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ASSESSING DIFFERENT LEVELS OF ZINC AND COPPER IMPACTS ON MICRO- AND MACRO ELEMENTS ACCUMULATION AND TRANSLOCATION IN VARIOUS PARTS OF *Ocimum basilicum* L. PLANT

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ABSTRACT. *The present study was conducted to investigate the effects of different levels of copper (Cu; 0, 5 and 25 mg kg^{-1} and zinc (Zn; 0, 10 and 50 mg kg^{-1}) and their combinations on uptake of micronutrients (Cu, Zn, Fe and Mn) and macronutrients (K and P) sweet basil (Ocimum basilicum L.) in a pot experiment with calcareous soil under greenhouse conditions. It was observed that high concentration of Cu and Zn in alone or in combination treatment ($Cu_{25}Zn_{50}$) had an adverse effect on Cu, Zn, Fe, Mn and P concentration in shoot. Low levels of the employed metals enhanced concentration of micro- and macro elements as compared to the control. The increase of Cu and Zn in soil had positive effect on concentration of all elements except K when compared to control. The accumulation of Cu, Zn, Fe, Mn and P were greater in root than those of shoot under all treatments. The results indicated that antagonist interaction between Cu and Zn took place with together and with Fe, Mn, P and K uptake in plants where excess Cu and Zn was applied in soil. Generally, increasing Cu and Zn concentration in above ground plant parts, increased the amount of these elements in the infusion, decoction and residual from distillation as well.*

KEYWORDS: *Accumulation, Copper, Mineral nutrients, Sweet basil, Translocation, Zinc.*

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INTRODUCTION

The contamination of soils with concern metals in agricultural production affects adversely food quality, crop growth and environmental health (Chaiyarat et al. 2011). Copper (Cu) and Zinc (Zn) are environmental pollutant that may come from parent materials in pedogenic processes or from anthropogenic activities such as mining and smelting of metalliferous ores, usage of organic and mineral fertilizers, pesticides, municipal wastes, sewage sludge (Yuan & Lavkulich 1997, Singh et al. 2009). However, anthropogenic activities are the main sources which increased concentration of these metals in agricultural soils (Sarma et al. 2012). On the other hand, Cu and Zn are the micronutrient cations necessary in small amounts for vital functions such as enzymatic processes, cell elongation, formation of protein, oxidation phase of respiration, and photosynthesis, but in high concentrations and when their bioavailability becomes high, toxicity will occur (Yuan & Lavkulich 1997). Adequate micronutrients depends largely on several factors including ability of plant species to uptake and translocation, availability of Zn and Cu to plants physicochemical characteristics of soil such as pH, electrical conductivity (EC), organic carbon (OC), $CaCO_3$, cation exchange capacity (CEC), clay content, time of contact and concentration of other micronutrient and macronutrients (Olowoyo et al. 2012, Shahriaripour & Tajabadipour 2010). Such interactions may take place in the soil and within the plant. These interactions may affect the availability of different nutrients from soils and their status in plants through the process of growth or absorption, distribution and /or utilization (Marschner & Marschner 2012, Shahriaripour & Tajabadipour 2010, Kabata-Pendias 2010). Plant uptake is one of the major routes of food chain exposure to trace elements in the soil (Prasad et al. 2011). The selection of metal-tolerant crops with no contaminated products could be an alternative for solving the problem. Previous studies have suggested that some essential aromatic and medicinal plants might be capable for accumulating heavy metals from contaminated soils (Zheljazkov et al. 2006). Sweet basil (*Ocimum basilicum* L.) is a tolerant plant to high concentration of Cu and Zn (Prasad et al. 2011). This is a vegetable and economically important medicinal plant belonging to the family *Lamiaceae* (Copetta et al. 2006)). Basil leaves are consumed as freshly and or as flavour in food industries (Lee et al. 2005). Basil is also used as a traditional natural medicine for the treatment of headaches, coughs, constipation, warts, worms, and kidney malfunction (Simon et al. 1999). To the our knowledge, no study has been carried out on interaction effects of Cu and Zn on the concentration of elements and even pass these metal into infusion and decoction of sweet basil. Therefore, the present study was undertaken to investigate the interactive effect of Cu and Zn on concentration of micro- and macro elements in plant tissues and accumulation of Cu and Zn in infusion and decoction of sweet basil.

MATERIALS AND METHODS

Soil characteristics

A pot experiment was conducted at the greenhouse subjected to natural conditions (25 ºC day/17 ºC night, 16 h light: 8 h dark and 75% relative humidity with a soil obtained from surface layer to 30 cm depth of an experimental farm in the campus of agriculture and natural resources, university of Tehran, Karaj, Iran. The soil was air-dried and then ground to pass through a 2 mm sieve. Characteristics of the soil are presented in Table 1. Organic carbon (OC) was determined by the method of Shaw (2006), total N by Kjeldhal method (Bremner & Mulvaney1982), available P by Olsen method (Olsen et al. 1954), available K by an flame photometer after extraction with NH_4OAC (Sparks et al. 1996), cation exchange capacity (CEC) by Bower method (Sumner et al. 1996), available Cu, Zn, Fe and Mn by DTPA-extraction following Lindsay & Norvell (1978) technique, equal calcium carbonate by Bouyoucos method (Bouyoucos 1962), texture of the soil by Bouyoucous hydrometer (Bouyoucos 1962), electrical conductivity (EC) by the Rhoades method (Rhoades 1996) and pH on saturated extract was determined by Potentiometric method with pH meter (Thomas 1996).

Experimental set up and treatments

Plastic pots with 6 inch in diameter and 18 cm of height were used. Air-dried, threshing and sieved (<4 mm) soil (4 kg) was used and then filled each plastic pot. A basal dose of 4 mg phosphorus (P) and 70 mg potassium (K) per kg of dry soil

Table 1. Physical and chemical properties of the soil used in this study before adding Cu and Zn.

Characteristic	Quantity	Characteristic	Quantity
Soil texture	Sandy loam	CEC (Cmol(c)kg^{-1})	10.7
(%)Clay	17.4	(%)Total nitrogen	0.04
(%)Silt	18	Available Phosphate (mgkg^{-1})	8.7
(%)Sand	64.5	Available Potassium(mgkg^{-1})	180
pH	7.4	*mgkg^{-1}) (Fe	12.3
EC(dS/m)	1.2	*mgkg^{-1} (Mn)	9.3
$CaCO_3$%	6.7	*mgkg^{-1} (Cu)	0.6
% OC	0.6	*mgkg^{-1} (Zn)	0.7
%SP	29.1		

* DTPA-Extractable

were applied uniformly to each pot by adding triple superphosphate, and potassium sulphate, respectively. P and K were mixed homogenously with soil before sowing. The treatments were consisted of different levels of Cu and Zn and there combinations (Table 2). The soil in each pot was spiked and the required amounts of metal salts were dissolved in 200 ml distilled water and then sprayed over each pot layer by layer as evenly as possible. After carefully mixing the metals solution with soil, pots were kept at 70% of water-holding capacity (WHC) and allowed to equilibrate for 60 days before sowing. During incubation, the soils were air dried and were then remoisturized using distilled Water. The wet/dry cycle was repeated two or three times during the incubation period. Basil seeds (*Ocimum basilicum* L. var. keshkeni levello) were provided from Zardband Pharmaceutical Company, Tehran, Iran. Seeds were cultivated in chest and after reaching to three-leaf stage, four uniform plants were transplanted into the each pot. The pots were arranged in a complete randomized design (CRD). All pots were rotated on a weekly basis on the benches to avoid any potential localized effect on plants. The soil water content in each pot was maintained at field capacity (FC) throughout the experiment. The pots were maintained under natural light and temperature conditions. Thereafter, plants were harvested at the full flowering growth stage (85 days after sowing). At harvest, plants were carefully cut at the soil surface and half of the shoots were dried in a shady well-aerated location with normal temperature (20–25 ºC) for 14 days to preserve essential oil and minimize oil losses (Topalov 1962). After

Table 2. Employed treatments with different Zn and Cu levels.

No.	Treatment ($mgKg^{-1}$)	Abbreviation
1	Control	Cu_0Zn_0
2	Zn at 10 mg Kg^{-1} and Cu zero	Cu_0Zn_{10}
3	Zn at 50 mg Kg^{-1} and Cu zero	Cu_0Zn_{50}
4	Cu at 5 mg Kg^{-1} and Zn zero	Cu_5Zn_0
5	Cu at 5 mg Kg^{-1} and Zn at 10 mg Kg^{-1}	Cu_5Zn_{10}
6	Cu at 5 mg Kg^{-1} and Zn at 50 mg Kg^{-1}	Cu_5Zn_{50}
7	Cu at 25 mg Kg^{-1} and Zn zero	$Cu_{25}Zn_0$
8	Cu at 25 mg Kg^{-1} and Zn at 10 mg Kg^{-1}	$Cu_{25}Zn_{10}$
9	Cu at 25 mg Kg^{-1} and Zn at 50 mg Kg^{-1}	$Cu_{25}Zn_{50}$

extraction of essential oil by hydro-distillation for 3 h in a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia (2010), the residual distillation water was collected at the end of each distillation and refrigerated until for elemental analysis. However, the plant biomass after the extraction of the essential oil oven dried at 70 ºC to a constant weight. The remaining half of the shoot washed thoroughly with tap water twice and rinsed again with deionized water. Then, they were oven-dried (70 °C) for 2 days to a constant weight. The plant roots samples were collected by emptied pots on a clear plastic sheet, and the roots were washed three times with distilled water and dried between two layers of paper tissues, and then kept at 70 ºC for 2 days to a constant weight.

Preparation of infusion and decoction

The infusion and decoction samples were prepared as follows: 200 ml of double distilled boiling water was added to 2 g dried plant material and left to cool during the 10 min preparation. For preparing decoction, 200 ml of double distilled boiling water was added to 2 g dried plant material and boiled for 10 min. The resulting water extract was filtered and considered for further elemental analyses (Pytlakowska et al. 2012).

Plant analysis

Dried root and shoot samples were ground in a stainless steel mill to pass through the 1mm sieve. Ground samples were stored at room temperature in paper bags for chemical analysis. The fine ground plant samples (1 g) were combusted in an electrical furnace at 550 ºC for 5 h, and digested with 10 ml of 2 N hydrochloric acid (HCl), and impurities were removed by filtration through a Whatman filter paper (No. 42). The final volume was made to 100 ml with deionized water (Cottenie 1980). The concentrations of mineral elements in the digest, such as Cu, Zn, Fe and Mn were determined with atomic adsorption spectroscopy (Shimadzu AA 6400), potassium (K) concentration was measured by flame photometer (Corning flame photometer 410) and Phosphorus (P) concentration was extracted with the vanado-molybdate yellow method and determined using spectrophotometer (Shimadzu U 73100). Plant extracts (infusion and decoction) were dry ash using 10 ml of plant extract. Briefly, these samples were evaporated to carbonization on a hot plate until smoking has stopped, and then it was heated in a muffle furnace at 400 ºC for 4 h. After cooling, 2 ml of concentrated HNO_3 was poured over the residue and the mixture was put in the sand bath to allow acid evaporation, after the samples were heated at 400 ºC for 1 h in the electrical furnace, cooled, and then 2 ml of hydrochloric acid was added. The samples were then poured into 10 ml volumetric flasks and brought to volume with double distilled water (Zheljazkov & Nielsen 1996).

Translocation factor

The translocation factor (TF) shows the plant's ability to translocate metals from the roots to the harvestable aerial parts (Mattina et al. 2003). It was calculated on a dry weight basis.

$$TF=\frac{\text{Metal concentration in shoot (mgkg} - 1)}{\text{Metal concentraion in root (mgkg} - 1)}$$

Statistical analysis

The results of the measured parameters were subjected to analysis of variance (ANOVA) using SAS (SAS Institute Inc 1999) program, and Duncan's multiple range test (P ≤ 0.05) was used to determine the significant difference between treatment means.

RESULTS AND DISCUSSION

Cu and Zn concentration in plants tissue, infusion and decoction

Sweet basil plant was accumulated the metals (Cu and Zn) in root more than shoot (Table 3). At all employed treatments, in root a significant and positive response was observed in concentration of Cu and Zn compared to the control. $Cu_{25} Zn_{50}$ and control treatments resulted in the highest and the lowest accumulation of Cu and Zn in roots, respectively. Roots accumulated significantly more Cu and Zn than shoots, which is in accordance with the results recorded by some authors such as Kovacik et al. (2008). Heavy metals tend to remain in root tissues of the most horticultural crops (Kim et al. 1988, Paivoke 2003), however, in basil plant of current study showed a similar trend. Previous studies have suggested that basil is tolerant to higher concentration of Cu and Zn (Prasad et al.

Table 3/A. Effect of different levels of Cu on concentration of Cu and Zn (mg kg-1) in various parts of *Ocimum basilicum* plant*

Treatment	Cu Concentration (mg Kg^{-1})						TF
	root	shoot	waste	water	infusion	decoction	
Cu_0Zn_0	9.0 ±0.5 h	6.2±0.3 fg	6.5±0.7 e	0.8±0.02 f	0.43±0.07 f	0.59±0.05 e	0.6 c
Cu_0Zn_{10}	11.4±0.4 g	9.9±0.4 e	9.0±0.4 d	0.7±0.02 f	0.32±0.01 g	0.45±0.03 e	0.8 ab
Cu_0Zn_{50}	17.6±0.4 e	5.4±0.1 g	6.3±0.4 e	0.6±0.04 f	0.31±0.01 g	0.44±0.02 e	0.3 d
Cu_5Zn_0	13.6±0.4 f	12.3±0.4 d	10.2±0.3 cd	1.7±0.06 e	0.81±0.04 d	1.26±0.04 cd	0.9 a
Cu_5Zn_{10}	18.4±0.4 e	14.1±0.3 c	11.7±0.6 c	2.0±0.05 d	0.93±0.07 c	1.42±0.05 c	0.7 bc
Cu_5Zn_{50}	24.2±0.2 d	7.4±0.4 f	5.4±0.1 e	1.5±0.05 e	0.66±0.06 e	1.17±0.02 d	0.3 d
$Cu_{25}Zn_0$	26.2±0.4 c	20.9±1 b	14.3±0.5 b	3.4±0.13 b	1.45±0.09 a	3.22±0.13 a	0.7 bc
$Cu_{25}Zn_{10}$	35.1±0.8 b	25.5±0.6 a	17.9±0.3 a	4.4±0.12 a	1.10±0.02 b	2.61±0.11 b	0.7 d
$Cu_{25}Zn_{50}$	42.2±1.1 a	14.6±0.4 c	10.3±0.3 cd	2.7±0.08 c	0.99±0.04 c	2.44±0.03 b	0.3 d

*Different letters indicate significant differences at P < 0.05. Values are given as mean ± SD (standard deviation). TF: transfer factor

Table 3/B. Effect of different levels of Zn on concentration of Cu and Zn (mg kg-1) in various parts of *Ocimum basilicum* plant*

Treatment	Zn Concentration (mg Kg^{-1})						TF
	root	shoot	waste	water	infusion	decoction	
Cu_0Zn_0	123.3±2.4 f	81.2±1.1 c	60.2±1.2 f	14.0±0.19 ef	7.33±0.4 ef	8.88±0.2 e	0.6 cd
Cu_0Zn_{10}	147.3±2 e	105.7±3 b	69.1±2.3 e	18.1±0.36 c	8.50±0.5 cd	11.25±0.1 c	0.7 bc
Cu_0Zn_{50}	184.7±2.6 c	150.4±2.6 a	106.1±1.9 b	25.6±0.79 a	10.26±0.1 a	18.87±0.3 a	0.8 a
Cu_5Zn_0	131.7±2.8 f	82.9±3.6 c	57.5±0.5 fg	13.2±0.35 f	7.67±0.3 def	9.27±0.2 ed	0.6 d
Cu_5Zn_{10}	155.3±4.8 e	109.7±3.9 b	86.7±2.2 c	15.6±0.22 d	8.29±0.3 cde	10.09±0.2 d	0.7 bc
Cu_5Zn_{50}	202.7±2.8 b	153.4±0.9 a	121.7±2.9 a	20.6±0.35 b	9.59±0.2 ab	12.34±0.4 b	0.7 ab
$Cu_{25}Zn_0$	145.6±4.3 e	69.3±2.2 d	54.3±1.3 g	10.9±0.19 g	5.92±0.1 g	7.48±0.1 f	0.4 e
$Cu_{25}Zn_{10}$	170.8±4.3 d	87.4±0.4 c	77.3±0.4 d	14.5±0.27 de	6.74±0.1 fg	9.80±0.4 d	0.5 e
$Cu_{25}Zn_{50}$	263.3±4.9 a	106.5±2.5 b	84.5±2.4 c	18.2±0.45 c	8.73±0.1 bc	12.62±0.2 b	0.4 f

*Different letters indicate significant differences at $P < 0.05$. Values are given as mean ± SD (standard deviation). TF: transfer factor

2011). The accumulation and distribution of Cu in roots may be major reason that basil can tolerate higher concentrations of the metals. In addition, it also resulted in Zn and Cu application at greater rates (50 and 25 mg Kg^{-1}) had an adverse effect on these metals concentration in shoot, while increased with increasing low levels of Zn and Cu (10 and 5 mg Kg^{-1}).The maximum concentration of Cu and Zn in shoot were found in $Cu_{25}Zn_{10}$ and Cu_5Zn_{50} treatments with value of 25.5 and 153.4 mg kg^{-1}, respectively. However, the minimum concentration of Cu (5.4 mg kg^{-1}) and Zn (69.3 mg kg^{-1}) in shoot were recorded for Cu_0Zn_{50} and $Cu_{25}Zn_{50}$ treatments. Cu and Zn concentration in plants to a great extend copied these elements concentration in the soils. It may be attributed to the physical and chemical properties of the soil. Availability of heavy metals to the plants depends upon various physicochemical properties of the soil, such as pH, OC, CEC, and soil texture (Singh et al. 2009). Gurpreet-Kaur et al. (2012) reported that the adsorption processes of metals by soils affected the availability of Zn and Cu to plants, and these processes increased with increasing in pH, CEC and clay content. Sing et al. (2009) showed that pH was negatively correlated with phyto- available heavy-metal concentrations in soil. Therefore, regarding the soil characteristics (low clay content and CEC) low Cu and Zn adsorption and immobilization by soil could be explained. On the other hand, Anguelova et al. (2004) demonstrated that the mobile and water-soluble forms of Zn and Cu in the soil had significant correlations with concentration of these metals in plant tissues. It is reported that light soil such as sandy loam (which is also used in our current study), due to lower mechanical strength does not limit the growth of plant roots (Taylor et al. 1966). Therefore, increase of metals concentration in shoot tissues of sweet basil with increasing metals concentration in soil was expected. Cu and Zn are essential micronutrient for normal plant growth and metabolism, but it can be phytotoxic at high concentrations. According to Kabata-Pendias (2010), the toxicity limit of Zn in plants ranged from 300 mg kg^{-1} to 400 mg kg^{-1}DW and Cu from 20 to 30 mg Kg^{-1} DW depends on plant species and growth stage. Only, the concentration of Cu in edible part of basil (shoot) in $Cu_{25}Zn_{10}$ treatment was beyond or within the critical value, however, no toxicity symptoms were found. The present study shows that the application of Zn and Cu at high levels depressed their concentrations in plant tissues because of antagonistic effect. These results were similar to those obtained by Kabata-Pendias (2010) and Marschner & Marschner (2012) who showed that variations in availability of the metals to plant could be due to differences in the uptake mechanism of individual ions and competition among the heavy metals for the absorption. In general, concentration of Cu and Zn in infusion and decoction of *O. basilicum* plant was related to the amount of these metals in above ground plant parts. Cu and Zn concentration in decoction was higher than that of infusion, however, Zn level in all treatments are relatively higher than Cu level. Cu levels in the infusion and decoction ranged from 0.31- 1.45 mg kg^{-1} and 0.44-3.22 mg kg^{-1}, respectively. Zn content in herb infusions and decoction were in the range of 5.92-10.26 mg kg^{-1} and 7.48-18.87 mg kg^{-1}, respectively. The highest and the lowest Cu value in both infusion and decoction were observed in $Cu_{25}Zn_0$ and Cu_0Zn_{50} treatments, respectively. However, the minimum and maximum Zn concentrations were determined in Cu_0Zn_{50} and $Cu_{25}Zn_0$, respectively. The results clearly indicated that increasing amounts of Zn and Cu in infusion and decoction were relative with increasing concentration of these metals in the above ground plant parts. The extraction efficiency or releasing of each element was estimated on the basis of comparison of metal concentrations in shoots and its concentration in herb infusions and decoction (% of the total content). We have noticed that the highest extraction efficiency for Cu in infusion and decoction, 8.91 and 16.67% were obtained in Cu_5Zn_{50} and $Cu_{25}Zn_{50}$ treatment, respectively, and the lowest extraction efficiency, 3.21 and 4.41% were gained in Cu0Zn10 treatment. The mean percentage of Cu released in herb infusion and decoction were 6.20 and 11.16%, respectively. The highest extraction efficiency for Zn in infusion and decoction, 9.25 and 12.54%, were observed in Cu_5Zn_0 and Cu_0Zn_{50}, respectively, whereas the lowest value of them, 6.25 and 8.04%, were achieved in Cu_5Zn_{50}. The mean percentage of Zn released to the herb infusion and decoction were 7.92 and 10.70%, respectively. We found that Cu and Zn may be taken up by basil plant, but translocated in small concentration to infusion and decoction. Previous studies have shown that the elements concentration in infusion and decoction may be affected by a number of parameters such asorganic matrix of the herbs in water, extraction deficiencies of the constituents under brewing conditions as well as total concentrations of the elements in herb shoots, solubility characteristics of mineral, natural pH of water used and time of brewing herb to prepare the infusion (Basgel & Erdemoglu 2006). Extraction efficiency for Zn and Cu has been reported for various aromatic and medicinal plants (Basgel & Erdemoglu 2006, Pytlakowska et al. 2012). Natesan & Ranganathan (1990) due to the extraction deficiencies of analyze, the elements in herb leaves were classied into three groups:

highly extractable (>55%), moderately extractable (20–55%) and poorly extractable (<20%). Our results showed that Cu and Zn belonging to the poorly extractable groups of elements and this is contrast with the finding of Pytlakowska et al. (2012) and Natesan & Ranganathan (1990) who reported that Cu and Zn is a moderately extractable element from herb leaves. The comparison of the results is sometimes difficult due to the lack of uniformity in the conditions used to prepare to the infusion and decoction. It has been recognized that infusion and decoction prepared from the medicinal herbs may be a good source of microelements necessary to human health, but toxicity or imbalance of these elements could cause physiological disorders (Pytlakowska et al. 2012). The elements concentrations in the herbs and their infusion and decoction were given for 1 kg of the corresponding herb. It is not expected that one directly consumes 1 kg of any medicinal herbs in a day. These medicinal herbs are generally in tea bags containing average 2 g herb leaves. This means that only 10 g of herb is to be consumed if herbal tea is drunk 5 times in a day and there is no danger mainly for toxic levels of elements originating from herbal infusion and decoction. However, it is better that due to the undisclosed harvest locations of medicinal plants, potential exposure to a variety of environmental contaminants, consumption rate of the herbal teas should be under strict control. Overall, the concentration of each metal in the raw material was compared with the sum content in the water from distillation and in separated solid (distilled plant material). Cu and Zn concentration in the sum water from distillation and distilled plant material was similar to Cu and Zn concentration in the above ground plant parts of the respective treatment. Our results demonstrated that the heavy metals were not removed from the tissues during the process of oil extraction by steam distillation. This result strongly supports the use of aromatic plants as alternative crops for Cd, Pb, and Cu enriched soils, which could yield a heavy metal free product (Zheljazkov et al. 2008, Zheljazkov et al. 2006, Scora & Chang 1997).

Fe and Mn concentration in plant tissues

It was obvious from the data that at each Cu and Zn levels more Fe and Mn accumulated in root than in shoot. Zn and Cu application had a decreasing trend of Mn and Fe concentration in shoot with elevated Cu and Zn additions (25 mg Kg^{-1} and 50 mg Kg^{-1} and their combination), whereas in shoot at low levels (5 mg Kg^{-1} and 10 mg Kg^{-1} and their combination) and in root at all employed treatments Mn and Fe were increased. Maximum concentration of Fe and Mn in root and shoot was obtained in $Cu_{25}Zn_{50}$ andCu_5Zn_{10} treatment. So that, the increase in the concentration of Fe and Mn of roots were 126.27% and 74.21% through application of $Cu_{25}Zn_{50}$ treatment and in the shoot were 9.36% and 36.13% by application of Cu5Zn10 treatment over the control. In current study, increase in Fe and Mn content in root could be due to positive effects of these elements with Cu and Zn. The decrease in Fe and Mn in shoot by application of Cu and Zn could be because of antagonistic effects of these elements. These results are comparable with reports of previous studies (Peralta-Videa et al. 2002, Adiloglu 2006). Basil plants accumulated high amounts of Fe and Mn in their roots, indicating a slow rate of translocation (Baker 1981). Kosegarten & Koyro (2001) concluded that restricted translocation of Fe toward the root tissue and the upper plant parts may be attributed to high amounts of Fe are trapped in the epidermal root apoplast (apoplastic Fe inactivation), probably because of a high apoplastic pH.

P and K concentration in plant tissues

In root, a significant and positive response in concentration of P compared to control was observed as the Cu and Zn content of the soil increased (Table 4). Also, root samples contain more P than shoot. Application of Cu levels (5 and 25 mg Kg^{-1}) in alone treatment increased the concentration of P in shoots compared to the control, although differences were not significantly. On the contrary, the addition of all levels of Zn in alone or in combination with Cu depressed P concentration in shoot compared to control. Maximum P concentration in shoot and root were obtained in Cu_5Zn_{10} and $Cu_{25}Zn_{50}$ treatments, respectively. Our findings are in a good line with the previous results reported by several authors that indicated the presence of high concentration of Cu and Zn in soil contribute a decreasing trend in shoot, whereas in roots P content was increased (Alloway 2004).

Table 4/A. Effect of different levels of Cu and Zn on root and shoot concentration of micro nutrient such as Fe and Mn (mg kg^{-1}) and macro element including P and K (%) in *Ocimum basilicum* plant*.

Treatment	Fe Concentration (mg Kg^{-1})		Mn Concentration (mg Kg^{-1})	
	root	shoot	Root	shoot
Cu_0Zn_0	1037.9±17.1 e	153.8±3.3 bc	105.1±2.7 d	77.2±2.5 a
Cu_0Zn_{10}	1354.1±48.7 d	161.9±4 ab	117.8±2.8 cd	82.1±3.9 a
Cu_0Zn_{50}	2027.4±66.9 b	137.1±3.6 d	149.3±4.9 b	60.5±0.5 bcd
Cu_5Zn_0	1060.7±48.1 e	159.4±2.3 ab	114±2.6 cd	80.9±4.4 a
Cu_5Zn_{10}	1425.3±61.1 d	168.2±3.1 a	123.6±3.3 c	105.1±1.8 a
Cu_5Zn_{50}	2254.3±25 a	124.6±3.4 e	156.5±3.5 b	83.6±0.5 a
$Cu_{25}Zn_0$	1831.8±37.5 c	144.6±3.5 cd	146.2±2.8 b	57.8±0.4 cd
$Cu_{25}Zn_{10}$	1907.2±30.9 bc	135.4±3.5 d	156.5±3.8 b	61.3±0.5 bc
$Cu_{25}Zn_{50}$	2348.5±37.3 a	112.3±2.8 f	183.1±2.2 a	53.5±1.6 d

*Different letters indicate significant differences at $P < 0.05$. Values are given as mean ± SD (standard deviation)

Alloway (2004) reported that antagonism effect between Zn and P may be occur, which inhibit translocation of P from root to shoot, as binding of Zn by phosphorus-containing phytate cause leakage of P from membranes. The K concentration in root and shoot of the basil plant had a positive effect with application of Zn (10 and 50 mg Kg^{-1}) in alone or in combination with low levels of Cu (5 mg Kg^{-1}). Whereas, concentration of K was significantly decreased in both root and shoot tissues when Cu applied at high levels (25 mg kg^{-1}) in alone or combinations with Zn. The highest content of K in

Table 4/B. Effect of different levels of Cu and Zn on root and shoot concentration of micro nutrient such as Fe and Mn (mg kg^{-1}) and macro element including P and K (%) in *Ocimum basilicum* plant*.

Treatment	P Concentration (%)		K Concentration (%)	
	root	Shoot	root	shoot
Cu_0Zn_0	0.18±0.001 f	0.163±0.006 abc	1.04±0.01 c	2.2±0.05 c
Cu_0Zn_{10}	0.205±0.004 de	0.158±0.003 c	1.24±0.02 ab	2.4±0.01 abc
Cu_0Zn_{50}	0.278±0.008 b	0.141±0.002 e	1.31±0.01 a	2.5±0.02 ab
Cu_5Zn_0	0.193±0.001 ef	0.174±0.002 a	1.14±0.08 b	2.4±0.1 bc
Cu_5Zn_{10}	0.210±0.004 d	0.160±0.000 bc	1.26±0.01 a	2.6±0.1 a
Cu_5Zn_{50}	0.281±0.001 b	0.153±0.003 cd	1.29±0.01 a	2.5±0.1 ab
$Cu_{25}Zn_0$	0.243±0.004 c	0.170±0.005 ab	0.82±0.00 d	1.9±0.05 d
$Cu_{25}Zn_{10}$	0.276±0.004 b	0.153±0.003 cd	0.87±0.01 d	1.8±0.07 d
$Cu_{25}Zn_{50}$	0.326±0.005 a	0.147±0.002 de	1.01±0.04 c	1.2±0.08 e

*Different letters indicate significant differences at $P < 0.05$. Values are given as mean ± SD (standard deviation)

root and shoot were obtained at Cu0Zn50 andCu5Zn10 treatment, respectively. These findings are comparable with the reports of previous studies (Kovacik et al. 2008, Ke et al. 2007). They reported that Cu, a redox-active metal, generates reactive oxygen species (ROS) by Fenton reaction, which may result in oxidative stress leading, e.g. to peroxidation of membrane lipids; this lipid peroxidation leads to the loss of membrane integrity and the leakage of K^+ from the cells in the culture medium. However, It is may be that in comparison to the other metals such as Cu and Fe; Zn due to lack of participation in Fenton reaction and lower ability to cause lipid peroxidation, ROS generation and oxidative stresses, leakage of K^+ less occur.

Transfer factor

The transfer factor is calculated as the concentration of heavy metal in plant parts to the concentration present in the soil. At each level of applied Cu and Zn treatment, transfer factor value was less than 1, indicating a poor ability of this plant to translocate Cu and Zn from root to shoot. Olowoyo et al. (2012) reported that ratios higher than 1 indicated that plants are enriched in elements from soil (accumulator), ratios around 1 indicated that plants are not influenced by elements (indicator) and ratios less than 1showed that plants exclude the element from soil (excluder). Generally, the transfer factor was less than 1, indicating the basil did not have a significant phytoextraction potential. Zheljazkov et al. 2008 reported that transfer factor for various aromatic and medicinal plants such as coriander, dill, chamomile, peppermint, basil, hyssop, lemon balm, and sage was less than 1 and these plants had not phytoremediation potential as the known metals hyperaccumulator plants.

CONCLUSION

It has been established that plants with high concentration of elements that are within the permissible levels might play an important role in the maintenance of human health. It was found from this study that Cu and Zn content of basil plants (shoot) are within the allowable limit with the exception of Cu value in $Cu_{25}Zn_{10}$ treatment. These findings indicate a potential public health threat with the safety of patients being compromised because of the lack of WHO permissible levels for Cu and Zn. Medicinal plants should be picked in areas free of any contamination sources. Adequate and necessary precautions should be taken while supplementing the trace elements through such medicinal plants in order to avoid other complications of metal toxicity. If medicinal plants are used for infusion and decoction preparation, only the extractable component of heavy metals is available to humans. Due to low extraction efficiency from raw materials to water, only a small fraction of the total content of Cu and Zn in the raw material can be found in infusions and decoction prepared from *O. basilicum* in comparison to levels of these metals in raw materials themselves.

REFERENCES

Adiloglu, S. (2006): The effect of increasing nitrogen and zinc doses on the iron, copper and manganese contents of maize plants in calcareous and zinc deficient soils. Asian Journal of Plant Sciences 5 (3): 504 -507.

Alloway, B.J. (2004): Zinc in soils and crop nutrition, International Zinc Association Brussels, Belgium.

Anguelova, I., Anguelov, G., Atanassov, I., Bailey, N. (2004): Heavy metals accumulation in biomass of some crop plants. Bulgarian Journal of Agricultural Science 10: 107-112.

Baker, A. (1981): Accumulators and excluders□strategies in the response of plants to heavy metals. Journal of plant nutrition 3: 643-654.

Basgel, S., Erdemoglu, S. (2006): Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. Science of the Total Environment 359(1): 82-89.

Bouyoucos, G.J. (1962): Hydrometer method improved for making particle size analyses of soils. Agronomy Journal 54 (5): 464-465.

Bremner, J., Mulvaney, C. (1982): Nitrogen-total. Methods of soil analysis. Part 2. Chemical and microbiological properties (methods of soil an 2): 595-624.

Chaiyarat, R., Suebsima, R., Putwattana, N., Kruatrachue, M., Pokethitiyook, P. (2011): Effects of soil amendments on growth and metal uptake by *Ocimum gratissimum* grown in Cd/Zn-contaminated soil. Water, Air, & Soil Pollution 214: 383-392.

Copetta, A., Lingua, G., Berta, G. (2006): Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. Genovese. Mycorrhiza16(7): 485-494.

Cottenie, A. (1980): Soil and plant testing as a basis of fertilizer recommendations. FAO Soils Bulletin 38(1): 1-250.

Gurpreet-Kaur, B.D., Sharma, P., Sharma, P. (2012): Zinc adsorption as affected by concentration, temperature, and time of contact in the presence of electrolytic and aqueous medium in benchmark soils of Punjab in Northwest India. Communications in Soil Science and Plant Analysis 43(4): 701-715.

Kabata-Pendias, A. (2010): Trace elements in soils and plants, CRC press.

Ke, W., Xiang, Z., Chen, S. (2007): Effects of copper and mineral nutrition on growth, copper accumulation and mineral element uptake in two *Rumex japonicus* populations from a copper mine and an uncontaminated field sites. Environmental and Experimental Botany 59(1): 59-67.

Kim, S.J., Chang, A.C., Page, A.L., Warneke, J.E. (1988): Relative concentrations of cadmium and zinc in tissue of selected food plants grown on sludge-treated soils. Journal of environmental quality 17(4): 568-573.

Kosegarten, H., Koyro H.W. (2001): Apoplastic accumulation of iron in the epidermis of maize (*Zea mays*) roots grown in calcareous soil. Physiologia plantarum 113(4): 515-522.

Kovacik, J., Gruz, J., Backor, M., Tomko, J., Strnad, M., Repcak, M. (2008); Phenolic compounds composition and physiological attributes of *Matricaria chamomilla* grown in copper excess. Environmental and Experimental Botany 62(2): 145-152.

Lee, S.J., Umano, K., Shibamoto, T., Lee, K.G. (2005): Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chemistry 91: 131-137.

Lindsay, W., Norvell, W.A. (1978): Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Science Society of America Journal 42(3): 421-428.

Marschner, H., Marschner, P. (2012): Marschner's mineral nutrition of higher plants. London, Academic press.

Mattina, M.I., Lannucci-Berger, W., Musante, C., White, J.C. (2003): Concurrent plant uptake of heavy metals and persistent organic pollutants from soil. Environmental Pollution 124(3): 375-378.

Natesan, S., Ranganathan, V. (1990): Content of various elements in different parts of the tea plant and in infusions of black tea from southern India. Journal of the Science of Food and Agriculture 51(1): 125-139.

Olowoyo, J.O., Okedeyi, O.O., Mkolo, N.M., Lion, G.N., Mdakane, S.T.R. (2012): Uptake and translocation of heavy metals by medicinal plants growing around a waste dump site in Pretoria, South Africa. South African Journal of Botany 78: 116-121.

Olsen, S.R. (1954): Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dep. of Agric. Circ. 939.

Paivoke, A.E. (2003): Mineral elements and phytase activity in *Pisum sativum* grown at different Zn supply levels in the greenhouse. Environmental and Experimental Botany 49(3): 285-294.

Peralta-Videa, J.R., Gardea-Torresdey, J.L., Gomez, E., Tiemann, K.J., Parsons, J.G., Carrillo, G. (2002): Effect of mixed cadmium, copper, nickel and zinc at different pHs upon alfalfa growth and heavy metal uptake. Environmental Pollution 119(3): 291-301.

Prasad, A., Kumar, S., Khaliq, A., Pandey, A. (2011): Heavy metals and arbuscular mycorrhizal (AM) fungi can alter the yield and chemical composition of volatile oil of sweet basil (*Ocimum basilicum* L.). Biology and Fertility of Soils 47: 853-861.

Pytlakowska, K., Kita, A., Janoska, P., Połowniak, M., Kozik, V. (2012): Multi-element analysis of mineral and trace elements in medicinal herbs and their infusions. Food

Chemistry 135(2): 494-501.

Rhoades, J.D. (1996): Salinity: Electrical conductivity and total dissolved solids. P. 417-435. In D.L. Sparks (ed.) Methods of soil analysis: Chemical methods, Part 3. ASA and SSSA, Madison, WI.

Sarma, H., Deka, S., Deka, H., Saikia, R.R. (2012): Accumulation of heavy metals in selected medicinal plants. Reviews of Environmental Contamination and Toxicology 214: 63–86.

SAS Institute Inc (1999): The SAS system for Windows, SAS Institute. Inc Cary North Carolina

Scora, R., Chang, A. (1997): Essential oil quality and heavy metal concentrations of peppermint grown on a municipal sludge-amended soil. Journal of Environmental Quality 26(4): 975-979.

Shahriaripour, R., Tajabadipour, A. (2010): Zinc nutrition of Pistachio: Interaction of zinc with other trace elements. Communications in soil science and plant analysis 41(15): 1885-1888.

Shaw, K. (1959). Determination of organic carbon in soil and plant material. Journal of Soil Science 10(2): 316-326.

Simon, J.E., Morales, M.R., Phippen, W.B., Vieira, R.F., Hao, Z. (1999): Basil: a source of aroma compounds and a popular culinary and ornamental herb. pp. 499-505. In: Janick, J. (ed.), Perspectives on new crops and new uses. ASHS Press, Alexandria, VA.

Singh, A., Bhati, R.K., Sharma, M., Agrawal, F., Marshall, F. (2009): Effects of wastewater irrigation on physicochemical properties of soil and availability of heavy metals in soil and vegetables. Communications in Soil Science and Plant Analysis 40: 3469-3490.

Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soluanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E. (1996): Methods of soil analysis. Part 3-Chemical methods, Soil Science Society of America Inc.

Sumner, M., Miller, W.P. (1996): Cation exchange capacity and exchange coefficients. pp. 1201-1229. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H. (eds), Methods of soil analysis: Part 3-chemical methods. Soil Science Society of America, American Society of Agronomy, Madison, Wisconsin.

Taylor, H.M., Robertson, G.M., Parker, J.J. (1966): Soil strength-root penetration relations for medium-to coarse-textured soil materials. Soil Science 102(1): 18-22.

Thomas, G.W. (1996): Soil pH and soil acidity. pp. 475-490. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H. (ed.), Methods of Soil Analysis – Part 3 - Chemical Methods. Soil Science Society of America and American Society of Agronomy, Madison, Wisconsin.

Topalov, V., Topalov, V. (1962): Essential oil and medicinal plants. Hr. G, Danov Press, Plovdiv, Bulgaria.

Yuan, G., Lavkulich, L. (1997): Sorption behavior of copper, zinc, and cadmium in response to simulated changes in soil properties. Communications in Soil Science and Plant Analysis 28(6-8): 571-587.

Zheljazkov, V.D., Nielsen, N.E. (1996): Effect of heavy metals on peppermint and cornmint.

Plant and Soil 178(1): 59-66.
Zheljazkov, V.D., Craker, L.E., Xing, B. (2006): Effects of Cd, Pb, and Cu on growth and essential oil contents in dill, peppermint, and basil. Environmental and Experimental Botany 58(1): 9-16.
Zheljazkov, V.D., Craker, L.E., Xing, B., Nielsen, N.E., Wilcox, A. (2008): Aromatic plant production on metal contaminated soils. Science of the Total Environment 395: 51-62.