanoid pathway. The differences among the various species of a genus for TPC were also found in other medicinal plants (Fattahi et al., 2019). Flavonoids are an important group of plant bioactive molecules occurring virtually in all plant parts. They are responsible for pigmentation and aroma in flowers also protects plants against UV damage. Therefore UV radiation increases strongly flavonoid synthesis (Asgari et al., 2019). There were significant differences among the studied species for TFC. Variation in TFC may be explained based on of difference in the genetic background of mullein species.

## CONCLUSION

Proper use of medicinal plants requires accurate scientific information and understanding of the chemical compounds present in them. Heavy metals are one of the types of chemical pollutants in the environment. Attention to soil contamination is an issue that should be pay

attention. The present study was carried out to investigate the effect of heavy metals (Cd, Cu, Pb, and Zn) concentration on phytochemical and quantitative analysis of dill (*Anethum graveolens* L.), and basil (*Ocimum basilicum* L.). The qualitative analysis showed the higher amounts of TFC, TPC, and radical scavenging activity in the leave parts of plants grown in polluted soil conditions than steam. The concentration of heavy metals in tissues of Dill were higher than Basil. Copper had the highest concentration in both studied plants followed by Pb, Zn, and Cd respectively. The results show the effect of soil contamination on the phytochemical composition of the plant. It is necessary to develop laws for the cultivation and operation of medicinal plants in environments with heavy metals and to set national standards for medicinal plants and their products.

## Declarations of interest

The authors declare that they have no conflict of interest.

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