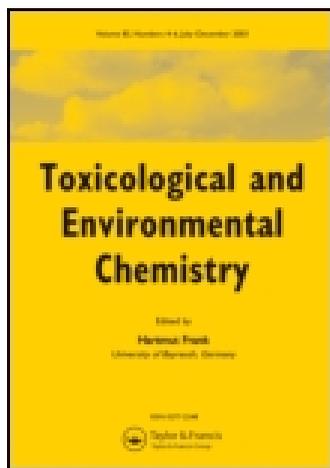


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### Metals in agricultural soils and plants in Egypt

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## Metals in agricultural soils and plants in Egypt

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Since analysis of both soil and plants are useful to assess contamination of a geographic area, concentrations of five representative metals: copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), and iron (Fe) in soil and associated plants were measured by atomic absorption spectroscopy. Samples were collected from four different Egyptian regions (El-mehala El-kobra, Kafr El-Sheikh, Kafr El-zayat, and Al-fayoum) during spring and summer 2010. Concentrations of the selected metals in agricultural soils were significantly different among locations and seasons. Concentrations of Cd and Fe in soils at the four locations exceeded the maximum allowable concentrations for Cd (8 mg/kg, dry mass (dm)) and Fe (1000 mg/kg, dm). Accumulation was different for clover and cotton. Clover blossoms grown in soil from Kafr El-zayat contained the greatest concentrations of Cu, Zn, Pb, and Fe. Cotton flowers from El-mehala El-kobra accumulated the highest levels of Cd. Concentrations of Cd and Pb in both clover and cotton flowers from the four locations exceeded maximum allowable concentrations (3 mg/kg, dm) for both Cd and Pb. Using such agricultural soils for cultivation of edible crops for consumption may result in chronic hazards to human health.

**Keywords:** bioaccumulation; soil; plants; environmental pollution; Africa

### Introduction

Pollutants may produce primary damage with direct identifiable effects on the environment, or secondarily damage in the form of minor perturbations with imbalance of the biological food web that are detectable only over long periods (Sharma 2012). Due to mobilization by activities of humans, including mining, smelting, manufacturing, use of agricultural fertilizers, pesticides, municipal wastes, traffic emissions, industrial effluents and industrial chemicals, pollution of soils by transition metals, such as cadmium (Cd), nickel (Ni), zinc (Zn), lead (Pb), copper (Cu), has increased dramatically during the last few decades (Chibuike and Obiora 2014). Contamination of the environment, including Egypt, by metals is now widespread (Hannigan et al. 2006; Al-Naggar et al. 2013) resulting in toxicity (Nordberg et al. 2011). Dispersion of metals in irrigated soils and plants growing there-on might result in contamination of food that may be hazardous to

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domestic animals and humans (Jolly, Islam, and Akbar 2013). Since elements including metals cannot be degraded these tend to accumulate in soils and remain available with subsequent accumulation in plants (Allsopp, Kenneth, and Christine 2004). Some of the more toxic elements are Pb, Cd, and arsenic (As). Contamination of soils and sediments by metals such as mercury (Hg), Cd, chromium (Cr), and Pb is of concern for human health (Chibuike and Obiora 2014). Although these metals are naturally occurring in soil, contamination also arises from industries, irrigation with polluted water, pesticides, atmospheric input from waste incineration, combustion of fossil fuels, petrochemical and refinery sector, road traffic, fertilizers, as well as biosolids from sewage sludge, and contaminated manure containing metals (Duruibe, Ogwuegbu, and Egwurugwu 2007).

Transfer of metals from soils to plants is one of the key pathways for exposure of humans via the food chain. In order to assess risks to health associated with metals in soils, it is necessary to predict transfer of metals from soil to tissues of plants for subsequent use in simulation models (Hough, Young, and Crout 2003). Metals from soil enter plants primarily through the root system and concentrate in tissues of plants which are often proportional to levels in soils on which they are cultivated. Differences in accumulation among plants are dependent on sensitivities of plants to adverse effects and magnitude and duration of exposure, presence or absence of nutrients and other chemical species in soil that are determinants of the bioavailable fraction (Smical et al. 2008).

For more reliable predictions by these models, site-specific rates of accumulation by specific species are needed. Soils have been used to detect the deposition, accumulation, and distribution of metals in various locations (Alirzayeva et al. 2006; Onder et al. 2007), but little quantitative knowledge is available on the contamination of agricultural soils in Egypt by metals, bioavailability, and associated health risk for consumers. To close this knowledge gap, this study investigated contamination of agricultural soils with metals and subsequent accumulation by plants in urban agricultural areas in Egypt. In addition, this study was part of a larger project conducted to assess effectiveness of a bioindicator-based method involving analysis of local honey bee workers and its products (honey and pollen) for determination of environmental pollution with metals through transfer of metals from contaminated soils to plants then to honey bees through nectar and pollen (Al Naggar et al. 2013).

## Materials and methods

### Study areas

This study was performed in spring and summer during 2010 at four locations in Egypt (Figure 1). Site one was in Kafr El-Sheikh, which is an agricultural governorate located in the Nile Delta, representing a road-side location close to Tanta Kafr El-Sheikh highway. The second and third sites represented two major cities in El Gharbia Governorate in the center of the Nile Delta. These are located in industrial areas of the cities of El-Mahalla Al-Kobra, where effluents from industries raw municipal effluents and sewage are used for irrigation of crops and Kafr El-zayat. The area also includes petrochemical, insecticide, and fertilizer industries. The fourth site was in the Al-Fayoum Governorate, which is the largest oasis in Egypt that is located in a depression or basin in the desert to the west of the River Nile in the south of Cairo. It represents a remote area from pollution sources and selected for comparison. These locations were selected to represent three different potential sources of metals.



Figure 1. Satellite view showing the four sampling locations used in this study in Egypt (Google Earth Gold Pro).

## ***Experimental method***

### ***Sampling materials***

At each site, three samples of soil were collected at least 1 km from each other. Each sample was collected from surface soil to a depth of 30 cm by use of a shovel. Samples were placed into polyethylene bags and shortly after collection transported to the lab. Three synoptic samples of blossoms of clover (during spring) or cotton flowers (during summer) were collected by hand. Clover blossoms and cotton flowers were selected for this study because of their importance as the main foraging and nectar source for bees during spring and summer, respectively, in these locations. Egyptian clover, berseem, is the major forage crop cultivated in the Nile Valley and Delta and occupies 1.2 million hectares. Egyptian clover is planted by the 1st of September until the 1st of June and flowering starts by the 1st of April. Cotton has traditionally been the most important fiber crop in Egypt and the leading agricultural export crop. Egyptian cotton is planted by 1st May till the end of October and flowering starts by the mid of July. Clover blossoms were collected by mid-May, while cotton flowers were collected by mid-August. Collected samples were cleaned and packed into polyethylene bags separately and brought to the lab the same day.

*Preparation of samples*

Soil was spread over sheets of paper and air-dried at room temperature for 5–7 days. Soils were sieved with nylon mesh (2 mm) to separate gravel and debris from finer soils. Particles less than 2 mm fraction was ground into fine powder using a grinder and then packed into polyethylene bags. Cotton and clover flowers were spread over sheets of paper and air dried for 3–5 days. Samples were ground, and packed in paper bags and stored until extraction of metals for quantification by the use of atomic absorption spectrophotometer (AAS).

**Extraction of metals***Soil samples*

A sample of 0.5 g air-dried ground soil was transferred and digested with 1 ml 60% HClO<sub>4</sub>, 5 ml concentrated HNO<sub>3</sub>, and 0.5 ml concentrated H<sub>2</sub>SO<sub>4</sub>. The sample was digested 10–15 min. After the appearance of white fumes, the content was cooled at room temperature (the digested cooled sample was usually colorless or occasionally pink). After cooling, the content was filtered using Whatman No. 4 filter paper and diluted to volume with distilled water. Extracts were analyzed for metal contents by the use of flame atomic absorption spectrophotometer (FAAS), by use of previously published methods (Allen et al. 1974).

*Flower samples*

One gram of each finely ground sample of flowers was transferred and digested with 20 ml HNO<sub>3</sub> / HClO<sub>4</sub> (3:1) acid mixture. Contents of the flask were heated for 3 hr until a transparent solution appeared, and evaporation continued to near dryness. The cooled ash was dissolved in 5 ml 2 N HCl and diluted to 50 ml using bi-distilled water. In the resulting solutions, concentrations of metals were quantified by use of FAAS (AOAC 1984).

**Metal analysis**

Concentrations of metals in soils and flowers were quantified at Atomic Absorption Unit, Central Laboratory, Tanta University, Egypt by the use of the FAAS a Model 2380 AAS (Perkin-Elmer, Cairo, Egypt). Specific conditions for each element were taken from the instrumental manual (Price 1979; Welz and Sperling 1999). Output from the device was converted to concentration of metals in samples (Equation 1).

$$\text{Metal conc.} \left( \frac{\text{mg}}{\text{kg}} \right) = \text{R.conc.} \frac{\text{S.Wt}}{\text{S.V.}} \text{D.f,} \quad (1)$$

where:

R.conc. = reading concentration

S.Wt = sample weight

S.V. = sample volume

D.f = dilution factor

**Transfer factor (TF) of metals from agricultural soils to cotton and clover flowers**

The transfer factors (TF) of metals Cu, Zn, Cd, Pb, and Fe from soils to flowers were calculated (Equation 2) (Cui et al. 2004).

$$TF = \frac{\text{Metal conc. in plant tissue (dry wt.)}}{\text{Metal conc. in the soil (dry wt.)}} \quad (2)$$

**Maximum allowable concentrations of metals**

Concentrations of Cu, Zn, Cd, Pb, and Fe in agriculture soils, clover and cotton flowers were compared to their maximum allowable concentrations (MAC) in soil and plant tissues according to Allen et al. (1974) and Kabata-Pendias and Pendias (1984, 1992).

**Statistical analysis**

Statistical analyses were performed by use of SAS software for windows version (6.12) (SAS 1985). Normality of each data-set was assessed using the Kolomogrov–Smirnov test and homogeneity of variance was determined using Levene’s test. Since variation in concentrations of metals in soils and flowers meet the assumptions of parametric statistics, differences among locations and seasons were assessed by use of two-way analysis of variance (ANOVA) without transformation. When there were significant interactions between the main effects of season and location, multiple range tests were not conducted. The criterion for significance was set at  $p < 0.05$ .

**Results****Concentrations of metals in agricultural soils**

Concentrations of Cu, Zn, Pb, and Fe were significantly different among seasons and levels of Cu and Zn were significantly different among locations. There were significant interactions between main effects of season and location for Cu and Zn (see Supplemental data, Table S1). Mean concentrations of metals in surficial agricultural soils collected during spring and summer 2010 at the four locations in Egypt ranging between 42.65–13.43, 53.10–28.50, 28.88–11.35, 88.67–32.22, and 6308.33–2208 mg/kg, dry mass (dm) for Cu, Zn, Cd, Pb, and Fe, respectively (Table 1). The greatest concentration of Cu, 42.65 mg/kg, dm was observed during spring in agricultural soil near a drainage canal and a highway in El-mehala El-kobra, while the lowest level, 13.43 mg/kg, dm was detected in agricultural soil at Al-fayoum collected during summer. The highest concentration of Zn, 53.10 mg/kg, dm was observed in agricultural soil from El-mehala El-kobra. Levels of Zn in soil near a fertilizer factory in Kafr El-zayat was 52.45 mg/kg dm and in soil near the highway in Kafr El-sheikh 46.08 mg/kg dm during summer were close to the highest concentration of Zn reported in El-mehala El-kobra. The lowest amount of Zn, 28.50 mg/kg, dm was found in agriculture soil collected from Kafr El-zayat during spring (Table 1).

The highest concentration of Cd, 28.88 mg/kg, dm was observed in agricultural soil collected from El-mehala El-kobra, during spring while the lowest detected was 11.35 mg/kg dm in agricultural soil collected from Al-fayoum during spring. Agricultural soil collected from adjacent to a highway in Kafr El-Sheikh, during summer contained the greatest quantity of Pb, which was 88.67 mg/kg dm. The lowest level of Pb,

Table 1. Concentrations (mg/kg, dry mass, dm) of metals (Mean  $\pm$  SD) in agricultural surface soils in El-mehala El-kobra, Kafr El-Sheikh, Kafr El-Zayat, and Al-fayoum during spring and summer 2010.

Season	Location	Concentration (mg/kg, dm)				
		Copper ( $n = 3$ )	Zinc ( $n = 3$ )	Cadmium ( $n = 3$ )	Lead ( $n = 3$ )	Iron ( $n = 3$ )
Spring	El-mehala El-kobra	42.65 $\pm$ 5.55	53.10 $\pm$ 1.30	28.88 $\pm$ 27.95	53.75 $\pm$ 1.21	2365.5 $\pm$ 19.66
	Kafr El-Sheikh	19.27 $\pm$ 0.58	30.95 $\pm$ 4.41	12.58 $\pm$ 1.73	52.68 $\pm$ 0.65	2271.00 $\pm$ 9.18
	Kafr El-zayat	23.65 $\pm$ 1.71	28.50 $\pm$ 8.53	11.82 $\pm$ 2.42	44.20 $\pm$ 0.48	2318.83 $\pm$ 6.45
	Al-fayoum	30.68 $\pm$ 4.25	31.68 $\pm$ 0.45	11.35 $\pm$ 0.70	32.22 $\pm$ 1.35	2208.0 $\pm$ 279.9
Summer	El-mehala El-kobra	32.05 $\pm$ 5.69	30.13 $\pm$ 9.69	11.43 $\pm$ 0.40	83.67 $\pm$ 8.61	6308.33 $\pm$ 52.04
	Kafr El-Sheikh	35.53 $\pm$ 0.94	46.08 $\pm$ 4.01	12.80 $\pm$ 1.05	88.67 $\pm$ 7.32	6145.0 $\pm$ 295.5
	Kafr El-zayat	26.80 $\pm$ 4.35	52.45 $\pm$ 3.66	12.82 $\pm$ 0.67	79.17 $\pm$ 8.95	6018.3 $\pm$ 235.1
	Al-fayoum	13.43 $\pm$ 3.36	36.93 $\pm$ 3.54	12.58 $\pm$ 1.08	74.50 $\pm$ 5.89	6108.3 $\pm$ 87.80

32.22 mg/kg, dm was detected in agricultural soil collected from Al-fayoum during spring. Concentrations of Pb in soils collected during summer were greater than those observed in soils collected during spring. The agricultural soil collected during summer at El-mehala El-kobra contained higher amounts of Fe, 6308.3 mg/kg, dm, while lower levels of Fe, 2208 mg/kg, dm were noted in agricultural soil collected during spring at Al-fayoum. Concentrations of Fe in soils collected at all four locations were greater during summer than those collected during spring (Table 1).

Concentrations of Cu, Pb, and Zn in agricultural soils from Kafr El-Sheikh, El-mehala El-kobra, Al-fayoum, and Kafr El-zayat were less than the MAC of 60 mg Cu/kg dm, 100 mg Pb/kg dm, and 200 mg Zn/kg dm. However, concentrations of Cd and Fe in agricultural soils from the four localities exceeded the MAC of 8 mg Cd/kg dm and 1000 mg Fe/kg dm.

### **Metals concentrations in flowers of clover, *Trifolium alexandria*, blossoms and cotton, *Gossypium* spp.**

Significant differences in concentrations of metals were observed between seasons and among locations. Concentrations of Cu, Cd, Pb, and Fe in flowers were significantly different between spring and summer. Concentrations of Cu, Zn, Pb in flowers were significantly different among locations. There was a significant interaction between main effects of season and location. Thus, multiple range tests were not conducted (see Supplemental data, Table S2).

Mean concentrations of Cu, Zn, Cd, Pb, and Fe in clover blossoms and cotton flowers were 13.82–3.50, 27.37–11.88, 32.53–8.13, 86.17–33.22, and 708.50–314.17 mg/kg, dm, respectively (Table 2). Clover blossoms collected from fields in El-mehala El-kobra contained the highest concentration of Cu, 13.82 mg/kg, dm. Cotton flowers collected from fields at Al-fayoum contained lower levels of Cu, 3.5 mg/kg, dm. Concentrations of Cu in clover blossoms were greater than those observed for flowers of cotton. Cotton flowers from Kafr El-zayat contained the highest quantities of Zn, 27.37 mg/kg, dm, while lower amounts of Zn, 11.88 mg/kg, dm was found for clover blossoms from Kafr

Table 2. Concentrations (mg/kg, dry mass, dm) of metals (Mean  $\pm$  SD) in clover, *Trifolium alexandria*, blossom and cotton, *Gossypium spp.*, flower at El-mehala El-kobra, Kafr El-Sheikh, Kafr El-Zayat, and Al-fayoum during spring and summer 2010.

Season	Location	Concentration (mg/kg, dm)					
		Copper ( $n = 3$ )	Zinc ( $n = 3$ )	Cadmium ( $n = 3$ )	Lead ( $n = 3$ )	Iron ( $n = 3$ )	
Spring (clover blossoms)	El-mehala El-kobra	13.8 $\pm$ 1.46	23.33 $\pm$ 2.01	12.60 $\pm$ 3.84	48.48 $\pm$ 2.35	465.67 $\pm$ 38.76	
	Kafr El-Sheikh	6.77 $\pm$ 0.85	11.88 $\pm$ 0.83	12.07 $\pm$ 1.06	35.85 $\pm$ 4.90	528.50 $\pm$ 50.09	
	Kafr El-zayat	9.38 $\pm$ 0.68	23.35 $\pm$ 1.62	10.03 $\pm$ 0.41	55.10 $\pm$ 1.27	708.50 $\pm$ 88.84	
	Al-fayoum	9.32 $\pm$ 0.56	14.32 $\pm$ 1.38	11.87 $\pm$ 3.21	33.22 $\pm$ 1.18	521.17 $\pm$ 4.16	
Summer (cotton flowers)	El-mehala El-kobra	4.42 $\pm$ 0.79	14.20 $\pm$ 2.82	32.53 $\pm$ 5.97	55.18 $\pm$ 1.90	320.83 $\pm$ 7.52	
	Kafr El-Sheikh	4.05 $\pm$ 0.84	18.37 $\pm$ 4.16	11.33 $\pm$ 4.16	81.83 $\pm$ 9.61	314.17 $\pm$ 18.64	
	Kafr El-zayat	5.42 $\pm$ 0.57	27.37 $\pm$ 0.85	12.58 $\pm$ 1.00	86.17 $\pm$ 5.01	407.83 $\pm$ 33.65	
	Al-fayoum	3.50 $\pm$ 1.32	18.25 $\pm$ 2.90	8.13 $\pm$ 0.55	53.22 $\pm$ 6.40	635.50 $\pm$ 55.01	

El-Sheikh. Zn levels in clover and cotton flowers were significantly different from each other at all the four different locations (Table 2).

The highest concentration of Cd, 32.53 mg/kg, dm was detected in cotton flowers at El-mehala El-kobra. Lower amounts of Cd, 8.13 mg/kg, dm was also reported in cotton flowers at Al-fayoum. Cd levels in clover blossoms were approximately similar at the four locations. Concentration of Cd in cotton flowers varied among the locations. The highest level, 86.17 mg/kg, dm of Pb was found in cotton flowers collected from Kafr El-zayat. The lowest amount of Pb, 33.22 mg/kg, dm was noted in clover blossoms at Al-fayoum. Concentrations of Pb in cotton flowers were greater than those reported in clover blossoms obtained from all the four locations. Clover blossoms collected at Kafr El-zayat contained higher levels, 708.5 mg/kg, dm of Fe. Cotton flowers collected at Kafr El-Sheikh contained the lowest amount of Fe, 314.17 mg/kg, dm. The greatest concentration of Fe was found in clover blossoms which had levels comparable with those in cotton flowers at all locations except at Al-fayoum (Table 2).

In general, concentrations of Cd and Pb in both clover blossoms and cotton flowers at the four different locations exceeded the MAC of 3 mg /kg, dm for both Cd and Pb. Concentrations of Fe in clover blossoms exceeded the MAC of 500 mg Fe/kg dm at all the locations except at El-mehala El-kobra. In cotton flowers, concentrations of Fe were less than the MAC at all the four locations except at Al-fayoum.

#### *Transfer factor (TF) of metals from soils to cotton and clover flowers*

Accumulation ratios for metals from agricultural soils to cultivated plants are of interest because metals exert adverse effects both on plants and animals that ingest them. Plants can be used to monitor the accessible fraction of metals. In this study, the TF for Cu, Zn, Cd, Pb, and Fe varied between species and among locations and given in Table 3. Maximum TF values for Cu 0.39, Zn 0.81, Pb 1.24, and Fe 0.3 were observed in clover blossoms collected in fields at Kafr El-zayat. The highest TF value for Cd was noted for cotton flowers grown in fields at El-mehala El-kobra. Lower TF for both Zn and Pb which were 0.17 and 0.39, respectively, were found for clover blossoms from Al-fayoum. Clover blossoms grown in Kafr El-zayat fields accumulated larger amounts of Cu, Zn, Pb, and Fe. Cotton flowers collected from El-mehala El-kobra were considered to be

Table 3. Transfer factors (TF) of metals from agricultural soils to cotton or clover flowers grown at Al-fayoum, Kafr El-zayat, El-mehala El-kobra, and Kafr El-Sheikh during 2010.

Season	Location	Transfer factor (TF)				
		Copper	Zinc	Cadmium	Lead	Iron
Spring (clover blossoms)	El-mehala El-kobra	0.32	0.43	0.43	0.90	0.019
	Kafr El-Sheikh	0.35	0.38	0.95	0.68	0.23
	Kafr El-zayat	0.39	0.81	0.84	1.24	0.30
	Al-fayoum	0.30	0.17	1.03	0.39	0.23
Summer (cotton flowers)	El-mehala El-kobra	0.13	0.47	2.80	0.65	0.05
	Kafr El-Sheikh	0.11	0.39	0.88	0.92	0.05
	Kafr El-zayat	0.20	0.52	0.98	1.08	0.06
	Al-fayoum	0.26	0.49	0.64	0.71	0.10

Table 4. Average concentrations (mg/ kg, dry mass, dm) of metals detected in agricultural soils during spring and summer 2010, maximum allowable concentrations (MAC) in soil and toxic soil for plants.

Metal	Mean concentration (mg/kg, dm) in soil		MAC (mg/kg, dm)	Toxic soil for plants* (mg/kg, dm)
	Spring	Summer		
Cu	29	28	100	60–125
Zn	36	41	300	70–400
Cd	16	12	0.3	3–8
Pb	46	79	20	100–400
Fe	2285	6197	1000	–

\* (Ross 1994; Singh and Steinnes 1994).

accumulators of large quantities of Cd. Average concentration of Cd in agricultural soils examined during spring and summer was greater than its MAC in soil, therefore these agricultural soils are toxic soils to plants (Table 4).

## Discussion

The city of El-mehala El-kobra is densely populated and is the capital of the local textile industry. Large amounts of industrial and urban waste water are discharged directly with no treatment into irrigation canals which often contain metals that contributed to metals enrichment in soil (Fakayode and Onianwa 2002). Rice and wheat ash fertilization is carried out in Egypt on a large scale (Abou-Sekkina et al. 2010). Application of ash to agricultural soils contributes significantly to the greater concentration of Cd in agricultural soil from El-mehala El-kobra. Application of pesticides is another source of Zn pollution in agricultural soils from El-mehala El-kobra. Metal construction works, welding metals, iron bending, auto repair shops which are common practice might be the main reason for the higher concentration of Fe observed in agricultural soils from El-mahala El-kobra (Abdiem 2011). Application of products that contain Cu such as organic or mineral fertilizers, crop protection products, sewage sludge, and plant nutritional supplements might also be the direct route for the higher concentration observed for Cu in agricultural soils from El-mahala El-kobra (Singh 1994). Pb levels were enriched in agricultural soil from Kafr El-Sheikh adjacent to highway road indicating that vehicular emission was the source of pollution (Panek and Zawodny 1993; Mmolawa, Likuky, and Gaboutloeloe 2011).

Lower concentrations of Cu, Cd, Pb, and Fe detected in agricultural soils from Al-fayoum may be attributed to low industrialization and limited anthropogenic activities at this location (Aksoy and Ozturk 1997). Concentrations of both Cd and Pb in agricultural soils observed during this study were greater than those reported by Nassef et al. (2006). Both Cd and Pb levels in soils measured during this study were less than those reported by Suciú et al. (2008). These differences might be related to different anthropogenic activities and concentrations of urbanization at each site. It is conceivable that environmental pollution with metals differs from area to area as the application of fertilizers and other human activities vary at each site (Zhou, Wu, and Xiong 1994).

In this study, metal concentrations in flowers varied among species and soil level contamination. Variation in metal uptake and accumulation between clover and cotton plant types exist. It might be due to sources of contamination and different accumulation of

metals among plants (Adriano 1986). Results of this study demonstrate that the concentrations of Fe, Cu, and Zn in clover blossoms were less than those detected in soil, indicating that these metals did not accumulate in the flowers (Nirmal et al. 2008). Concentrations of Cd in clover blossoms cultivated at Al-fayoum were greater than those in synoptically collected soils, which indicated that Cd accumulated in the plant. Crops differ significantly in the amount of Cd that they contain (Akinola, Njoku, and Ekeifo 2008; Toth et al. 2009)

Accumulation and exclusion have been viewed as two fundamental mechanisms by which plants respond to greater concentrations of metals in soils (Yanqun et al. 2004). Excluders are plants that limit concentrations of metal translocation in their tissues and contain relatively small quantities in their shoot over a wide range of soil concentrations. In this study, concentrations of metals detected in clover flowers cultivated in El-mehala El-kobra and Kafr El-zayat were 5–40-fold greater than those reported in Upper Egypt by Rashed and Soltan (2004). This might be due to more activities of humans that lead to increasing metals concentrations in soil, irrigation water, air, and consequently in cultivated plants. The higher concentrations of metals observed in clover blossoms and cotton flowers at both El-mehala El-kobra and Kafr El-zayat might be attributed to several factors. First, at Kafr El-zayat emissions of industry, e.g., from electric generators or exhaust contribute to Pb enrichment in soil. Moreover, combustion of tetraethyl and tetramethyl Pb in gasoline was the main source which increased concentrations of Pb in soil (IPCS 1995; Fakayode and Onianwa 2002). In El-mehala El-kobra, chemicals, such as dyes and other finishes used in the textile industry increased concentrations of Cd in soil. Plants growing in such polluted soil accumulate Cd. Cadmium emitted as particulate matter by tires attrition, Pb and some other metals reached water, soil, and plants as a direct result of the atmospheric pollution from automotive emissions (Lagerwerff and Specht 1970).

The higher concentration of Zn observed might be due to abrasion of tires, barks, and Zn-containing compounds, which are used in some manufactured goods, such as paints, cosmetics, automobile tires, batteries, and electrical components (Imperato et al. 2003; Micó et al. 2006). Concentrations of metals in soil and plant samples were consistent with those previously reported (Abdul Hakeem and Singh 1999; Fakayode and Onianwa 2002).

Concentrations of metals measured in soils demonstrate that TF values differed greatly according to the agricultural site, plant species, and metal type. The difference in TFs between locations might be related to soil nutrient management, soil properties, and accumulation of metals, which depends on variety and age of plants, metal levels, and duration of effect (Vassilev and Yordanov 1997). Transfer of metals from soils to plants is dependent on three factors: the total amount of potentially available elements (quantity factor), the activity as well as the ionic ratios of elements in the soil solution (intensity factor), and the rate of element transfer from solid to liquid phases and to plant roots (reaction kinetics) (Greger 1999).

Concentrations of Cd, Pb, and Fe measured in flowers were greater than the MAC reported by Kabata-Pendias and Pendias (1992) and Allen et al. (1974). In Egypt, cattle and milk buffalo depends mainly on clover as fodder; and therefore, contaminated clover might be responsible for milk contamination by metals (Malhat et al. 2012). Concentrations of Cd and Pb measured in samples of honey collected from bee hives in the studied areas during spring and summer were greater than MAC (Al Naggar et al. 2013). This might be due to contamination of clover blossoms and cotton flowers with metals since, honey bees depend mainly on clover and cotton during spring and summer, respectively, for honey production in Egypt. Average concentration of Cd in agricultural soils studied during spring and summer was greater than its MAC in soil therefore, these agricultural

soils are Cd-mediated toxic soils to plants and vegetables grown on this soil and accumulate high concentrations of Cd in their edible parts (Mapanda et al. 2007, Khan et al. 2008).

In conclusion, using metal-contaminated agricultural soils in cultivation of edible crops for human consumption may result in health hazards. Moreover, concentrations of the toxic elements Pb and Cd detected in cotton flowers and clover blossoms might be a potential contamination source of these metals in honey produced by bee hives in these locations.

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### Supplemental data

Supplemental data for this article can be accessed here.

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**Table S1.** Two- way ANOVA results of concentrations of metals in soil (test between subjects) for seasons, locations and the interaction between them (independent variables). F values and their probabilities are indicated

Dependent variable	Source of variation ( independent variables)					
	Season		Location		Interaction	
	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
Copper	12.1	0.0001	6.61	0.0021	8.97	0.0001
Zinc	14.16	0.0001	17.98	0.0001	7.23	0.0002
Cadmium	1.79	0.189	0.82	0.4961	1.24	0.3219
Lead	15.52	0.0001	0.65	0.5928	0.71	0.6423
Iron	3211.6	0.0001	0.87	0.4723	1.12	0.3829

**Table S2.** Two- way ANOVA results of metals concentrations in flowers (test between subjects) for seasons, locations and the interaction between them (independent variables). F values and their probabilities are indicated.

Dependent variables	Source of variation ( independent variables)					
	Season		Location		Interaction	
	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
Copper	190.13	0.0001	15.86	0.0001	13.55	0.0001
Zinc	1.93	0.1839	23.01	0.0001	13.74	0.0001
Cadmium	4.26	0.0557	1.06	0.3936	4.34	0.0203
Lead	165.99	0.0001	33.14	0.0001	17.14	0.0001
Iron	54.16	0.0001	25.730	25.73	0.0001	23.30