Effect of pollution on the chemical content and secondary metabolites of *Zygophyllum coccineum* and *Tamarix nilotica*

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Objectives
This study investigated the uptake and translocation pattern of trace metals from two medicinal plant species namely: *Zygophyllum coccineum* and *Tamarix nilotica* from two contaminated sites and a noncontaminated (NC) site. The effects of heavy metals on the amino acids and secondary metabolites of the tested plant species were assessed.

Materials and methods
Medicinal plant samples and soil samples were collected from three different sites: two contaminated and one NC site. The concentration levels (mg/kg) of the selected trace metals (Al, B, Cr, Cu, Fe, Mn, Mo, Pb, V, and Zn) were estimated in the tested plant species and associated soil.

Results
Heavy metal contents in the investigated plant species reflected the metal concentration in the soil samples. The highest content of the determined heavy metals were detected in both tested plants from contaminated sites in comparison with those from the NC site.
The concentrations of free amino acids in *T. nilotica* and *Z. coccineum* plants from the contaminated sites were higher compared with those in plants from the NC site. Moreover, the concentration of free amino acids in plants from the wastewater-contaminated sites was higher compared with that in plants from the Suez industrial emission site.
The content of secondary metabolites (tannins, saponins, and alkaloids) was decreased in plants from polluted sites compared with those from the NC site. The concentration of tannins ranged from 0.07 to 0.33 g, saponins from 9.99 to 8.22%, and alkaloids from 7.95 to 1.00%. Moreover, the maximum tannins and alkaloid content was detected in *Z. coccineum* from the noncontaminated site.

Conclusion
The plants collected from the investigated sites pose a serious danger. However, a periodical assessment of plants used for traditional medicine should be encouraged as this will assist in ensuring their quality and safety in herbal use, especially for people living in urban areas where the level of pollution may be very high.

Keywords:
free amino acid, heavy metals, medicinal plant, secondary metabolites, *Tamarix nilotica*, *Zygophyllum coccineum*

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Introduction

Medicinal plants are widely used as home remedies and raw materials for pharmaceutical industries. The past decade has seen a significant increase in the use of herbal medicine. The environmental conditions in developing countries; pollution in irrigation water, atmosphere, and soil; sterilization methods; and storage conditions all play an important role in the contamination of medicinal plants by pesticides and heavy metals. The sources of environmental pollution with toxic metals are quite varied, ranging from industrial and traffic emissions to the use of purification mud and agricultural expedients, such as cadmium-containing dung, organic mercury fungicides, and the insecticide lead arsenate [1].

Heavy metal contamination in agricultural environments can result from an atmospheric fallout, pesticide formulations, contamination by chemical fertilizers, and irrigation with water of poor quality [2]. Heavy metals rank high among the chief contaminants of leafy vegetables and medicinal plants [3].

Uptake of trace elements by plants varies and depends largely on several factors such as soil pH and organic matter content. Plant uptake is one of the major routes of exposure of the food chain to trace elements in the soil [4].

Trace elements play an important role in the chemical, biological, metabolic, and enzymatic reactions in the living cells of plants, animals, and human beings [5]. However, the release of trace metals through human activities into the environment has increased over the years, and the excess of these metals in the environment has been reported to be extremely dangerous to human health [6]. The accumulation of trace metals by plants is one of the most serious environmental concerns. This is as a result of the harmful effects of toxic metals on animal and human health [7].

Evidence of severe poisoning caused by some metal compounds and the proven carcinogenicity of some metal ions has fostered intensive research into the different uptake and translocation patterns in food crops [8]. The broad use of traditional medicines by rural communities because of the accessibility and affordability of herbal medicine has also necessitated a further research into the uptake and translocation pattern of trace metals by some medicinal plants from urban areas [3].

Zygophyllum coccineum belongs to the Zygophyllaceae family. The leaves, stems, and fruits of this plant are used in folk medicine as a drug active against rheumatism, gout, asthma, and hypertension. It is also used as a diuretic, local anesthetic, antihistaminic, and antidiabetic agent [9].

Several species of plants belonging to the genus *Tamarix* (Family: Tamaricaceae) have been used in traditional medicine. Antioxidant and antimicrobial activities of *T. hispida* [10] and *T. aphyla* [11] have also been described. Tamaricaceous plants produce a unique class of hydrolysable tannins with diverse structures [12].

The environmental conditions, atmosphere, pollution, soil, and harvesting and handling are some of the factors that may play important roles in the contamination of medicinal plants by metals and microbial growth [3]. It is therefore of major interest to evaluate the composition of some metallic elements in herbal plants, because at elevated levels, these metals can be dangerous and toxic [13,14].

Although some trace metals may have both curative and preventive roles in combating diseases, it has been established that an overdose or prolonged ingestion of medicinal plants may lead to chronic accumulation of different elements that may cause various health problems [15].

The overall objectives of this research were to determine the concentrations of the 10 tested heavy metals in *Tamarix nilotica* and *Z. coccineum* plant biomass from contaminated and noncontaminated (NC) sites and to determine the effect of heavy metal contamination from industrial emissions or by wastewater irrigation on the content of secondary metabolites and amino acids of both tested plant species.

Materials and methods

Site description

This study was carried out at three sites: two contaminated and one NC. The NC site was located at Sokhna Road, 35 km from Cairo governorate.

The first contaminated site is a wastewater-contaminated (WWC) site near the domestic wastewater channel. This site is located at El-Saff, Cairo governorate, which is south of the industrial complex of Helwan (including the Iron and Steel Factory and Weaving, Coke, and fertilizer industries). These industrial activities produce large amount of wastes that are usually dumped into an artificial canal extending over a large area behind the factories. The source of irrigation in this site is the sewage effluent, which comes from the sewage treatment station at Helwan since the past 23 years (according to the report of the committee preparing the Egyptian code for reuse of wastewater, 2004). The second contaminated site, the Suez industrial emission (SIE) contaminated site (SEC), is located near the fertilizer and ceramic factories in Ain Sokhna, Suez governorate. The fertilizer plant of the Egyptian Fertilizers Company (EFC) manufactures granulated urea.

Soil and plant sampling

During June 2009, *Z. coccineum* and *T. nilotica* plant samples, based on their coverage at the site, together with the associated soil samples were collected. The tested medicinal plants were collected from their natural habitats. The plants were not exposed to any agricultural treatments. Five random samples were collected from each site to obtain a comprehensive profile of the site for statistical analysis.

The soil samples were collected from a depth of 0–60 cm. The collection of plant samples was based on plant coverage at the site and plant health.

Soil and plant analysis

Soil samples were air dried at room temperature and then sieved using a 2-mm stainless steel sieve. The soil : water extracts (1 : 2.5) were prepared and used in the determination of pH, electrical conductivity, and cationic and anionic compositions according to the methods described by Richards [16] and by Jackson [17]. The total carbonates were determined according to the methods described by Piper [18]. The organic matter was determined according to the method described by Nelson and Sommers [19]. The available nitrogen in the soil was extracted using a solution of 2 mol/l KCl according to the method described by Keeney and Nelson [20]. The available phosphorus was extracted using a solution of 0.5 mol/l $NaHCO_3$, pH 8.5, according to the method described by Watanabe and Olsen [21].

The soil samples were analyzed for the total content of the studied elements in the filtered soil extracts obtained from samples digested by HNO_3, H_2SO_4, and 60% $HClO_4$, as outlined by Hesse [22]. Total tested heavy metals were determined by inductively coupled plasma optical emission spectrometry (ICP).

The plant samples were washed with distilled water to remove any adhering soil. After washing, the plant samples were oven dried at 65°C and then ground to a powder. The plant samples were digested with H_2O_2 and H_2SO_4 [23] and then subjected to analysis of nitrogen and phosphorus. The nitrogen content was determined using a modified Micro-Kjeldahl method, as described by Peach and Tracey [24]. The phosphorous content was determined according to the method described by Rowell [25]; this method depends on the formation of a blue complex between phosphate and ammonium molybdate in the presence of ascorbic acid (reducing agent). The samples were measured with a spectrophotometer at an absorbance of 880 nm. The plant samples were analyzed for the total content of the studied elements using the digested extracts, which were obtained with 0.5 g of concentrated HNO_3 and H_2O_2 [26]. The heavy metal content in all the samples was determined by aspirating directly to ICP. The alkaloid content was determined according to the method described by Jenkins *et al.* [27]. The saponin content was determined according to the method described by Wall *et al.* [28]. The tannin content was determined according to the method described by Claus [29]. The free amino acid content was determined according to the method described by Block *et al.* [30].

Metal translocation factor

The root-to-shoot translocation factor (TF) was described as the ratio of heavy metals in the plant shoot to that in the plant root [31]. The TF is determined according to the equation: BF = C [HM in shoot]/C [HM in root].

Statistical analysis

The experiment was laid out in a randomized complete block design with three replications. There were two factors in the study: three sites (NC, WWC, and SEC) and two types of plant species (*Z. coccineum* and *T. nilotica*). Data were subjected to analyses using M-STATC., as described by Russell [32]. The mean values were compared using the Duncan New Multiple range test as described by Waller and Duncan [33]. Mean values indicated by the same alphabetical letters in the same column are not significantly different at $P = 0.05$.

The data on the TF, alkaloid content, tannin content, and saponin content of the samples were presented as mean ± SD of the three replicates and were analyzed using Excel 2007 for Windows.

Results and discussion

Soil properties and heavy metal concentrations

Chemical properties of the soil from the three tested sites are presented in Table 1. The data shows that salinity of the saturated extract from the soil, as evidenced by the EC values, was very high in soil from the WWC site (11.28 mMho). The values of soil pH ranged from 8.83 in the soil from the WWC site to 8.71 in that from the industrial emission site, indicating that the soils are alkaline in these locations. The soil from the NC site was slightly alkaline with a pH of 7.97. Schipper *et al.* [34] reported that after long-term wastewater irrigation, the soil pH increased and that this may be due to the high content of cations such as Na, Ca, and Mg in the wastewater.

The organic matter content was high in the soil from the contaminated sites; it was 1.24% at the WWC site and 0.69% at the SIE site compared with 0.43% at the NC site. The cationic composition of the total salts is mostly dominated by Na^+, followed by Ca^{2+} and Mg^{2+}, and then by K^+. The most dominant anion was SO_4^{2-}, followed by Cl^-, and then by HCO_3. The highest OM, Ca^{2+}, Mg^{2+}, Na^{2+}, K^+, Cl^-, and SO_4^- concentrations were detected in the WWC sample, whereas the highest HCO_3 content was detected in the SIE sample.

Accumulation of K in the soil with wastewater application was attributed to the original content of this nutrient in the wastewater applied [35]. Irrigation with wastewater increased the total cation concentration of Ca and Mg [36].

As shown in Table 2, the available N and P content in the soil samples from the contaminated sites is significantly higher compared with those from the NC site as a result of contamination with wastewater at the WWC site and

Table 1 Electrical conductivity (EC), pH, concentration organic matter content (OM) and some anions and cations (mEq/l) in the studied soil samples from the noncontaminated (NC) site, El-Saff wastewater-contaminated (WWC) site, and Suez industrial emission (SIE) site

				Cations (mEq/l)				Anions (mEq/l)			
Sites	EC (mMho)	pH	OM (%)	Ca^{2+}	Mg^{2+}	Na^+	K^+	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}
NC	1.206	7.97	0.43	5.5	3	3.65	1.01	0	0.8	3.125	9.235
WWC	11.28	8.83	1.24	32	22	140.50	2.05	0	0.8	86.25	109.5
SIE	0.468	8.71	0.69	3	2.5	9.90	0.75	0	1.2	1.25	13.7

Table 2 Interaction effects of the site and plant species on nitrogen, phosphorus, and heavy metal contents (mg/kg) of the studied soil samples from the noncontaminated (NC) site, El-Saff wastewater-contaminated (WWC) site, and Suez industrial emission (SIE) site

Site	Plant	N	P	Al	B	Cr	Cu	Fe	Mn	Mo	Pb	V	Zn
NC	*T. nilotica*	24.4 d	4.6 b	449.4 d	51.1 d	23.0 d	32.4 d	163.4 c	22.8 c	1.4 d	21.7 b	3.3 b	25.5 b
	Z. coccineum	21.0 d	2.7 b	368.0 d	42.8 d	17.3 d	35.8 d	119.5 c	30.4 c	1.6 d	21.7 b	3.9 b	25.3 b
WWC	*T. nilotica*	65.1 c	5.5 b	3221.0 b	111.7 b	54.8 a	117.9 a	1533.5 a	131.0 ab	4.1 c	123.3 a	16.3 a	122.5 a
	Z. coccineum	194.5 b	4.9 b	3493.1 a	128.7 a	49.2 ab	112.3 a	1215.1 b	127.6 ab	5.0 b	121.9 a	15.1 a	124.6 a
SIE	*T. nilotica*	228.9 a	18.8 a	2306.6 c	94.4 c	36.8 c	96.2 b	1264.3 ab	116.6 b	6.1 a	113.9 a	12.6 a	135.2 a
	Z. coccineum	34.9 d	5.0 b	2303.3 c	103.3 bc	41.6 bc	83.9 c	1510.7 a	141.3 a	5.3 b	115.6 a	14.3 a	125.8 a

Mean values for each column having common letters are not significantly different at the 0.05 level.

with fertilizer factory effluent at the SIE site. These elements are essential nutrients for plant growth.

Heavy metal contents of the three sites are represented in Table 2. The total heavy metal contents were increased significantly many folds in the samples from the contaminated sites compared with those from the NC site. Heavy metal concentrations of the contaminated sites were increased by 8.21, 2.56, 2.58, 3.38, 9.72, 4.86, 3.14, 5.65, 4.36, and 4.86 times at the WWC site, whereas they were increased by 5.64, 2.11, 1.95, 2.64, 9.81, 4.84, 3.94, 5.29, 3.74, and 5.14 times at the SIE site for Al, B, Cr, Cu, Fe, Mn, Mo, Pb, V, and Zn, respectively compared with the NC site.

The results show a great variability in the heavy metal content according to site of plant collection. The maximum concentrations of Al, B, Cr, Cu, Mn, Pb, and V were found at the WWC site: significantly for Al, B, Cr, and Cu and nonsignificantly for Mn, Pb, and V. Meanwhile, the maximum but not significant concentrations of Fe, Mo, and Zn were detected in plants from the SIE site.

Soils, especially those found in or near the metalliferous sites and metal smelters, are highly contaminated with heavy metals, including Cd, Cr, Cu, Pb, Ni, and Zn. For example, soils sampled from a former Zn/Cd smelter site contained up to 99 500 mg/kg Zn in addition to 1005–7220 mg/kg Pb, 2500–4500 mg/kg Cu, and 28–578 mg/kg Cd [37].

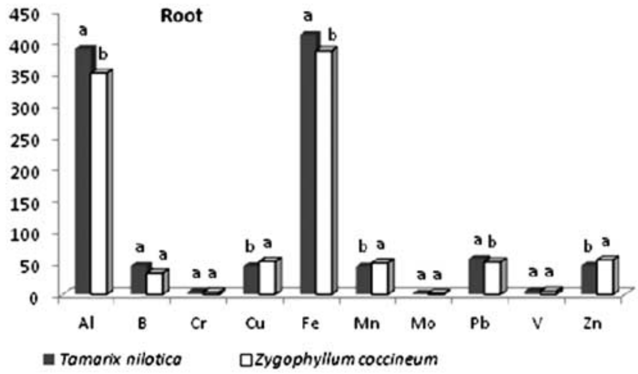
Heavy metal concentrations in plants

Metal concentrations in plants vary with plant species [38]. Plant uptake of heavy metals from soil occurs either passively with the mass flow of water into the roots or through active transport across the plasma membrane of root epidermal cells. Under normal growing conditions, plants can potentially accumulate certain metal ions an order of magnitude greater than the surrounding medium [39].

The plant species has a considering effect on the heavy metal content in both roots and shoots of *T. nilotica* and *Z. coccineum* plants. The contents of Al, B, and Fe in *T. nilotica* roots and those of Al, B, Cr, Cu, Fe, Pb, and Zn in *T. nilotica* shoots were significantly higher compared with those in *Z. coccineum* roots and shoots, respectively. Meanwhile, the contents of Cu, Mn, and Zn in *Z. coccineum* roots were higher compared with those in *T. nilotica* roots (Figs 1 and 2). The contents of B, Cr, Mo, and V and Mn, Mo, and Zn in roots and shoots, respectively for both plants were the same.

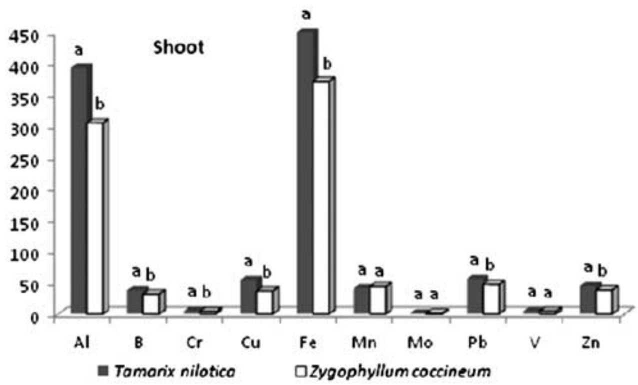
The effect of the site on the heavy metal concentrations in both *T. nilotica* and *Z. coccineum* plants are depicted in Figs 3 and 4. The results showed that, in most cases, the concentrations of the tested heavy metals in plants from the WWC site were significantly higher compared with those in plants from the SIE site. The increase in Al, B, Cr, Cu, Fe, Mn, Mo, Pb, V, and Zn concentrations was 7.74, 3.10, 4.36, 3.81, 4.17, 7.42, 4.22, 9.30, 6.10, and 5.30-fold, respectively in plant shoots from the WWC site and was 6.57, 1.96, 3.39, 2.73, 3.91, 5.35, 6.31, 7.35, 5.55, and 4.39-fold, respectively in plants from the SIE site compared with that in plants from the NC site.

Figure 1



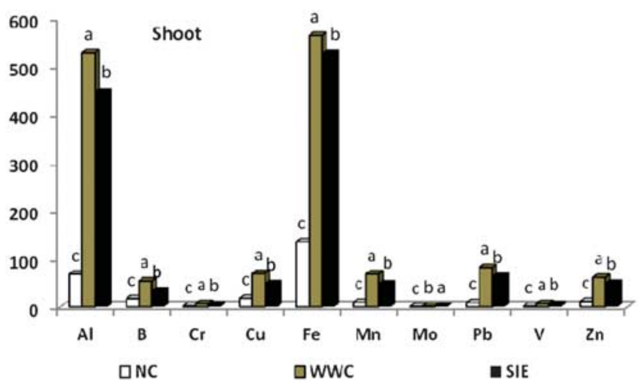
Effect of plant species on root heavy metal content (mg/kg) of *Tamarix nilotica* and *Zygophyllum coccineum*. Values followed by different letters within columns are significantly different at the 0.05 probability level.

Figure 2



Effect of plant species on shoot heavy metal content (mg/kg) of *Tamarix nilotica* and *Zygophyllum coccineum*. Values followed by different letters within columns are significantly different at the 0.05 probability level.

Figure 3



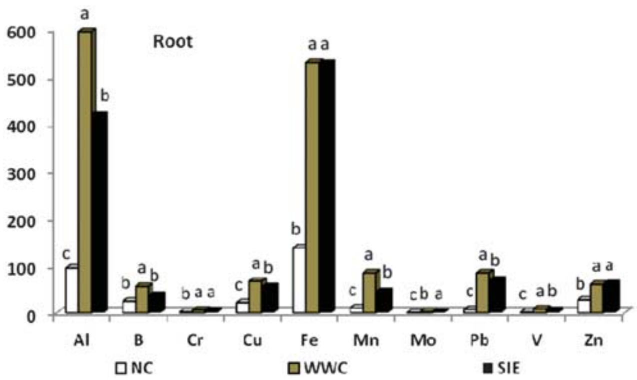
Effect of the site on shoot heavy metal content (mg/kg) of *Tamarix nilotica* and *Zygophyllum coccineum*. Values followed by different letters within columns are significantly different at the 0.05 probability level.

On comparing the two contaminated sites, mostly there was a significant increase in the determined heavy metal content in plants from the WWC site compared with plants from the SIE site (Figs 3 and 4).

The data in Table 3 shows the interaction effect of the plant species and site on the tested heavy metal contents for *T. nilotica and Z. coccineum*. The high heavy metal contents for both roots and shoots, mostly, were detected in plants from the WWC site.

The content of heavy metals in industrialized regions were determined by Januz *et al.* [40], who reported that the plants growing in an industrialized region have higher contents of heavy metals compared with plants growing in a second less industrialized region. Some metals such as Cu, Mn, and Zn are the natural essential components of enzymes and coenzymes and are important for growth, photosynthesis, and respiration, although other metals such as Pb and Cd have no biochemical or physiological importance, therefore they are considered as very toxic pollutants.

Figure 4



Effect of the site on root heavy metal contents (mg/kg) of *Tamarix nilotica* and *Zygophyllum coccineum*. Values followed by different letters within columns are significantly different at the 0.05 probability level.

Although the concentrations of the tested heavy metals in soils at contaminated sites were above the critical concentrations in soil for these elements [41], no visual phytotoxicity symptoms on both tested plants were observed.

The Al, Cr, Cu, Fe, Mn, Mo, and Pb concentrations were all above the normal range for roots and shoots of both tested plants from the contaminated sites, whereas the concentrations of B and Zn were within the permissible level (Table 3).

The variation in the elemental content from plant to plant is mainly attributed to the differences in the botanical structure and mineral composition of the soil in which the plants are cultivated. Other factors responsible for a variation in the elemental content are preferential absorbability of the plant, use of fertilizers, irrigation water, and climatic conditions [38].

Translocation factor of heavy metals

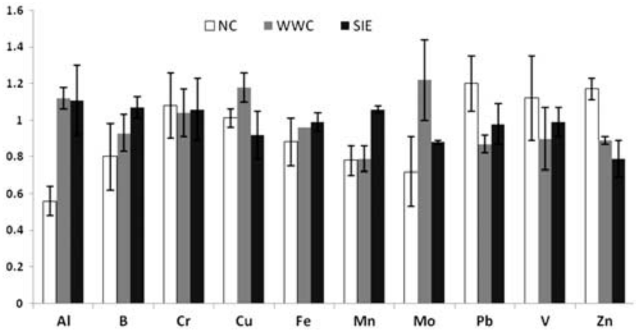
A plant's ability to translocate metals from the roots to shoots is measured using the TF, which is defined as the ratio of metal concentration in the shoots to that in the roots. The TF index showed that the both tested plant species most efficiently translocated the tested heavy metals to the shoot system. The mean TF (average TF values for each metal in different sites for both tested plants) values revealed that *T. nilotica* showed great efficiency for translocating metals from the roots to shoots. The TF values for *T. nilotica* for all tested metals under study were higher than 1, except for B and V (Figs 5 and 6). The trends of the TF values for heavy metals in *T. nilotica* were in the order of Cr > Cu > Mo > Fe > Pb > Zn > Mn > Al > V > B. Meanwhile, *Z. coccineum* had a TF higher than 1 for Cr, Cu, Pb, and V. The results in Figs 5 and 6 show that TF of *Z. coccineum* for these considered metals were in the order of Cr > Cu > Pb > V > Zn > Fe = Mo > Al = B > Mn. A TF higher than 1 indicated a very efficient ability to transport metals from the roots to shoots, most likely due to efficient metal transport systems [43].

Table 3 Interaction effect of the site and different plant species on heavy metal contents (mg/kg) in roots and shoots of *T. nilotica* and *Z. coccineum* plants from the noncontaminated (NC) site, El-Saff wastewater-contaminated (WWC) site, and Suez industrial emission (SIE) site

Site	Plant	Al	B	Cr	Cu	Fe	Mn	Mo	Pb	V	Zn
Root											
NC	*T. nilotica*	84.83 d	24.37 b	1.29 c	19.68 c	113.6 d	8.32 d	0.33 d	6.92 c	0.75 d	21.64 d
	Z. coccineum	104.00 d	24.12 b	1.66 c	23.84 c	161.1 c	11.7 d	0.37 d	6.88 c	1.47 d	32.25 c
WWC	*T. nilotica*	644.20 a	67.26 a	6.02 a	58.88 b	564.8 a	82.97 a	1.84 b	82.24 a	8.49 a	58.23 b
	Z. coccineum	545.10 b	44.28 ab	5.19 b	74.65 a	494.4 b	85.28 a	1.39 c	86.38 a	7.59 b	62.21 ab
SIE	*T. nilotica*	438.80 c	43.75 ab	5.65 ab	55.55 b	556.2 a	41.08 c	1.7 b	81.25 a	6.62 c	56.55 b
	Z. coccineum	402.20 c	33.78 b	5.01 b	68.31 b	502.9 b	52.32 b	2.45 a	60.54 b	6.55 c	69.80 a
Shoot											
NC	*T. nilotica*	77.80 e	15.49 d	1.47 c	20.38 e	131.6 d	10.71 d	0.45 e	8.55 d	0.74 e	11.39 d
	Z. coccineum	58.92 e	19.11 d	1.77 c	16.00 e	139.30 d	8.17 d	0.26 f	9.04 d	1.62 d	11.79 d
WWC	*T. nilotica*	570.00 a	66.18 a	8.70 a	79.72 a	655.8 a	69.28 a	1.37 d	88.89 a	7.60 a	67.84 a
	Z. coccineum	488.20 c	41.04 b	5.42 b	58.79 c	474.50 c	67.54 a	1.67 c	74.84 b	6.71 bc	55.05 b
SIE	*T. nilotica*	530.5 b	31.79 c	5.73 b	63.88 b	559.5 b	43.27 c	2.39 a	71.84 b	6.92 ab	53.88 b
	Z. coccineum	368.30 d	35.97 bc	5.24 b	35.52 d	498.90 c	55.30 b	2.15 b	57.43 c	6.55 c	47.98 c
PL		135[a]	14–78[a]	5[b]	1.1–33.1[a]	450[b]	44.25[b]	Up to 1[a]	0.3–18.8[a]	–	6–126[a]

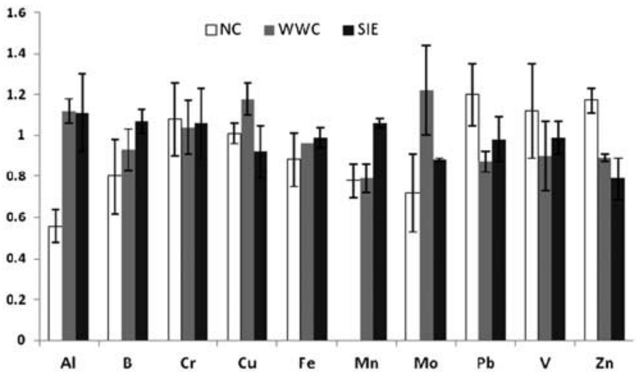
PL, permissible limits according to Kabata Pendias & Pendias [41][a] and FAO/WHO [42][b] standards for metal concentrations in consumable vegetables and edible parts.
Mean values for each column having common letters are not significantly different at the 0.05 level.

Figure 5



Translocation factors with SDs of Al, B, Cr, Cu, Fe, Mn, Mo, Pb, V, and Zn in *Tamarix nilotica* from the noncontaminated (NC) site, El-Saff wastewater-contaminated (WWC) site, and Suez industrial emission (SIE) site. Error bars represent ± SE of the mean values for three separate plant extractions.

Figure 6



Translocation factors with SDs of Al, B, Cr, Cu, Fe, Mn, Mo, Pb, V, and Zn in *Zygophyllum coccineum* plants from the noncontaminated (NC) site, El-Saff wastewater-contaminated (WWC) site, and Suez industrial emission (SIE) site. Error bars represent ± SE of the mean values for three separate plant extractions.

The mean TF for the tested heavy metals ranged from 0.62 to 1.21 and 0.83 to 1.21 for *T. nilotica* and *Z. coccineum*, respectively. According to Baker [44], there are three basic types of tolerance strategies to heavy metals (accumulation, exclusion, and indication), which describe the relationship between the total soil and plant metal concentration and that excluder and accumulator plants could grow together in the same environment. The relationships between the soil and plant metal concentrations should be thoroughly tested for each plant species separately to understand the physiological mechanisms.

Accumulation and exclusion are two basic strategies by which plants respond to elevated concentrations of heavy metals [45]. In metal accumulator species, TFs greater than 1 were common, whereas in metal excluder species the TFs were typically lower than 1 [44].

Nitrogen and phosphorus content in plants

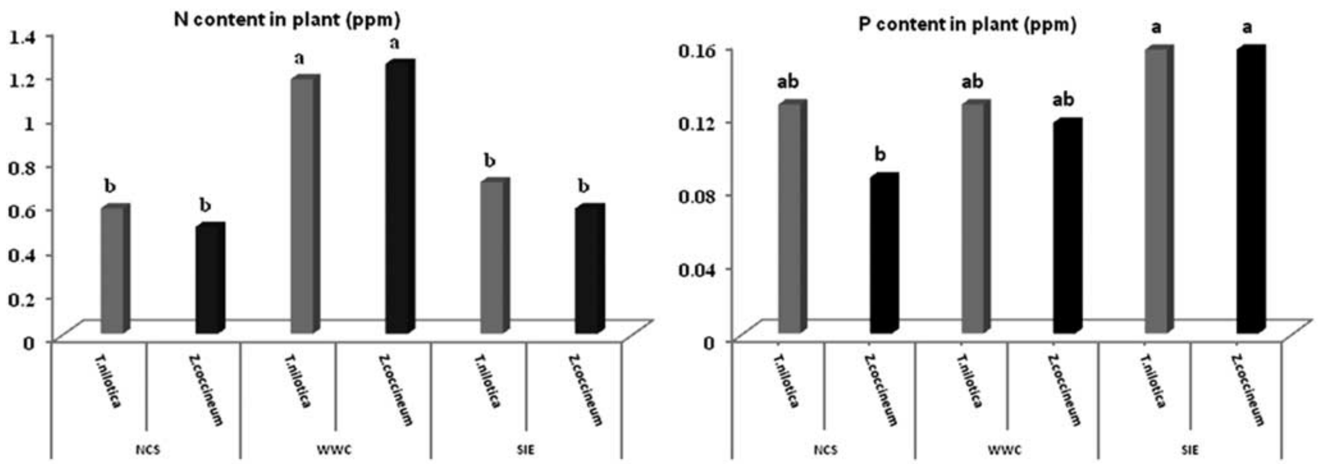
Nitrogen (N) is the essential mineral element required in the greatest amount by plants, comprising 1.5–2% of plant dry matter [46]. Phosphorus (P) is the second nutritional element after nitrogen that limits plant growth, having a concentration of about 0.2% of the total plant dry weight [47]. P is a macronutrient that is a key component in many molecules (i.e. nucleic acids, phospholipids, and ATP) that participates in basic plant processes [48].

The concentration of nitrogen and phosphorus were significantly higher in tested plants from the contaminated sites compared with those from the NC site. The highest content of N was detected in plants from the WWC site, whereas the highest P content was detected in plants from the SIE site (Fig. 7).

Amino acid contents

Under heavy metals stress, plants exhibit a number of physiological changes in their cells [49,50]. Several mechanisms allow plants to tolerate the presence of

Figure 7



Interaction effect of the site [noncontaminated (NC) site, El-Saff wastewater-contaminated (WWC) site, and Suez industrial emission (SIE) site] and plant species (*Tamarix nilotica* and *Zygophyllum coccineum*) on nitrogen and phosphorus contents (ppm) in plants. Values followed by different letters within columns are significantly different at the 0.05 probability level.

Table 4 Mean free amino acid (FAA) contents of *Tamarix nilotica* and *Zygophyllum coccineum* from the noncontaminated (NC) site, El-Saff wastewater-contaminated (WWC) site, and Suez industrial emission (SIE) site

		NC		WWC		SIE	
FAA (%)		*T. nilotica*	*Z. coccineum*	*T. nilotica*	*Z. coccineum*	*T. nilotica*	*Z. coccineum*
Aspartic	Acidic	0.3109	0.3637	0.4752	0.6929	0.3893	0.6369
Glutamic		0.3549	0.4244	0.6011	0.9076	0.4433	0.6742
Histidine	Alkali	0.1642	0.2343	0.2725	0.3422	0.2005	0.2515
Arginine		0.1828	0.2866	0.3877	0.3321	0.4278	0.2527
Lysine		0.1658	0.2139	0.2912	0.4333	0.2768	0.2422
Threonine	Neutral	0.1224	0.1481	0.2433	0.3333	0.1931	0.1568
Serine		0.1446	0.1756	0.2816	0.3608	0.1926	0.2127
Proline		0.4923	0.4405	0.6181	0.8159	0.5669	0.6279
Glycine		0.1314	0.1756	0.2669	0.2898	0.2017	0.2086
Alanine		0.1796	0.1914	0.2899	0.4014	0.2510	0.2735
Valine		0.1193	0.1455	0.2327	0.3260	0.2070	0.1451
Methionine		0.0005	0.0026	0.0005	0.0210	0.0147	0.0014
Isoleucine		0.0947	0.1145	0.1336	0.2393	0.1453	0.1125
Leucine		0.1860	0.2191	0.3622	0.4993	0.3056	0.2357
Tyrosine		0.0543	0.0601	0.1538	0.1623	0.0868	0.0794
Phenylalanine		0.1192	0.1400	0.2240	0.3264	0.1488	0.1596

Table 5 Correlation coefficients between the contents of free amino acids and heavy metals in shoots of *Tamarix nilotica*

Amino acid	Al	B	Cr	Cu	Fe	Mn	Mo	Pb	V	Zn
Aspartic	0.888	0.984	0.992*	0.958	0.930	0.996*	0.449	0.940	0.896	0.952
Threonine	0.938	0.955	1.00**	0.986	0.968	0.999*	0.556	0.975	0.944	0.983
Serine	0.813	0.999*	0.963	0.907	0.667	0.972	0.315	0.881	0.823	0.898
Glutamic	0.818	0.999*	0.965	0.911	0.872	0.975	0.325	0.886	0.828	0.903
Proline	−0.958	−0.586	−0.803	−0.887	−0.924	−0.779	−0.944	−0.913	−0.953	−0.897
Glycine	0.909	0.975	0.997*	0.971	0.947	0.999*	0.491	0.956	0.916	0.966
Alanine	0.960	0.932	0.998*	0.996*	0.984	0.995*	0.613	0.988*	0.965	0.993*
Valine	0.989*	0.873	0.980	0.999*	0.999*	0.971	0.716	1.00**	0.992	1.00**
Methionine	0.436	−0.202	0.103	0.260	0.343	0.064	0.881	0.315	0.420	0.280
Isoleucine	0.957	0.582	0.800	0.885	0.923	0.776	0.945	0.911	0.951	0.895
Leucine	0.969	0.918	0.995*	0.998*	0.989*	0.990*	0.641	0.993*	0.974	0.997*
Tyrosine	0.797	1.00**	0.955	0.896	0.854	0.966	0.290	0.869	0.807	0.886
Histidine	0.767	0.999*	0.940	0.873	0.827	0.952	0.243	0.844	0.778	0.863
Lysine	0.802	1.00**	0.958	0.900	0.959	0.968	0.299	0.873	0.813	0.891
Arginine	0.955	0.577	0.797	0.883	0.920	0.773	0.947	0.908	0.950	0.892

*Correlation is significant at the level 0.05.
**Correlation is significant at the level 0.01.

Table 6 Correlation coefficients between the contents of free amino acids and heavy metals in shoots of *Zygophyllum coccineum*

Amino acid	Al	B	Cr	Cu	Fe	Mn	Mo	Pb	V	Zn
Aspartic	0.993	0.998*	0.993	0.915	0.976	0.999*	0.919	0.995	0.998*	1.00**
Threonine	0.744	0.709	0.572	0.908	0.483	0.693	0.313	0.732	0.615	0.658
Serine	0.835	0.806	0.687	0.960	0.608	0.792	0.450	0.825	0.725	0.762
Glutamic	0.974	0.961	0.896	0.998*	0.845	0.954	0.731	0.969	0.918	0.939
Proline	0.969	0.955	0.887	0.999*	0.834	0.948	0.717	0.964	0.910	0.932
Glycine	0.883	0.858	0.753	0.982	0.680	0.846	0.532	0.874	0.786	0.820
Alanine	0.931	0.911	0.822	0.997*	0.759	0.901	0.625	0.924	0.851	0.880
Valine	0.714	0.677	0.536	0.889	0.445	0.661	0.271	0.701	0.580	0.625
Methionine	0.677	0.638	0.491	0.864	0.397	0.620	0.220	0.663	0.536	0.583
Isoleucine	0.706	0.669	0.526	0.884	0.434	0.652	0.260	0.693	0.570	0.615
Leucine	0.752	0.717	0.581	0.913	0.493	0.701	0.324	0.739	0.623	0.666
Tyrosine	0.828	0.799	0.679	0.957	0.598	0.785	0.440	0.818	0.717	0.755
Histidine	0.779	0.746	0.616	0.930	0.530	0.731	0.364	0.768	0.656	0.698
Lysine	0.811	0.780	0.657	0.948	0.574	0.766	0.413	0.801	0.696	0.735
Arginine	0.993	0.985	0.940	0.984	0.899	0.981	0.802	0.991	0.956	0.971

*Correlation is significant at the level 0.05.
**Correlation is significant at the level 0.01.

heavy metals inside the cells, and synthesis of phytochelatins has been particularly concerned, as phytochelatins may chelate heavy metals, leading to detoxification of these metals in cells [51]. The interaction of heavy metals with sulfhydryl-containing amino acids and peptides/proteins plays a major role in their environmental and biochemical behavior [52].

Sixteen types of amino acids were detected in the shoots of the tested plant species from the three sites (NC, WWC, and SIE) (Table 4). Amino acids are divided into three types (i.e. acidic, alkali, and neutral) on the basis of their characters [53].

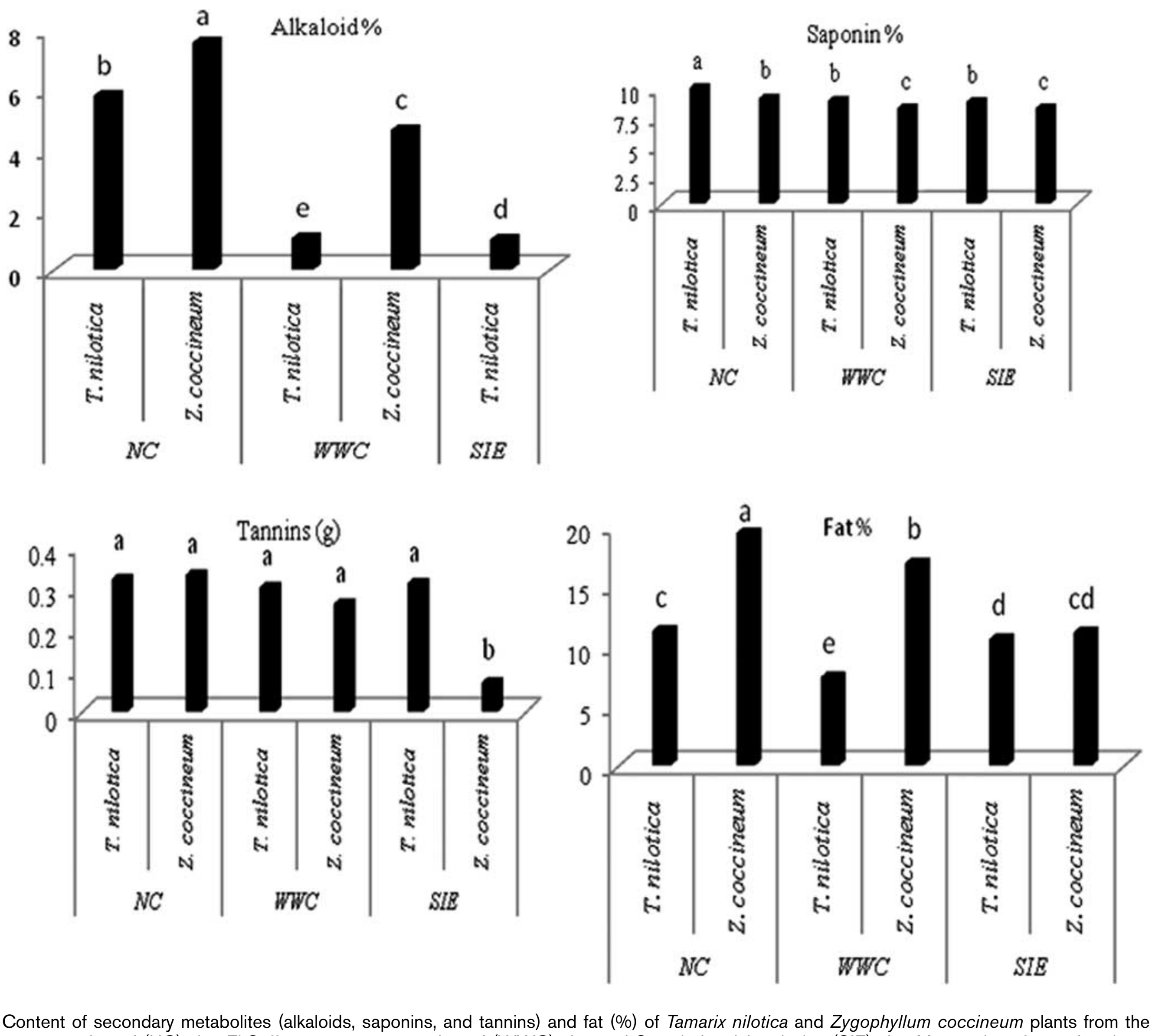
The concentrations of amino acids in *T. nilotica* and *Z. coccineum* plants from the contaminated sites were higher compared with those in plants from the NC site. The most abundant amino acid in all the plant tissues was glutamic acid. Moreover, the concentration of amino acids in plants from the domestic wastewater site was higher compared with that in plants from the SIE site for both tested plants. These results are in agreement with those of Wu *et al*. [54] and of Kováčik *et al*. [55].

On computing correlation coefficients it was revealed that levels of aspartic acid and threonine in shoots of *T. nilotica* were significantly positively correlated with their respective Cr and Mn concentrations (Table 5). As regards the levels of serine, glutamic acid, tyrosine, histidine, and lysine, only boron (B) showed a positive relationship. In case of levels of proline, methionine, isoleucine, and arginine, no correlations were detected. Levels of valine, alanine, and leucine were positively and significantly correlated with more than one metal. Concentrations of Al, Cu, Fe, Pb, and Zn; Cr, Cu, Fe, Mn, Pb, and Zn; and Cr, Cu, Mn, Pb, and Zn, respectively were correlated with levels of valine, leucine, and alanine, respectively.

In *Z. coccineum*, a significant positive correlation was detected between levels of aspartic acid and concentration of B, Mn, V, and Zn in the shoot, whereas levels of glutamic, proline, and alanine correlated with shoot concentrations of Cu (Table 6).

In most agricultural soils, nitrate (NO_3^-) is the most important source of N for plants [56]. For nitrogen metabolism, the nitrate must be taken up across the plasma membrane. Once inside the symplast of a plant,

Figure 8



Content of secondary metabolites (alkaloids, saponins, and tannins) and fat (%) of *Tamarix nilotica* and *Zygophyllum coccineum* plants from the noncontaminated (NC) site, El-Saff wastewater-contaminated (WWC) site, and Suez industrial emission (SIE) site. Mean values for each column having common letters are not significantly different at the 0.05 level.

NO_3^- is reduced to NO_2^- by nitrate reductase (NR), and NO_2^- is converted to NH_4-N by nitrite reductase. The resulting NH_4-N is then assimilated into amino acids, nucleic acids, proteins, chlorophylls, and other metabolites [57]. Factors influencing the enzymatic regulation responsible for N assimilation include: contents of Mo [58] and Cu [59].

The content of amino acids in shoots of *T. nilotica* and *Z. coccineum* plants from the three tested sites were in the order of WWC > SIE > NC, in line with the nitrogen and phosphorus concentrations in plants. The amino acid content (acidic, alkali, and neutral amino acids) showed an increase in plants from the WWC site compared with those from the other sites, which may be due to an elevation of nitrogen, phosphorus, Mo, and Cu concentrations in shoots of the plants (Table 3).

Cruz *et al*. [60] reported that activities of nitrogen metabolism-related enzymes such as nitrate reductase are considerably lower in a low nitrate supply compared with a high supply of nitrates.

Mo, one of the essential microelements for plant growth and the metal component of the Mo cofactor, is responsible for the catalytic activity of NR, aldehyde oxidase, xanthine dehydrogenase, and sulfite oxidase. Mo promotes N accumulation and utilization in wheat plants, which is directly related to nitrate reductase. A higher Mo status also results in higher accumulation and utilization of plant N [58]. Cu exposure results in increase in the concentration of free amino acids [59]. It can be observed that there is superiority of *Z. coccineum* plants in terms of amino acid content compared with *T. nilotica*; this may be due to the higher content of shoot Mo in *Z. coccineum* compared with *T. nilotica* and a genetic variation between the two plants.

Effect of heavy metals on secondary metabolites

Phytochemicals are divided into two main groups according to their function in the plant body: primary and secondary constituents. The primary constituents are sugars, amino acids, proteins, and chlorophyll and the secondary constituents consist of alkaloids, terpenoids, saponins, flavonoids, tannins, and phenolic compounds [61].

The content of secondary metabolites (tannins, saponins, and alkaloids) and fat were lower in plants from the polluted sites compared with those from the NC site. The tannin content ranged from 0.07 to 0.33 g, saponin from 9.99 to 8.22%, and alkaloids from 7.95 to 1.00%. Moreover, the maximum tannin and alkaloid contents were detected in *Z. coccineum* from the NC site (Fig. 8).

Heavy metal-induced changes in the phenolic compounds may further affect their functions in plant cells. Phenolic compounds, including tannins, are often involved in responses to different kinds of abiotic and biotic stresses [62].

Cobbett and Goldsbrough [63] hypothesized that secondary metabolism may be an integral part of the plant's capacity to modify metabolic processes to survive and grow in adverse conditions, including in the presence of phytotoxic metals.

Individual plant species differ in their capacity to modify their metabolism to tolerate or accumulate heavy metals. The modifications may involve sequestration of the metals in vacuoles, biosynthesis of organic compounds that detoxify these metals, or synthesis of modified tissues to exclude the contaminant [64]. These processes often alter the uptake and distribution of other metal ions, as was seen in the present study with altered heavy metal concentrations in both tested plant tissues. A consequence of this modified metabolism may include the loss of specific enzymes or nonessential biomolecular synthetic processes such as secondary metabolite biosynthesis.

Conclusion

These results prove that industrial pollutants and their metal contamination can change the chemical composition of the soil and its properties, which reflects on some medicinal plants, thereby, seriously impacting the quality, safety, and efficacy of natural plant products produced by medicinal plant species. The plants from polluted areas cannot be used as herbal medicine. It is also important to implement good quality control practices for screening of herbal medicines to protect consumers from toxicity. The data presented in this study provide the evidence of the detrimental effects of naturally occurring or industrially generated metal contamination in *T. nilotica* and *Z. coccineum.*

The plants collected from the investigated sites pose a serious danger; however, a periodical assessment of plants used for traditional medicine should be encouraged as this will assist in ensuring their quality and safety in herbal use, especially for people living in urban areas where the level of pollution may be very high.

Amino acids are well-known biostimulants that have positive effects on plant growth and yield. The higher content of amino acids in the studied plant species from the contaminated sites led us suggest extraction of amino acid and their usage as foliar sprays for different plant species (agricultural uses), especially plants of *Z. coccineum* that have a short life cycle. Further studies are warranted to extract these amino acids and to ensure the safety and heavy metal-free status of these amino acids for their use.

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Conflicts of interest

There are no conflicts of interest.

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