ASSESSING MACRO-NUTRIENT REMOVAL POTENTIAL OF NINE NATIVE PLANT SPECIES GROWN AT A SEWAGE SLUDGE DUMP SITE

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Abstract. Searching for new plants that could phytoextract soil macro-nutrients is one of the main research interests nowadays. In the present study, nine native plant species were collected to determine their potential to uptake six macro-nutrients from the soil of a sewage sludge dump site (SS) in comparison with a reference site (RS). The results showed that the studied native plants can accumulate the macro-nutrients in their tissues regardless of their size or vegetative stages. The concentrations of all macro-nutrients (except K) in the tissues of the most studied plant species were positively correlated with those in the soil. The stoichiometric ratios vary among species and depend on many limiting factors. The values of bioaccumulation and translocation factors were > 1.0, indicating the high tendency of the plants to accumulate the macro-nutrients. *Portulaca oleracea* growing in the SS accumulated larger quantities of macro-nutrients compared to two perennials growing at the same place. *Phragmites australis, Rumex dentatus, Portulaca oleracea, Bassia indica, Amaranthus viridis* and *Pluchea dioscoridis* from the SS; and *Portulaca oleracea, Rumex dentatus, Pluchea dioscoridis* and *Solanum nigrum* from the control site showed the highest element accumulation indices, which could be recommended for the phytoextraction of macro-nutrients. We recommend using the studied species in mitigation of the eutrophic status of agricultural soil amended with sewage sludge.
Keywords: *bioindicator, biosolids, Nile delta, nutrient elements, phytoextraction, wastewater treatment plants, wild plants*

Introduction

Sewage sludge is defined as an organic byproduct of the treatment of municipal and/or industrial wastewater. It contains high amounts of macro-nutrients and heavy metals (Eid and Shaltout, 2016). It is used as fertilizers in many agricultural lands and it has significant positive influences on soil fertility and plant macro-nutrients (Aráujo et al., 2007). On the other hand, the presence of toxic levels of heavy metals in the sludge

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due to the contribution of industrial effluents may have adverse effects on agriculture and food chain (Singh and Agrawal, 2007; Eid et al., 2018a). Moreover, availability of nitrogen and phosphorus from sludge-amended soils and their transfer in runoff may lead to eutrophication of soil and downstream surface water (Quilbé et al., 2005). The worldwide production of sewage sludge is increasing with the populations, as well as their use as soil amendments on agricultural lands (Moreno-Caselles et al., 1997; Eid et al., 2018a). Numerous studies on the use of sewage sludge as soil amendments have been conducted on agricultural crops, wood- and wetlands (e.g., Fuentes et al., 2007; Yu et al., 2014; Eid et al., 2017a, b, 2018b, 2019, 2020a, b, c). Few studies were conducted on wild native plants (e.g., Ho and Wong, 1994; Korboulewskya et al., 2002; Farahat and Linderholm, 2013; Eid and Shaltout, 2016).

Native plants are key species in the ecosystems and play important roles in degrading and removing macro-nutrients, heavy metals, and other pollutants from the environment (Eid and Shaltout, 2016). This green technology is called "Phytoremediation" (Peng et al., 2008). It includes many methods, one of them is phytostabilization, where plants are used to immobilize metals and store them in their below-ground tissues and/or soil. In addition, plants may play an important role in element removal (phytoextraction) through many ways, such as absorption and cation exchange (Chandra et al., 2017).

Many studies were conducted to investigate the influences of sewage sludge on the growth, yield and solute contents in crops and forest trees (e.g. Henry et al., 1994; Antolín et al., 2010; Singh and Agrawal, 2010). Some other studies tried to identify the health risks that could be associated with using sewage sludge and/or sewage wastewater in agriculture (e.g., Singh and Agrawal, 2007; Galal, 2016; Farahat et al., 2017; Eid et al., 2018a). Most of these studies were interested in evaluating the role of plants in phytoremediation of heavy metals, but not macro-nutrients. The soil pollution by macro-nutrients is unusual, but especially problematic when involves eutrophication. These levels of high concentrations of macro-nutrients in the soil will adversely affect the biodiversity of native species and it may introduce only the species that grow well on eutrophic soil (El-Sheikh et al., 2012; Shaltout et al., 2019). In addition, over-use of sewage sludge in agricultural practices may lead to over concentrations of macro-nutrients that lead to eutrophication of soil and downstream surface water (Quilbé et al., 2005). The application of sewage sludge in high quantities to the agricultural lands without prior knowledge to its chemical structure may lead to presence of high non-desirable concentrations of macro-nutrients (Quilbé et al., 2005). Presence of native species that able to uptake extra concentrations of macro-nutrients and reduce the macro-nutrient loads in soil will represent a good service to the ecosystem.

Wild plants play an important role in macro-nutrient cycling through uptake, sequestering and release of macro-nutrient elements (Eid et al., 2012a). Utilization of these wild plants as phytoremediators in eutrophic habitats requires emphasis on the effect of macro-nutrient loadings on these plants, but this effect has received little attention and is still not well understood (Eid et al., 2010; Zhao et al., 2013). According to the authors' knowledge, so far no studies have been carried out in Egypt on the grown plant of native species at a sewage sludge dump site in comparison with the reference site in the context of their usefulness for macro-nutrients phytoremediation. Therefore, the wider objective of this study was to assess the removal efficiency of macro-nutrients by nine native plant species grown at a sewage sludge dump site (SS) in Egypt. The specific objectives of the present study were to: (1) to assess the ability of nine Tropical and Mediterranean native plant species grown on SS to accumulate

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macro-nutrients in their tissues compared to a reference site (RS); and (2) to examine the roles of these nine species as potential bioindicators of macro-nutrients. The results of the present study could be useful when designing a phytoremediation system.

Materials and methods

Study area

The study area is the dump site of Kafr El-Sheikh Wastewater Treatment Plant in the northern Nile Delta, Egypt (Lat. 31° 05′ 05.42″ N, Long. 30° 57′ 43.24″ E), and the adjacent agricultural farms are the reference site (RS) (Lat. 31° 04′ 53.46″ N, Long. 30° 57′ 31.60″ E). The RS is located about 1 km away from the dump site and its soil type is clay. The climatic conditions are warm summers (20 to 30 °C) and mild winters (≥10 °C) (EMA, 1980).

Field sampling process

The sampling process was carried out at six locations, three of which represent each of the SS and RS (*Fig. 1*). Nine native plant species dominating the study area were chosen at each site (*Amaranthus viridis* L.: *Amaranthaceae*, slender amaranth, annual herb, often 60-80 cm tall; *Bassia indica* (Wight) A. J. Scott: *Amaranthaceae*, kochia, annual richly branched herb, often 2 m tall; *Conyza bonariensis* (L.) Cronquist: *Asteraceae*, flax-leaf fleabane, annual herb, often 75 cm tall; *Portulaca oleracea* L.: *Portulacaceae*, common purslane, glabrous fleshy herb, may reach 40 cm in height; *Rumex dentatus* L.: *Polygonaceae*, toothed dock, annual or biennial herb, erect stem up to 70-80 cm in height; *Solanum nigrum* L.: *Solanaceae*, black nightshade, herb or short-lived perennial shrub, often 30-120 cm tall; *Lycopersicon esculentum* Mill.: *Solanaceae*, tomato, perennial in native habitat, but cultivated as annual, grows up to 1-3 m in height. *Phragmites australis* (Cav.) Trin. ex Steud.: *Poaceae*, common reed, perennial robust reed, erect stem up to 5 m in height; and *Pluchea dioscoridis* (L.) DC.: *Asteraceae*, camphorweeds, richly branched hairy shrub, often 2-3 m high). For each plant species, three mature and healthy replicates were collected from each sampling location during May 2014. Plants were identified following Boulos (1999, 2002, 2005). In the laboratory, samples were carefully washed with tap water over a 4 mm mesh sieve to minimize material loss, separated into leaves, stems and roots, oven dried at 85 °C to a constant weight, and then ground using a metal-free plastic mill. Roots were collected by excavated carefully around the root system of each plant. At each sampling location, a composite soil sample was collected as a profile from three holes, each about 20 cm deep. The soil samples were air dried and passed through a 2 mm sieve to separate gravel and debris.

Chemical analysis

Soil samples were analyzed for organic matter (OM) content using loss-on-ignition method at 550 °C for 2 h (Wilke, 2005). Soil-water extracts at 1:5 were prepared for the determination of pH and electric conductivity (EC). Macro-nutrients (except N) in soil and plant samples were extracted from 0.5 to 1 g using a mixed-acid digestion method. EC and pH were measured using conductivity and pH meters, respectively. Calcium, Mg, Na and K were determined by atomic absorption, while total P was determined by spectrophotometer using the ammonium-molybdate method. Total N was determined in

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plant and soil samples using a CHN Elemental Analyzer (Yanako CHN Corder MT-5, Japan). All these procedures are outlined in Allen (1989) and APHA (1998).

Figure 1. *Location map of study area showing the sampling sites. SS: sewage sludge dump site, RS: reference site*

Calculations

The bioaccumulation factor (BF) was calculated to determine the efficiency of the plant for accumulating a macro-nutrient from the soil (Xiao et al., 2011):

$$BF = \frac{\text{Concentration of a macronutrient in belowground tissues (mg/kg)}}{\text{Concentration of the same macronutrient in soil at the same site (mg/kg)}} \quad \text{(Eq.1)}$$

The translocation factor (TF) was calculated to depict the ability of plants to translocate a macro-nutrient from below- to above-ground tissues (Gupta et al., 2008):

$$TF = \frac{\text{Concentration of a macronutrient in aboveground tissues (mg/kg)}}{\text{Concentration of a macronutrient in belowground tissues (mg/kg)}} \quad \text{(Eq.2)}$$

The element accumulation index (EAI) was used to assess the overall performance of macro-nutrient accumulation in plants (Liu et al., 2007):

$$EAI = (1/N)\sum_{j=1}^{N} Ij;\ Ij = \frac{x}{\delta x} \quad \text{(Eq.3)}$$

where N is the total number of the analyzed macro-nutrients and I_j is the sub-index for a macro-nutrient j, obtained by dividing the mean concentration of a macro-nutrient (x) by its standard deviation (δx).

Statistical analysis

Before performing an analysis of variance (ANOVA), the data were tested for their normality of distribution (Shapiro-Wilk's W test) and homogeneity of variance (Levene's

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test), and when necessary, the data were log-transformed. Macro-nutrients data for nine plant species were subjected to a three-way ANOVA (the assumptions have been met) to identify the interactions in the independent variables (species, tissues, and sites). The significance of variation in the relative concentrations of the different macro-nutrients (considering whole plant), one species at a time at the two different sites (SS/RS) was assessed using a paired *t*-test, while one-way ANOVA was used to analyze the variation among species within the same site. The significant differences between means, among the nine plant species were identified using the Tukey HSD test at $P < 0.05$. The significance of variation in soil quality parameters between the SS and RS was assessed using a paired *t*-test. EAI data for nine plant species were subjected to a two-way analysis of variance (ANOVA-2) to test the differences between species and sites. Correlations between the concentrations of macro-nutrients in plant tissues and soil samples (the SS and RS) were evaluated using the Pearson's *r* coefficient. Statistical analyses were carried out using Statistica 7.1 (Statsoft, 2007). Stochiometric ratios were calculated for some of the analyzed macro-nutrients. To identify statistically significant differences in the stochiometric ratios among different sites, the paired *t*-test was performed.

Results

The soil of the SS was rich in OM, N, P, Ca and it was also slightly acidic, while EC, Mg, Na and K concentrations of the RS were significantly higher than those of the SS (*Table 1*). Macro-nutrient concentrations in the soil of the SS had the following sequence: Ca > N > Mg > Na > K > P; while in soil of the RS the sequence was: Ca > Na > Mg > K > N > P.

Table 1. *Mean ± standard error (n = 3) of soil physico-chemical characteristics for sewage sludge dump site and reference site, where the present study was carried out*

Characteristics	Sewage sludge dump site	Reference site	*t*-value	*p*
EC (mS cm^{-1})	0.04 ± 0.01	3.46 ± 0.08	38.9	0.001
pH	6.40 ± 0.08	7.76 ± 0.03	14.8	0.000
OM (%)	51.50 ± 0.23	6.74 ± 0.07	148.6	0.000
N (mg g^{-1})	30.27 ± 0.52	2.00 ± 0.23	37.9	0.001
P (mg g^{-1})	2.36 ± 0.09	0.89 ± 0.02	14.9	0.000
Ca (mg g^{-1})	53.36 ± 0.57	40.90 ± 1.39	10.1	0.000
Mg (mg g^{-1})	6.34 ± 0.18	8.29 ± 0.29	6.3	0.000
Na (mg g^{-1})	6.22 ± 0.26	8.50 ± 0.20	15.5	0.000
K (mg g^{-1})	4.69 ± 0.07	6.03 ± 0.13	14.7	0.000

t-values represent the paired t-test, EC: electric conductivity, OM: organic matter content

Regarding the studied plant species, concentrations of N, P and Na were significantly affected by site; those of N, Ca, Mg and Na were significantly affected by plant species, while those of N, Ca, Mg and K were significantly affected by plant tissue (*Table 2*). N, P and K concentrations were significantly higher in the plant species of the SS as compared to the RS (*Table 3; Appendix 1*). In addition, concentrations of N, P, Ca, Mg and Na were higher in the leaf; but K was higher in the stem. The highest N, P and K concentrations (48.2, 3.2 and 80.2 mg g^{-1}, respectively) was observed in *P. oleracea*

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grown at the SS, while the lowest (17.7, 0.3 and 17.7 mg g^{-1}, respectively) was observed in *P. dioscoridis*, *A. viridis*, *C. bonariensis* and *P. australis* grown at the RS (*Table 3*). The highest Ca and Mg concentrations (45.1 and 15.2 mg g^{-1}) were observed in *L. esculentum* and *P. oleracea* has grown at the RS, while the lowest (8.9 and 1.4 mg g^{-1}) were observed in *P. australis* has grown at the same site. The highest Na concentration (53.0 mg g^{-1}) was recorded in *B. indica* has grown at the SS, while the lowest (2.8 mg g^{-1}) was detected in *S. nigrum* has grown at the RS.

Table 2. *Results of three-way ANOVA (F-values) of macro-nutrients concentrations of nine native plant species in north Nile Delta, Egypt*

Effect	*df*	Dependent variables					
		N	**P**	**Ca**	**Mg**	**Na**	**K**
Site	1	55.4**	9.2*	0.1^{ns}	2.1^{ns}	9.3*	1.3^{ns}
Species	8	4.4*	0.7^{ns}	6.4**	5.5**	4.8**	2.2^{ns}
Tissue	3	63.1***	4.5^{ns}	16.7**	8.2**	2.6^{ns}	4.8*
Site × Species	8	1.3^{ns}	2.7*	4.3**	4.7**	1.9^{ns}	4.3**
Site × Tissue	3	0.8^{ns}	0.6^{ns}	0.2^{ns}	1.2^{ns}	1.2^{ns}	2.6^{ns}
Species × Tissue	16	2.0^{ns}	1.1^{ns}	1.9^{ns}	10.2***	4.9**	4.4**
Site × Species × Tissue	16	36.5***	20.9***	22.7***	16.3***	53.9***	18.9***

Site: Sewage sludge dump site/Reference site, Species: nine native plant species, Tissue: fruit/leaf/stem/root. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ns: not significant (i.e., $p > 0.05$), df: degree of freedom

All the investigated species were characterized by a bioaccumulation factor (BF) > 1.0 for some macro-nutrients (*Fig. 2*). *A. viridus* showed the higher BF value for K (14.4), *P. oleracea* showed higher BF values for N (1.3), P (1.6) and Mg (1.5), *R. dentatus* showed the higher BF value for Na (3.3). At the RS, *B. indica* showed the higher BF value for Na (2.8), *P. oleracea* showed higher BF values for N (12.3) and Mg (0.9), *L. esculentum* showed higher BF values for P (3.6) and Ca (1.1), and *P. dioscoridis* showed the higher BF value for K (6.9).

In the present study, the translocation factor (TF) varied among the studied sites, plant species and among the macro-nutrients (*Fig. 3*). Regarding the SS, *A. viridus* had the highest TF for Mg (3.8), *B. indica* for Na (5.2), *P. oleracea* for K (1.6), *S. nigrum* for N (2.2), *L. esculentum* for P (2.0) and *P. dioscoridis* for Ca (2.3). At the RS, *B. indica* had the highest TF for P (98.0), *C. bonariensis* for Mg (3.12), *P. oleracea* for Ca (1.9) and Na (3.4), and *R. dentatus* for N (4.1) and K (2.0).

Element accumulation index (EAI) were significantly higher in all plant species at the SS as compared to the RS (except *C. bonariensis* and *P. dioscoridis; Fig. 4*). The species with the highest EAI at the SS were *P. oleracea* (41.6), followed by *P. australis* (30.6), *R. dentatus* (27.7), *P. dioscoridis* (27.4), *A. viridis* (26.9) and *B. indica* (26.7). In contrast, *C. bonariensis* showed the lowest EAI value (19.2). In addition, *P. oleracea* (31.0), followed by *P. dioscoridis* (29.5), *R. dentatus* (24.7) and *S. nigrum* (24.5) that had the highest EAI in the RS, while *P. australis* (16.3) had the lowest. Positive linear correlations were detected between N, P, Ca, Mg and Na concentrations in all tissues of *A. viridus* and the soil of both sites (*Appendix 2*); N, P, Ca and Na for *B. indica*; N and P for *C. bonariensis*; N, P, Ca and Na for *P. oleracea*; Ca for *R. dentatus*; N, P, Mg and Na for *S. nigrum*; N and Na for *L. esculentum*; N, P, Ca and Na for *P. australis*; N for *P. dioscoridis*.

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Table 3. *Mean macro-nutrients concentrations ± standard errors in nine native plant species that grown at sewage sludge dump site (SS) and reference site (RS) in north Nile Delta, Egypt. (Detailed values for each tissue are present in Appendix 1)*

Species	Site	Macro-nutrient (mg g^{-1})					
		N	**P**	**Ca**	**Mg**	**Na**	**K**
A. viridis	SS	40.9 ± 4.5bc	2.0 ± 0.2bc	40.8 ± 3.0b	6.3 ± 1.5b	3.3 ± 0.2a	65.6 ± 6.8bc
	RS	23.7 ± 4.8a	0.3 ± 0.1ab	29.0 ± 1.9bc	11.5 ± 1.5c	7.0 ± 0.4a	49.9 ± 5.7b
	***t*-value**	**22.2*** **	**29.8*** **	**9.3*** **	**10.3*** **	**7.4*** **	**4.3** **
B. indica	SS	33.4 ± 4.4ab	2.8 ± 0.1cd	16.9 ± 1.5a	4.2 ± 0.6ab	37.5 ± 7.3d	44.3 ± 3.5a
	RS	25.5 ± 3.7a	0.7 ± 0.2abc	11.0 ± 0.8a	3.2 ± 0.2ab	53.0 ± 7.3c	46.5 ± 2.2b
	***t*-value**	**9.1*** **	**16.3*** **	**5.7*** **	**2.4***	**6.6*** **	**0.6^{ns}**
C. bonariensis	SS	29.4 ± 3.5ab	2.1 ± 0.4bc	13.9 ± 2.1a	2.8 ± 0.5ab	14.2 ± 1.3bc	39.0 ± 2.3a
	RS	22.7 ± 3.9a	0.3 ± 0.1a	17.6 ± 1.2ab	3.2 ± 0.6ab	14.6 ± 2.9ab	38.0 ± 2.5b
	***t*-value**	**3.4** **	**5.2** **	**2.2^{ns}**	**0.9^{ns}**	**0.2^{ns}**	**0.3^{ns}**
L. esculentum	SS	41.8 ± 1.9c	0.5 ± 0.2a	36.4 ± 5.8b	5.8 ± 0.7ab	9.6 ± 1.2abc	46.3 ± 2.5a
	RS	29.1 ± 1.9a	1.5 ± 0.3cd	45.1 ± 8.2d	5.9 ± 0.9ab	13.3 ± 1.5ab	46.8 ± 4.2b
	***t*-value**	**15.0*** **	**2.8***	**2.0^{ns}**	**0.2^{ns}**	**7.4*** **	**0.2^{ns}**
P. australis	SS	24.5 ± 3.1a	1.6 ± 0.3b	14.6 ± 1.7a	2.1 ± 0.4a	4.4 ± 0.3ab	33.2 ± 1.2a
	RS	18.9 ± 2.7a	0.4 ± 0.2ab	8.9 ± 0.2a	1.4 ± 0.3a	13.9 ± 2.0ab	17.7 ± 1.6a
	***t*-value**	**6.2*** **	**2.7***	**3.2** **	**1.0^{ns}**	**7.1*** **	**7.2*** **
P. dioscoridis	SS	27.8 ± 3.2ab	1.5 ± 0.3b	18.0 ± 3.9a	4.4 ± 0.7ab	14.4 ± 1.8bc	36.8 ± 2.4a
	RS	17.7 ± 3.9a	1.2 ± 0.3abcd	19.9 ± 2.3ab	3.7 ± 0.6ab	13.4 ± 2.3ab	48.6 ± 2.0b
	***t*-value**	**7.0*** **	**2.1^{ns}**	**1.2^{ns}**	**1.9^{ns}**	**1.1^{ns}**	**2.9***
P. oleracea	SS	48.2 ± 2.9c	3.2 ± 0.4d	27.5 ± 4.7ab	13.8 ± 1.9c	5.2 ± 0.5ab	80.2 ± 10.1c
	RS	30.6 ± 2.4a	1.6 ± 0.1d	17.5 ± 2.8ab	15.2 ± 2.5c	22.8 ± 4.2b	51.8 ± 6.5b
	***t*-value**	**4.4** **	**5.0** **	**5.3** **	**1.5^{ns}**	**4.8** **	**6.4*** **
R. dentatus	SS	24.6 ± 2.5a	1.6 ± 0.3b	20.0 ± 0.7a	4.9 ± 0.2ab	18.8 ± 0.6c	37.2 ± 1.2a
	RS	19.5 ± 5.2a	1.3 ± 0.3bcd	15.9 ± 1.0ab	6.4 ± 0.7b	26.3 ± 4.2b	37.1 ± 3.8b
	***t*-value**	**1.9^{ns}**	**0.7^{ns}**	**3.6** **	**2.8***	**1.7^{ns}**	**0.1^{ns}**
S. nigrum	SS	31.8 ± 5.2ab	2.2 ± 0.2bcd	22.4 ± 0.6a	2.6 ± 0.3ab	2.8 ± 0.4a	50.3 ± 3.0ab
	RS	22.5 ± 4.8a	1.4 ± 0.1cd	37.6 ± 0.8cd	5.1 ± 0.4ab	6.1 ± 0.1a	51.4 ± 3.3b
	***t*-value**	**6.0*** **	**7.6*** **	**19.3*** **	**24.4*** **	**10.3*** **	**1.1^{ns}**

t-values represent the paired t-test. Means in the same columns (for each site) followed by different letters are significantly different at $p < 0.05$ according to Tukey HSD test. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ns: not significant (i.e., $p > 0.05$)

Regarding the stoichiometric ratios, both SS and RS were only significantly different in K/Na and P/K ratios at $p < 0.01$ (*Table 4*). For individual plant species, it was noticed that the following stochiometric ratios were significantly different among sites: P/K in *B. indica*, N/P and K/Na in *A. viridus*, N/P in *C. bonarensis*, K/Na in *P. australis*. On the contrary, *S. nigrum*, *P. dioscoridis* and *L. esculemtum* showed no significant differences in the estimated stoichiometric ratios among sites.

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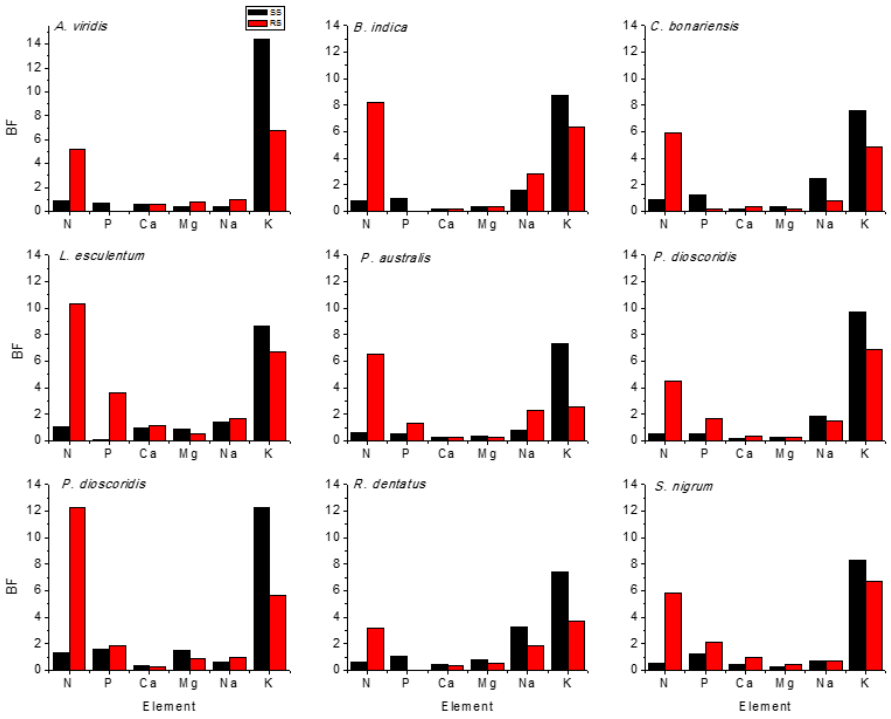

Figure 2. *Mean (n = 3) of bioaccumulation factors (BFs) from soil to below-ground tissues of macro-nutrients in nine native plant species that grown at sewage sludge dump site (SS) and reference site (RS) in north Nile Delta, Egypt*

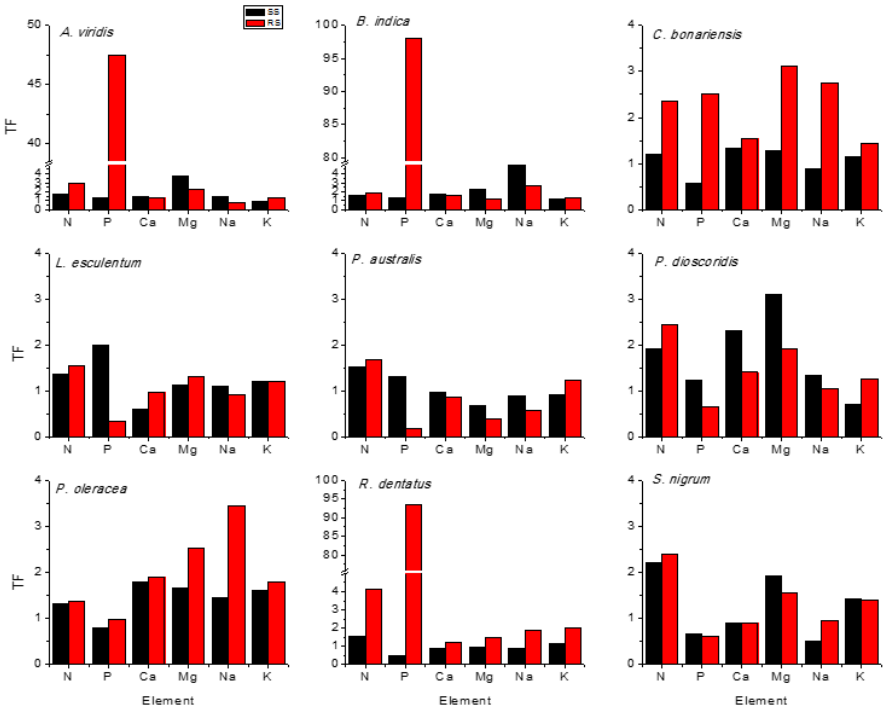

Figure 3. *Mean (n = 3) of translocation factors (TFs) from below- to above-ground tissues of macro-nutrients in nine native plant species that grown at sewage sludge dump site (SS) and reference site (RS) in north Nile Delta, Egypt*

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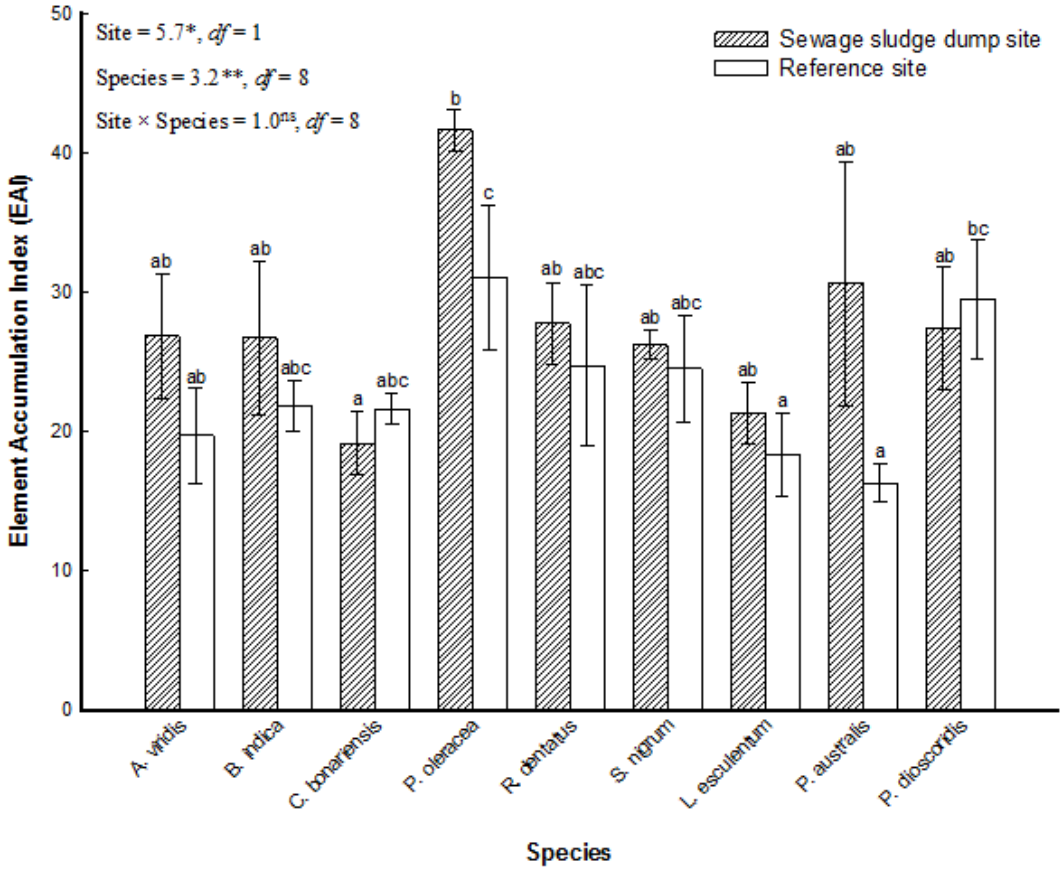

Figure 4. *Element accumulation index (EAI) in nine native plant species that grown at sewage sludge dump site and reference site in north Nile Delta, Egypt. Site: Sewage sludge dump site/Reference site, Species: 9 native plant species, *: $p < 0.05$, **: $p < 0.01$, ns: not significant (i.e., $p > 0.05$), df: degree of freedom. Means followed by different letters are significantly different at $p < 0.05$ according to Tukey HSD test*

Discussion

The collected soil from the SS was characterized by a higher N concentration than the RS, which could be ascribed to the high organic matter in its contents. The present study showed that the tissues of the studied plant species were characterized by high macro-nutrient concentrations at the SS more than at the soil of agricultural farms (control). This could be ascribed to the low pH value of the SS and its high concentrations of these elements. The results of some previous investigations indicated that the land application of sewage sludge increased soil N and P content (Bai et al., 2012), and plant uptake of N and P (Andres et al., 2010).

The uptake of macro-nutrients by plants varies among species and depends on the macro-nutrient demands of each species to apply their physiological and biochemical requirements. Plants differ both in their capacity to acquire macro-nutrients from the soil and in the amount of macro-nutrients they need per unit growth, the macro-nutrient concentrations in their tissue, and the time and extent to which they withdraw macro-nutrients during leaf senescence before leaf abscission (Kabata-Pendias, 2011). Lambers et al. (2008) reported that plants differ in the concentration of macro-nutrients in their

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tissue, depending on environment, allocation to woody and herbaceous tissues, developmental stage, and species. Moreover, N, P, and K are the macro-nutrients that most frequently limit plant growth. However, N tends to limit plant productivity on young soils, whereas P becomes increasingly limiting as soils age. The presence of high concentrations of N, P, Ca and K in the sampled plants from the SS compared to control site because these elements are essential for the plant growth. However, the presence of a specific mineral in plant tissues does not imply that the plant needs this mineral for growth. For example, high Na concentrations are not required for growth (Lambers et al., 2008).

Table 4. *Stoichiometric ratios (±standard deviations) of the studied nine plant species that grown at sewage sludge dump site (SS) and reference site (RS) in north Nile Delta, Egypt. Bold values of the ratios in each column are significantly different among sites at $p < 0.05$. F-values and significance level are shown for each column*

Species	Site	Ca/Mg	N/P	K/Na	N/K	P/K
A. viridus	SS	9.1 ± 5.0	**20.5 ± 3.1**	**20.4 ± 7.6**	0.7 ± 0.6	0.04 ± 0.02
	RS	2.8 ± 0.9	**73.1 ± 28.8**	**7.2 ± 2.4**	0.6 ± 0.5	0.01 ± 0.01
B. indica	SS	4.3 ± 0.7	12.1 ± 4.4	2.0 ± 1.9	0.8 ± 0.6	**0.1 ± 0.02**
	RS	3.6 ± 1.2	41.2 ± 6.7	1.0 ± 0.5	0.5 ± 0.2	**0.01 ± 0.0**
C. bonariensis	SS	5.1 ± 0.6	**17. 7 ± 9.4**	2.9 ± 0.7	0.7 ± 0.2	0.05 ± 0.03
	RS	6.9 ± 3.0	**95.3 ± 21.8**	3.4 ± 1.6	0.6 ± 0.3	0.01 ± 0.0
L. esculentum	SS	5.8 ± 3.1	183.2 ± 6.7	6.0 ± 4.0	0.9 ± 0.3	0.01 ± 0.0
	RS	6.8 ± 3.4	40.3 ± 6.5	4.6 ± 1.0	0.7 ± 0.4	0.04 ± 0.0
P. australis	SS	7.8 ± 2.1	17.4 ± 11.3	**7.6 ± 0.3**	0.8 ± 0.6	0.05 ± 0.01
	RS	7.5 ± 2.8	10.9 ± 0.0	**1.8 ± 1.7**	1.0 ± 0.2	0.03 ± 0.01
P. dioscoridis	SS	4.1 ± 1.6	24.3 ± 17.5	2.9 ± 1.3	0.8 ± 0.5	0.04 ± 0.0
	RS	5.7 ± 1.1	26.5 ± 5.5	5.0 ± 3.8	0.4 ± 0.2	0.02 ± 0.0
P. oleracea	SS	1.9 ± 0.2	18.3 ± 2.8	17.0 ± 9.7	0.6 ± 0.1	0.05 ± 0.01
	RS	1.2 ± 0.3	18.7 ± 3.9	2.8 ± 1.4	0.7 ± 0.3	0.04 ± 0.02
R. dentatus	SS	4.1 ± 0.4	29.2 ± 4.5	2.0 ± 0.3	0.7 ± 0.3	0.04 ± 0.0
	RS	2.6 ± 0.4	31.3 ± 9.1	1.5 ± 0.3	0.5 ± 0.3	0.03 ± 0.0
S. nigrum	SS	9.8 ± 4.9	15.5 ± 8.1	21.3 ± 10.4	0.6 ± 0.3	0.05 ± 0.0
	RS	7.9 ± 2.7	17.3 ± 11.6	8.5 ± 2.2	0.4 ± 0.02	0.03 ± 0.0
F-value		0.7	0.001	7.7	3.2	8.5
p		0.4	0.9	**0.008**	0.08	**0.005**

The presence of high macro-nutrients concentrations in leaves compared to the other tissues because leaves are the main sink for minerals in plants. Macro-nutrients associated with metabolism (e.g., N, P, and K) have their highest concentrations when a leaf or other tissue is first produced, then concentrations decline by dilution during cell wall formation and then by resorption of macro-nutrients during senescence, while the roots have intermediate concentrations. Species differ in the macro-nutrient requirement for growth but the physiological mechanisms for this are not always known (Woodward et al., 1984). The variations in macro-nutrient concentrations in various parts of plants have been ascribed to compartmentalization and translocation through the vascular

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system (Kim et al., 2003). As stem plays the role of a transferring tissue, minimum concentrations of macro-nutrients were found in stem (Planquart et al., 1999).

The evaluation of the bioaccumulation factor (BF) represents a simple method to characterize quantitatively the transfer of available macro-nutrients from the soil to the plant (Branzini et al., 2012; Farahat et al., 2017), while its ability to translocate them from the roots to the shoots is measured using the translocation factor (TF). Both BF and TF can be used to estimate a plant's potential for phytoremediation purposes (Galal and Shehata, 2015). According to Zu et al. (2005), BF > 1.0 were found in macro-nutrient- and heavy metal-accumulating plants, whereas they were typically < 1.0 in macro-nutrient- and heavy metal-excluding plants. TF > 1.0 indicate that the plant is effective in the translocation of macro-nutrients from root to shoot tissue (Ma et al., 2001). In the present study, all studied species were characterized by BF values > 1.0 for some of the macro-nutrients, showing that they can accumulate macro-nutrients and are therefore more suitable for phytoextraction purposes. In the present study, the accumulation of macro-nutrients was done regardless of their stem size or life form of the species. For instance, *P. oleracea* as short (20-40 cm) annual herbaceous plant tend to accumulate high concentrations of N, P and K more than other perennial tall species e.g., *P. dioscoridis* and *P. australis*. The same observations were reported by Farahat and Linderholm (2013) for medicinal native plants that irrigated by sewage wastewater. Although the BF values for some macro-nutrients were > 1.0, which means that our study species could accumulate these macro-nutrients, but none of them are hyper-accumulator (Kabata-Pendias, 2011).

The presence of TF values > 1.0 for N, P, Ca, Mg, and K mean that plants allocate the macro-nutrients in their vegetative parts because it is essential for growth. This behavior is opposite in many cases to the TF values for heavy metals that estimated in many aquatic and terrestrial plants (e.g., Galal and Farahat, 2015; Galal and Shehata, 2015; Eid et al., 2018a). This could be attributed to the fact that macro-nutrients are more essential for plant growth than heavy metals (Lambers et al., 2008). As a rule, when a plant has TF value > 1.0 for a certain mineral, this indicates the its suitability for the phytoextraction of this mineral, while TF value < 1.0 indicates its suitability for the phytostabilization of this mineral. For instance, *A. viridus* and *B. indica* are good phytoextractor for N, P, Ca, Mg and Na

Plant species accumulate different elements simultaneously, so the element accumulation index (EAI) was used to assess the overall performance of macro-nutrient accumulation in the studied plant species. Thus, in the present study, the highest EAI values of *P. oleracea*, *P. australis*, *R. dentatus*, *P. dioscoridis*, *A. viridis* and *B. indica* from the SS; and *P. oleracea*, *P. dioscoridis*, *R. dentatus* and *S. nigrum* from the cultivated field soil, indicates that these species are better able to accumulate macro-nutrients, and are therefore more suitable for phytoextraction purposes.

The stoichiometric ratios vary among species and depends on many limiting factors. For instance, N:P ratios are, on average, higher in graminoids than in forbs and higher in stress-tolerant species than in ruderals. This ratio declines with increasing growth rates (Lambers et al., 2008). The whole-plant biomass N:P ratios may vary up to 50-fold, due to differences in macro-nutrient uptake, biomass turnover, root allocation, and reproductive output (Aerts and Chapin, 2000). In the present study, there were few ratios that are significantly different among sites and species. In our opinion the availability of water and macro-nutrients in the studied sites interacts differently according to the species. This agrees with the findings of Lü et al. (2012) and Farahat

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and Linderholm (2015). They reported that in rich N habitats, water availability can modulate the plant nutritional and stoichiometric responses to increased N and other macro-nutrients.

The present study proved that the concentrations of all macro-nutrients (except K) in the tissues of the most studied plant species were positively correlated with those in the soil. Such correlations indicate that these species reflect the cumulative effects of environmental pollution from the soil, and thereby suggesting their potential use in the biomonitoring of most macro-nutrients examined. This indication is supported by several studies according to which the total quantity of some macro-nutrients in a soil is correlated with the quantity of macro-nutrients absorbed by plants (Bonanno, 2011, 2013; Bonanno and Lo Giudice, 2010; Eid et al., 2012a, b; Eid and Shaltout, 2014). Moreover, plants with macro-nutrient concentrations strongly correlated with those in the soil have been considered potential indicators of macro-nutrient availability (Alyemeni and Almohisen, 2014).

Conclusion and recommendations

In this study, it was found that some plant species were more effective in absorbing certain macro-nutrients compared to other species that grown in the same soil. *L. esculentum* can bioaccumulate many of the macro-nutrients from sewage sludge without excessive quantities of macro-nutrients being translocated into the edible portions of the plant (fruits). In the present study, establishing a pattern of translocation of macro-nutrients from root to shoot of a plant can be very useful in biological monitoring of macro-nutrient contamination as well as a selection of macro-nutrient accumulator species. The highest EAI values of *P. oleracea*, *P. australis*, *R. dentatus*, *P. dioscoridis*, *A. viridis* and *B. indica* growing in the SS; and of *P. oleracea*, *P. dioscoridis*, *R. dentatus* and *S. nigrum* growing in the cultivated field soil, indicate that they are better able to accumulate macro-nutrients and are therefore more suitable for phytoextraction purposes.

Due to the noticeable high concentrations of macro-nutrients in the different tissues of the studied species, we recommend it in mitigation of the eutrophic status of SS when applied to the agricultural lands. This will be achieved through leaving these weeds grow prior to cultivation of the agricultural crops. This needs further investigation to determine the best density for each native species that helps efficiently in reducing the macro-nutrients load of sewage sludge on the cultivated plants.

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APPENDIX

Appendix 1. *Macro-nutrient concentrations ± standard errors in nine native plant species grown at sewage sludge dump site (SS) and reference site (RS) in north Nile Delta, Egypt*

Species	Site	Tissue	Macro-nutrient (mg g^{-1})					
			N	P	Ca	Mg	Na	K
A. viridis	SS	L (*n* = 3)	57.5 ± 0.7	2.6 ± 0.0	51.9 ± 0.9	12.2 ± 0.2	3.4 ± 0.1	41.2 ± 0.7
		S (*n* = 3)	38.1 ± 0.7	1.7 ± 0.0	39.1 ± 0.8	4.5 ± 0.1	4.0 ± 0.1	88.0 ± 2.3
		R (*n* = 3)	27.0 ± 0.2	1.6 ± 0.0	31.3 ± 0.5	2.2 ± 0.0	2.5 ± 0.1	67.5 ± 1.5
		M (*n* = 9)	**40.9 ± 4.5bc**	**2.0 ± 0.2bc**	**40.8 ± 3.0b**	**6.3 ± 1.5b**	**3.3 ± 0.2a**	**65.6 ± 6.8bc**
	RS	L (*n* = 3)	42.2 ± 0.6	0.8 ± 0.0	36.6 ± 0.8	16.6 ± 0.3	5.5 ± 0.2	36.9 ± 0.4
		S (*n* = 3)	18.7 ± 0.3	0.2 ± 0.0	26.0 ± 0.6	11.6 ± 0.2	7.4 ± 0.2	72.2 ± 3.3
		R (*n* = 3)	10.3 ± 0.1	0.0 ± 0.0	24.4 ± 0.4	6.2 ± 0.1	8.0 ± 0.2	40.7 ± 0.9
		M (*n* = 9)	**23.7 ± 4.8a**	**0.3 ± 0.1ab**	**29.0 ± 1.9bc**	**11.5 ± 1.5c**	**7.0 ± 0.4a**	**49.9 ± 5.7b**
	***t*-value**		**22.2*** **	**29.8*** **	**9.3*** **	**10.3*** **	**7.4*** **	**4.3** **
B. indica	SS	L (*n* = 3)	50.9 ± 0.3	3.0 ± 0.0	21.9 ± 0.4	6.3 ± 0.1	59.4 ± 1.0	34.1 ± 0.6
		S (*n* = 3)	24.9 ± 0.5	2.9 ± 0.0	17.5 ± 0.4	4.0 ± 0.1	43.3 ± 1.5	57.6 ± 1.5
		R (*n* = 3)	24.4 ± 0.1	2.3 ± 0.0	11.4 ± 0.1	2.3 ± 0.0	9.9 ± 0.2	41.1 ± 1.0
		M (*n* = 9)	**33.4 ± 4.4ab**	**2.8 ± 0.1cd**	**16.9 ± 1.5a**	**4.2 ± 0.6ab**	**37.5 ± 7.3d**	**44.3 ± 3.5a**
	RS	L (*n* = 3)	40.3 ± 0.5	0.6 ± 0.0	11.9 ± 0.2	3.9 ± 0.1	68.1 ± 0.5	51.1 ± 1.6
		S (*n* = 3)	19.8 ± 0.2	1.3 ± 0.0	13.2 ± 0.1	2.7 ± 0.0	66.5 ± 4.4	50.2 ± 1.1
		R (*n* = 3)	16.4 ± 0.4	0.0 ± 0.0	7.9 ± 0.1	2.9 ± 0.0	24.4 ± 0.9	38.1 ± 1.1
		M (*n* = 9)	**25.5 ± 3.7a**	**0.7 ± 0.2abc**	**11.0 ± 0.8a**	**3.2 ± 0.2ab**	**53.0 ± 7.3c**	**46.5 ± 2.2b**
	***t*-value**		**9.1*** **	**16.3*** **	**5.7*** **	**2.4***	**6.6*** **	**0.6^{ns}**
C. bonariensis	SS	L (*n* = 3)	43.0 ± 0.2	2.6 ± 0.2	22.2 ± 0.4	4.7 ± 0.1	17.8 ± 0.4	48.0 ± 1.3
		S (*n* = 3)	19.3 ± 0.4	0.7 ± 0.0	8.0 ± 0.1	1.4 ± 0.0	9.3 ± 0.4	33.4 ± 0.9
		R (*n* = 3)	25.8 ± 0.3	2.9 ± 0.2	11.3 ± 0.1	2.4 ± 0.1	15.5 ± 0.3	35.7 ± 0.9
		M (*n* = 9)	**29.4 ± 3.5ab**	**2.1 ± 0.4bc**	**13.9 ± 2.1a**	**2.8 ± 0.5ab**	**14.2 ± 1.3bc**	**39.0 ± 2.3a**
	RS	L (*n* = 3)	38.0 ± 0.9	0.5 ± 0.0	21.4 ± 0.2	5.3 ± 0.1	26.3 ± 0.3	38.8 ± 1.0
		S (*n* = 3)	18.2 ± 0.1	0.2 ± 0.0	18.5 ± 0.2	2.8 ± 0.1	10.7 ± 0.2	45.8 ± 1.4
		R (*n* = 3)	11.9 ± 0.7	0.1 ± 0.0	13.0 ± 0.1	1.3 ± 0.0	6.7 ± 0.1	29.3 ± 1.1
		M (*n* = 9)	**22.7 ± 3.9a**	**0.3 ± 0.1a**	**17.6 ± 1.2ab**	**3.2 ± 0.6ab**	**14.6 ± 2.9ab**	**38.0 ± 2.5b**
	***t*-value**		**3.4** **	**5.2** **	**2.2^{ns}**	**0.9^{ns}**	**0.2^{ns}**	**0.3^{ns}**
L. esculentum	SS	F (*n* = 3)	43.1 ± 0.2	0.0 ± 0.0	5.0 ± 0.0	2.1 ± 0.0	4.1 ± 0.1	49.4 ± 2.2
		L (*n* = 3)	50.4 ± 0.7	1.5 ± 0.0	50.9 ± 1.1	8.4 ± 0.1	9.9 ± 0.2	37.3 ± 0.5
		S (*n* = 3)	40.6 ± 0.9	0.1 ± 0.0	37.0 ± 0.5	7.4 ± 0.1	15.6 ± 0.3	58.1 ± 1.2
		R (*n* = 3)	33.0 ± 0.4	0.3 ± 0.0	52.7 ± 0.7	5.3 ± 0.1	8.9 ± 0.1	40.5 ± 0.9
		M (*n* = 12)	**41.8 ± 1.9c**	**0.5 ± 0.2a**	**36.4 ± 5.8b**	**5.8 ± 0.7ab**	**9.6 ± 1.2abc**	**46.3 ± 2.5a**
	RS	F (*n* = 3)	27.5 ± 2.3	0.3 ± 0.0	4.7 ± 0.0	1.8 ± 0.0	5.8 ± 0.1	62.2 ± 2.4
		L (*n* = 3)	38.0 ± 0.6	1.5 ± 0.1	80.9 ± 2.4	8.3 ± 0.4	14.6 ± 0.7	27.9 ± 1.1
		S (*n* = 3)	30.3 ± 0.1	0.8 ± 0.0	49.0 ± 1.1	8.8 ± 0.2	18.7 ± 1.2	56.5 ± 3.9
		R (*n* = 3)	20.6 ± 0.4	3.2 ± 0.0	45.8 ± 0.6	4.8 ± 0.1	14.0 ± 0.5	40.7 ± 1.5
		M (*n* = 12)	**29.1 ± 1.9a**	**1.5 ± 0.3cd**	**45.1 ± 8.2d**	**5.9 ± 0.9ab**	**13.3 ± 1.5ab**	**46.8 ± 4.2b**
	***t*-value**		**15.0*** **	**2.8***	**2.0^{ns}**	**0.2^{ns}**	**7.4*** **	**0.2^{ns}**
P. australis	SS	L (*n* = 3)	42.0 ± 0.2	1.4 ± 0.0	16.6 ± 0.3	2.2 ± 0.0	3.5 ± 0.0	27.5 ± 0.7
		S (*n* = 3)	17.0 ± 0.2	2.1 ± 0.4	12.1 ± 0.1	1.2 ± 0.0	4.8 ± 0.1	36.5 ± 0.9
		R (*n* = 3)	19.6 ± 0.8	1.4 ± 0.5	14.8 ± 3.4	2.5 ± 0.8	4.7 ± 0.5	34.4 ± 1.0
		M (*n* = 9)	**24.5 ± 3.1a**	**1.6 ± 0.3b**	**14.6 ± 1.7a**	**2.1 ± 0.4a**	**4.4 ± 0.3ab**	**33.2 ± 1.2a**
	RS	L (*n* = 3)	29.5 ± 0.6	0.0 ± 0.0	8.7 ± 0.1	1.0 ± 0.0	6.2 ± 0.1	23.8 ± 1.1
		S (*n* = 3)	14.2 ± 1.3	0.0 ± 0.0	8.3 ± 0.1	0.9 ± 0.0	16.1 ± 0.5	14.1 ± 0.5
		R (*n* = 3)	13.1 ± 0.5	1.2 ± 0.0	9.8 ± 0.2	2.4 ± 0.1	19.5 ± 0.9	15.3 ± 0.6
		M (*n* = 9)	**18.9 ± 2.7a**	**0.4 ± 0.2ab**	**8.9 ± 0.2a**	**1.4 ± 0.3a**	**13.9 ± 2.0ab**	**17.7 ± 1.6a**

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	***t*-value**		**6.2*** **	**2.7***	**3.2****	**1.0^{ns}**	**7.1*** **	**7.2*** **
P. dioscoridis	SS	L (*n* = 3)	39.5 ± 0.5	2.6 ± 0.0	33.5 ± 0.8	6.7 ± 0.1	21.3 ± 0.4	30.3 ± 0.6
		S (*n* = 3)	26.7 ± 0.1	0.6 ± 0.0	10.8 ± 0.2	4.8 ± 0.1	10.0 ± 0.2	34.3 ± 0.9
		R (*n* = 3)	17.3 ± 0.3	1.3 ± 0.0	9.6 ± 0.2	1.9 ± 0.0	11.7 ± 0.3	45.7 ± 1.4
		M (*n* = 9)	**27.8 ± 3.2ab**	**1.5 ± 0.3b**	**18.0 ± 3.9a**	**4.4 ± 0.7ab**	**14.4 ± 1.8bc**	**36.8 ± 2.4a**
	RS	L (*n* = 3)	33.1 ± 0.2	1.8 ± 0.0	29.2 ± 0.5	6.2 ± 0.0	21.6 ± 1.0	51.5 ± 1.2
		S (*n* = 3)	11.0 ± 0.1	0.2 ± 0.0	14.8 ± 0.1	2.6 ± 0.0	5.6 ± 0.1	52.9 ± 2.0
		R (*n* = 3)	9.0 ± 0.1	1.5 ± 0.0	15.7 ± 0.2	2.3 ± 0.0	12.9 ± 0.2	41.4 ± 1.1
		M (*n* = 9)	**17.7 ± 3.9a**	**1.2 ± 0.3abcd**	**19.9 ± 2.3ab**	**3.7 ± 0.6ab**	**13.4 ± 2.3ab**	**48.6 ± 2.0b**
	***t*-value**		**7.0*** **	**2.1^{ns}**	**1.2^{ns}**	**1.9^{ns}**	**1.1^{ns}**	**2.9***
P. oleracea	SS	L (*n* = 3)	45.3 ± 0.1	4.1 ± 0.1	46.1 ± 0.9	21.4 ± 0.5	7.2 ± 0.1	63.7 ± 2.4
		S (*n* = 3)	59.7 ± 0.2	1.8 ± 0.0	18.2 ± 0.2	10.4 ± 0.1	4.3 ± 0.1	119.7 ± 6.0
		R (*n* = 3)	39.7 ± 0.1	3.7 ± 0.0	18.1 ± 0.2	9.6 ± 0.2	4.0 ± 0.0	57.4 ± 1.4
		M (*n* = 9)	**48.2 ± 2.9c**	**3.2 ± 0.4d**	**27.5 ± 4.7ab**	**13.8 ± 1.9c**	**5.2 ± 0.5ab**	**80.2 ± 10.1c**
	RS	L (*n* = 3)	40.1 ± 1.1	1.8 ± 0.0	28.8 ± 0.5	24.4 ± 0.5	37.5 ± 0.7	44.8 ± 1.1
		S (*n* = 3)	27.1 ± 0.1	1.4 ± 0.0	12.8 ± 0.1	13.6 ± 0.1	22.3 ± 0.6	76.8 ± 3.6
		R (*n* = 3)	24.6 ± 0.1	1.7 ± 0.0	11.0 ± 0.4	7.5 ± 0.4	8.7 ± 0.3	33.8 ± 0.9
		M (*n* = 9)	**30.6 ± 2.4a**	**1.6 ± 0.1d**	**17.5 ± 2.8ab**	**15.2 ± 2.5c**	**22.8 ± 4.2b**	**51.8 ± 6.5b**
	***t*-value**		**4.4****	**5.0****	**5.3****	**1.5^{ns}**	**4.8****	**6.4*** **
R. dentatus	SS	L (*n* = 3)	34.5 ± 0.1	0.5 ± 0.0	20.2 ± 0.3	5.6 ± 0.1	17.5 ± 0.6	35.3 ± 0.8
		S (*n* = 3)	21.6 ± 0.1	1.9 ± 0.0	17.7 ± 0.6	4.1 ± 0.1	18.7 ± 0.8	41.6 ± 1.3
		R (*n* = 3)	17.9 ± 0.8	2.5 ± 0.0	22.1 ± 0.3	5.0 ± 0.1	20.3 ± 1.0	34.8 ± 0.8
		M (*n* = 9)	**24.6 ± 2.5a**	**1.6 ± 0.3b**	**20.0 ± 0.7a**	**4.9 ± 0.2ab**	**18.8 ± 0.6c**	**37.2 ± 1.2a**
	RS	L (*n* = 3)	40.1 ± 0.3	1.6 ± 0.0	19.9 ± 0.4	9.1 ± 0.1	42.7 ± 0.7	47.7 ± 1.1
		S (*n* = 3)	12.1 ± 0.3	2.1 ± 0.0	13.9 ± 0.0	5.3 ± 0.1	20.3 ± 0.3	41.4 ± 0.9
		R (*n* = 3)	6.3 ± 0.4	0.0 ± 0.0	14.0 ± 0.4	4.8 ± 0.1	15.9 ± 0.2	22.3 ± 0.5
		M (*n* = 9)	**19.5 ± 5.2a**	**1.3 ± 0.3bcd**	**15.9 ± 1.0ab**	**6.4 ± 0.7b**	**26.3 ± 4.2b**	**37.1 ± 3.8b**
	***t*-value**		**1.9^{ns}**	**0.7^{ns}**	**3.6****	**2.8***	**1.7^{ns}**	**0.1^{ns}**
S. nigrum	SS	L (*n* = 3)	51.9 ± 0.7	2.4 ± 0.0	20.2 ± 0.4	3.7 ± 0.1	2.0 ± 0.1	53.4 ± 1.1
		S (*n* = 3)	25.9 ± 0.1	1.4 ± 0.2	22.8 ± 0.5	2.6 ± 0.0	2.1 ± 0.0	58.4 ± 1.5
		R (*n* = 3)	17.6 ± 0.2	2.8 ± 0.0	24.1 ± 0.5	1.6 ± 0.0	4.2 ± 0.1	39.0 ± 1.2
		M (*n* = 9)	**31.8 ± 5.2ab**	**2.2 ± 0.2bcd**	**22.4 ± 0.6a**	**2.6 ± 0.3ab**	**2.8 ± 0.4a**	**50.3 ± 3.0ab**
	RS	L (*n* = 3)	41.1 ± 4.4	1.4 ± 0.0	37.5 ± 0.3	6.4 ± 0.1	6.2 ± 0.2	50.8 ± 1.4
		S (*n* = 3)	14.8 ± 0.7	0.9 ± 0.0	35.0 ± 0.3	5.1 ± 0.1	5.8 ± 0.3	62.9 ± 1.9
		R (*n* = 3)	11.7 ± 0.1	1.9 ± 0.0	40.4 ± 0.4	3.7 ± 0.1	6.3 ± 0.0	40.5 ± 1.3
		M (*n* = 9)	**22.5 ± 4.8a**	**1.4 ± 0.1cd**	**37.6 ± 0.8cd**	**5.1 ± 0.4ab**	**6.1 ± 0.1a**	**51.4 ± 3.3b**
	***t*-value**		**6.0*** **	**7.6*** **	**19.3*** **	**24.4*** **	**10.3*** **	**1.1^{ns}**
F-value$_{Sewage\ sludge\ dump\ site}$			**5.7*** **	**9.6*** **	**8.3*** **	**14.9*** **	**19.8*** **	**12.4*** **
F-value$_{Reference\ site}$			**1.4^{ns}**	**5.9*** **	**11.1*** **	**16.5*** **	**17.7*** **	**7.5*** **

t-values represent the paired t-test. Means in the same columns (for each site) followed by different letters are significantly different at $p < 0.05$ according to Tukey HSD test. F: fruit, L: leaf, S: stem, R: root, M: mean of tissues, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ns: not significant (i.e., $p > 0.05$)

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Appendix 2. *Significant Pearson correlation coefficient ($p < 0.05$) between macro-nutrients concentrations in soil and nine native plant species in north Nile Delta, Egypt. Above diagonal represent the positive correlations and below diagonal represent the negative correlations*

Species		Soil	Leaf	Stem	Root
A. viridis	Soil	—	N, P, Ca, Mg, Na	N, P, Ca, Mg, Na	N, P, Ca, Mg, Na
	Leaf	K	—	N, P, Ca, Mg, Na, K	N, P, Ca, Mg, Na, K
	Stem	K		—	N, P, Ca, Mg, Na, K
	Root	K			—
B. indica	Soil	—	N, P, Ca, Na, K	N, P, Ca, Na	N, P, Ca, Mg, Na
	Leaf	Mg	—	N, P, Ca, Mg, Na	N, P, Ca, Na
	Stem	Mg, K	K	—	N, P, Ca, Na, K
	Root	K	Mg, K	Mg	—
C. bonariensis	Soil	—	N, P, Ca, Mg, Na	N, P, Mg, Na, K	N, P
	Leaf	K	—	N, P, Mg, Na	N, P, K
	Stem	Ca	Ca, K	—	N, P, Ca
	Root	Ca, Mg, Na, K	Ca, Mg, Na	Mg, Na, K	—
P. australis	Soil	—	N, P, Ca, Na	N, P, Ca, Na	N, P, Ca, Na
	Leaf	Mg, K	—	N, P, Ca, Mg, Na, K	N, P, Ca, Mg, Na, K
	Stem	Mg, K		—	N, P, Ca, Mg, Na, K
	Root	K			—
P. dioscoridis	Soil	—	N, P, Ca, Na, K	N, P, K	N, Mg, Na
	Leaf	Mg	—	N, P, Mg, K	N, Na
	Stem	Ca, Mg, Na	Ca	—	N, Ca
	Root	P, Ca, K	P, Ca, Mg, K	P, Mg, Na, K	—
P. oleracea	Soil	—	N, P, Ca, Mg, Na	N, P, Ca, Mg, Na	N, P, Ca, Na
	Leaf	K	—	N, P, Ca, Mg, Na, K	N, P, Ca, Na, K
	Stem	K		—	N, P, Ca, Na, K
	Root	Mg, K	Mg	Mg	—
R. dentatus	Soil	—	Ca, Mg, Na, K	N, Ca, Mg, Na	N, P, Ca
	Leaf	N, P, Ca	—	P, Ca, Mg, Na	Ca
	Stem	P	N	—	N, Ca
	Root	Mg, Na, K	N, P, Mg, Na, K	P, Mg, Na	—
S. nigrum	Soil	—	N, P, Mg, Na	N, P, Mg, Na, K	N, P, Mg, Na, K
	Leaf	Ca, K	—	N, P, Ca, Mg, Na	N, P, Ca, Mg, Na, K
	Stem	Ca		—	N, P, Ca, Mg, Na, K
	Root	Ca			—

		Soil	Fruit	Leaf	Stem	Root
L. esculentum	Soil	—	N, Ca, Na, K	N, Na	N, Mg, Na	N, Ca, Na, K
	Fruit	P, Mg	—	N, P, Na	N, P, Na	N, P, Ca, Mg, Na
	Leaf	P, Ca, K	Ca, K	—	N, P, Ca, Na, K	N, P, Na
	Stem	P, Ca, Mg	Ca, Mg, K		—	N, P, Na, K
	Root	P		Ca	Ca, Mg	—

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