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Bioaccumulation and distribution of some elements by *Eclipta Alba* (L.) Hassk as affected by the element level in soils

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Abstract

The present study presents an investigation of possible accumulation of some heavy metals in roots, stems and leaves of *Eclipta alba*, grown in a cultivated land irrigated with canal irrigation water (locality-1) and a cultivated land irrigated with drain water (locality-2) in El- Mahmodeya village near Zagazig city, Sharkia Governorate, Egypt. Soil and plants were collected from three sites per non-contaminated and contaminated localities. The considered elements were Al, Co, Ni, Pb, Cd, Ba, Cu, Zn, Mn, and Fe. Results revealed that *E. Alba* accumulated significantly high concentrations of the considered elements found in the corresponding soils in the studied localities in their different organs.

The results showed that the levels of elements with higher and lower values had the sequence of Fe > Cu > Mn > Al > Zn and Pb > Ni > Ba > Co > Cd respectively. Also, the considered heavy metals showed multifold higher concentrations in plant organs compared to corresponding soils.

Based on the values of bioaccumulation and translocation factors, *Eclipta Alba* displayed the potential to be used in phytoextraction of all considered metals, except for Ni. Moreover, it proved relevance for phytostabilization of Ni and Fe as it recorded TF >1.

Eclipta. Alba showed Al and Fe concentrations above 1000 mg kg^{-1} in its leaves; therefore, it is considered a hyperaccumulator of these metals. Significant positive correlations were found between concentrations of some heavy metals like (Al, Fe, and Cd) in soil and plant organs. The high bioaccumulation factor, reveals *E. Alba* as a promising phytoremediator. Also, the present results revealed that *E. Alba* could be considered a potential biomonitoring of the predominance of heavy metals in polluted localities.

Keywords: *Eclipta alba*, hyper accumulation, phytoremediation, polluted soil

Introduction

Over the past decades, soil contamination due to the elements, Ni, Cd, Zn, and Pb, increased severely due to traffic emissions, pesticides, mining, applying agricultural fertilizers, industrial effluents, and municipal wastes (Chibuike and Obiora, 2014) [14]. In Egypt, environmental contamination due to heavy metals has been recorded widely to be predominantly resulting in increased environmental toxicity (Al- Naggar *et al*. 2014) [3].

Mobilization of metals in soils leads to their adsorption by roots thus reaching to plants. In the plant body, heavy metals are trapped by cells or transport through the cell membrane (Singh *et al*. 2010) [59]. It was reported that plants grown in non-polluted irrigated soils showed significantly lower heavy metals compared with plants grown in wastewater irrigated soils (Khaled and Muhammad 2016) [32]. Moreover, long-time use of wastewater in irrigation has caused a significant increase of heavy metals in the soil (Khan *et al*. 2008; [33] Ullah *et al*. 2012) [66].

Wastewater is drained to agricultural lands, increase levels of some heavy metals like Mn, Fe, Zn, Cu, Pb, Cr, Ni, Co, and Cd, in the receiving soils (Rattan *et al*.2005) [54].

The mobility of heavy metals and their level in the soil of high pH, clay, and organic matter content are generally low (Roosselli *et al*. 2003) [57].

Phytoextraction is one of the processes utilized in phytoremediation of contaminated soil with metals, which involves the transfer of heavy metals through their continuous uptake from soils to the aerial plant parts Phytostabilization is a further strategy of phytoremediation and happens when plants are used to restrict transfer of soil elements to root-shoot,

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consequently the mobility of metals in contaminated soils is reduced. Plants which efficiently used for phytoextraction have to be able to accumulate more than 1000 mg kg^{-1}, should exhibit values of transfer and bioaccumulation factors above unity, and possess high biomass production (Reeves and Baker, 2000) [55].

Plants used for phytoextraction usually can tolerate heavy metals with high concentrations. Plants tolerant to high concentration of heavy metals have been listed by Marques *et al*. (2009) [39].

Hyperaccumulator plants can accumulate 10 to 500 times more metals than non-hyper accumulator plants of the same species growing in non-polluted sites (Mganga *et al*. 2011) [44]. This could either be the result of the exclusion of these metals from the plants or of compartmentalization and translocation of these metal ions out of the plant vascular system (Kim *et al*.2003) [35].

Few studies only have been investigated the potential uptake of metals of wild plants, growing at non-contaminated and contaminated soils of cultivated lands subjected to irrigation canal water or drain water that has been affected by wastewater channels.

It is known that, few types of research only have been conducted in Egypt to date, that investigates the ability of wild plants growing in a non-contaminated cultivated land irrigated with canal water and a contaminated cultivated land irrigated with drain water, to reduce concentrations of metals from the soil; nor have studies been published that provide an estimation of bioaccumulation and translocation factors of metals. Therefore, this study aimed to achieve three objectives: 1) To compare the potential of the wild plant *Eclipta alba* (collected from cultivated lands the first irrigated with water of irrigation canal and the second irrigated with water drains (non-contaminated and contaminated locations respectively) to accumulate metals from soil samples in roots, stems, and leaves. 2) To evaluate the following factors: bioaccumulation factor (BAF) and transfer factor (TF) from soil to plant organs. 3) To provide the relationship between metals in soil and different organs of *E. alba* grown at the studied localities.

Materials and Methods

Study species

Eclipta alba (L.) Hassk (Asteraceae) is an annual plant that thrives in moist places. In Egypt, it spreads along canals, swamps, wells, waste places, and at the edges of springs (Boulos, 2009; Tackholm, 1974) [9]. It is also one of the major components with higher importance value compared with other plants in the vegetation stands of irrigation and drainage canals of the River Nile (Mashaly and El-Amier, 2007).

Soil sampling and analysis

Sampling of soils were carried out at three sites per locality from three quadrats (0.5×0.5m) that were distributed randomly at each site.

Three composite soil samples were collected per site. They were air dried then sieved through 2mm sieve. Soil water extracts were diluted at 1:5 for the estimation of soil pH using a pH meter (Model 9107 BN, ORION type). Diethylene triamine penta acetic acid solution (DTPA) was used for the extraction of the tested elements (Al, Co, Ni, Pb, Cd, Ba, Cu, Zn, Mn and Fe) that were measured using the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) with Ultra Sonic Nebulizer (USN) (model: Perkin Elmeroptima 3000). In addition, soil mechanical analyses were conducted. All these analyses were conducted according to the methods of Allen *et al*. (1986).

Plant sampling and analysis

Plant samples collection was performed at the end of the vegetative period during the autumn. Sampling of plants were carried out at three sites per locality in Egypt: Along a non-contaminated cultivated land irrigated with canal water El- Mahmodeya village irrigation canal (locality-1) as a representative of a non-contaminated location and a contaminated cultivated land irrigated with El Mahmodeya village, drain water (locality-2) as a representative of a contaminated location. Both locations were in the vicinity of the Zagazig-city (45 ′39 °30″ N and 53 ′29 °31E) and (49 ′39 30″ N and 54 ′29 °31 E) respectively in El- Mahmodeya village near Zagazig city, Sharkia Governorate, Egypt. Elevated elements concentrations in the drain water (locality 2) resulted from the inputs of agricultural managements, such as uncontrolled application of fertilizers, pesticides, wastewater irrigation, dumping of refuse, and also human excreta. Water drainages are among the most common areas for the dumping of refuse between rural neighbors, regardless of whether these are farmers or not.

Samples of plant biomass were collected from the same sampling quadrats where soil samples were taken. Plant samples were separated into roots, stems and leaves in the laboratory and washed with deionised water to remove the soil particles adhering to plant parts. The air dried plant parts were oven dried at 60 ºC for 24 hrs, then the dry weight was determined**.** The values of biomass were calculated as gram dry matter per square metre (g DM m^{-2}).

For plant sample analysis; the grinding sample (0.5−1g) was digested in 20 ml tri-acid mixture of HNO3: HClO4: HF (1:1:2 V: V: V) until the solution turned transparent. Plant samples were filtered after digestion via filter paper of Whatman no. 1 and diluted to 25 ml using double de-ionized water. The concentrations of Al, Co, Ni, Pb, Cd, Ba, Cu, Zn, Mn, and Fe were measured using the Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) with Ultra Sonic Nebulizer (USN) using (Allen 1989) [5].

Estimation of bioaccumulation factor (baf) and translocation factor (TF)

Bioaccumulation factor (BAF) was utilized for determination of the ability of plant under investigation to accumulate elements from soils. It was calculated as follows: BAF=element concentration in the root (mg kg^{-1}) concentration of the same element in the soil at the same site (mg kg^{-1}) according to Ghosh and Singh (2005) [22]. The translocation factor (TF) was applied to determine the ability of a plant to translocate an element from the roots to the shoots of the plant Ghosh and Singh (2005) [22]. The TF was calculated as $TF = C_{shoot} / C_{root}$, where C_{shoot} and C_{root} represent element concentrations in the shoots and roots of the plants, respectively.

Statistical analysis

To assess the effect of the location of soil, independent sample t-tests were utilized and the data were tested via the Levene’s test for their normality of distribution and homogeneity of variances. Data were subjected to two-way analyses of variance with the type of soil and part of the plant and their interaction effects using the General Linear Model (GLM) procedure of SAS User's Guide (2005) [58]. Statistical analyses were conducted using the Statistical Package for Social Sciences version 22.0 (IBM Corp., Armonk, NY, USA). Pearson's correlation coefficient which indicated the evaluation of the correlation between element levels in soil and in each plant organ was calculated.

Results

The results obtained (Table 1) indicated a significant difference in pH of soil samples of locality -1 and locality -2 where it reached (7.16, 6.17), respectively. The soil texture of locality-2 showed significantly higher averages ($p < 0.0001$) for both coarse and fine sand (7.91 % and 19.27 %) compared to soil samples of the locality 1 (6.81 % and 13.57 %), respectively. Average silt and clay percentages of Locality- 1 soil samples showed high significant increase ($p < 0.0001$) compared with those of locality 2.Samples of soils of localities (1 and 2) are characterized by clay and clay loam textures, respectively.

Table 1: Selected physicochemical Characteristics of the investigated non-contaminated (locality 1) and contaminated (locality -2) soils

Variables		Locality-1	Locality-2	t-value	P-value
pH		7.16±0.15	6.17±0.12	8.99**	0.001
Coarse sand	%	6.81±0.03	7.91±0.05	- 32.36**	< 0.001
Fine sand		13.57±0.19	19.27±0.23	44.99**	< 0.001
Silt		39.35±0.08	37.25±0.16	20.79**	< 0.001
Clay		40.27±0.02	35.50±0.11	77.21**	< 0.001
Soil Texture		Clay	Clay loam		

*: indicate significant difference ($p < 0.05$) **: indicate high significant difference ($p < 0.01$) NS: indicate non-significant difference

Soil samples collected from contaminated (locality-2) contained high concentrations ($p<0.01$) of the heavy metal predominated in this soil except Cd and Fe where their concentration subjected to non-significant change, compared with locality-1 (Table 2).

Table 2: The soil elements content (mg kg^{-1}) in the investigated non-contaminated locality -1 and contaminated locality -2

Elements	Locality-1	Locality-2	t-value	P-value
Al	5.3±0.5	7.07±0.02	- 6.08**	0.004
Co	0.03±0.01	0.06±0	- 5.65**	0.005
Ni	0.4±0.1	1.01±0.01	- 10.42**	< 0.0001
Pb	1.09±0.01	1.51±0.01	- 61.48**	< 0.0001
Cd	0.01±0.01	0.04±0.02	- 2.19^{NS}	0.094
Ba	0.38±0.09	0.67±0.05	- 4.99**	0.008
Cu	4.71±0.3	9.76±0.14	- 26.6**	< 0.0001
Zn	3.23±0.36	6.56±0.11	- 15.30**	< 0.0001
Mn	3.99±0.21	7.51±0.1	- 26.69**	< 0.0001
Fe	9.11±0.1	11.52±0.58	-7.10*	0.017

*: indicate significant difference ($p<0.05$)
**: indicate high significant difference ($p<0.01$) NS: indicate non-significant difference

Table 3 showed the studied localities effects on overall concentrations of the considered elements in plant parts, effects of plant parts upon one another concerning elements concentrations in each plant part and the effects of interaction between type of locality (non-contaminated or contaminated) on the level of the considered elements. The present study showed significant differences concerning the overall mean concentrations of the considered elements of plant parts at the studied localities (except for Al and Ni). Regarding plant parts, the present study showed significant differences between plant parts for the whole considered elements and interaction between plant parts and the studied localities for the considered elements.

Table 3: Elements content (mg kg^{-1}) of soil and *E. alba* (roots, stems and leaves) as affected by locality and their interactions

Element	Al	Co	Ni	Ph	Cd	Ba	Cu	Zn	Mn	Fe
Studied localities										
Locality-1	523.19^{a}	0.47^{b}	1.48^{a}	1.67^{b}	0.86^{a}	7.74^{a}	15.21b	8.99^{b}	38.09^{b}	602.76^{b}
Locality-2	497.39^{a}	0.59^{a}	1.67^{a}	2.36^{a}	0.24^{b}	7.01^{b}	17.18a	19.22^{a}	45.13^{a}	710.27^{a}
F	4.03	35.58	3.86	34.42	105.48	19.76	12.78	75.84	56.75	25.01
p	0.07	0.00	0.07	0.00	0.00	0.001	0.01	0.00	0.00	0.00
LSD	28.64	0.04	0.22	0.26	0.13	0.36	1.22	2.61	2.08	47.90
Plant parts										
Root	959.50^{a}	0.79^{a}	1.62^{ab}	1.47^{b}	0.56^{ab}	6.54^{b}	11.44b	14.01^{b}	30.89^{b}	769.90^{b}
Stem	451.90^{b}	0.36^{c}	1.35^{b}	0.76^{c}	0.68^{a}	9.87^{a}	7.09c	10.49^{c}	15.84^{c}	119.20^{c}
Leaves	119.47^{c}	0.79^{a}	1.75^{a}	3.81^{a}	0.40^{b}	5.71^{c}	30.04a	17.81^{a}	78.09a	1080.50^{a}
F	1444.52	186.83	5.57	241.16	7.28	237.49	655.56	12.96	1609.05	694.28
p	0.00	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00
LSD	35.07	0.05	0.27	0.32	0.16	0.45	1.50	3.20	2.55	58.67
Interaction (L * P)										

Locality-1-root	539.7^{c}	0.70^{b}	2.47^{a}	0.11^{e}	0.82^{b}	4.44^{d}	13.09^{c}	12.55^{bc}	15.21^{d}	337.20^{d}
Locality-2-root	364.10^{d}	0.88^{a}	0.78^{d}	2.84^{c}	0.31^{c}	8.64^{b}	9.80^{d}	15.48^{b}	46.57^{c}	1162.60^{b}
Locality-1-stem	119.10^{e}	0.52^{c}	0.79^{cd}	0.71^{d}	1.17^{a}	15.08^{a}	9.60^{d}	5.02^{d}	17.48^{d}	122.90^{e}
Locality-2-stem	119.90^{e}	0.19^{d}	1.9s1^{b}	0.82^{d}	0.20^{c}	4.67^{d}	4.58^{e}	15.97^{b}	14.21^{d}	115.40^{e}
Locality-1-leaf	833.40^{b}	0.20^{d}	1.17^{c}	4.18^{a}	0.59^{b}	3.70^{e}	22.94^{b}	9.42^{cd}	81.58^{a}	1308.10^{a}
Locality-2-leafs	1085.65^{a}	0.70^{b}	2.34^{a}	3.44^{b}	0.21^{c}	7.72^{c}	37.15^{a}	26.21^{a}	74.61^{b}	1162.60^{b}
F	3.86	157.91	88.21	77.49	8.83	864.75	124.69	11.72	170.42	284.73
p	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
LSD 0.05	49.60	0.07	0.39	0.46	0.23	0.63	2.12	4.53	3.61	82.97

*: $P<0.05$ **: $P<0.01$ NS: $P>0.05$a, b, c, d, e : Means within columns with different superscripts differ ($P<0.05$)
Interaction(L * P):interaction between the studied locality and plant parts

Table 4: Bioaccumulation factor (BAF) and translocation factor (TF) of elements within *E. alba* naturally grown in non-contaminated and contaminated soils

Elements	**Non-contaminated locality1**		**Contaminated locality2**	
	BAF	**TF**	**BAF**	**TF**
Al	101.82	1.76	51.50	3.31
Co	23.33	1.03	14.67	1.01
Ni	6.18	0.79	0.77	5.45
Pb	0.10	44.45	1.88	1.50
Cd	82.00	2.15	7.75	1.32
Ba	11.68	4.23	12.90	1.43
Cu	2.78	2.48	1.00	4.26
Zn	3.89	1.15	2.36	2.72
Mn	3.81	6.51	6.20	1.91
Fe	41.41	3.79	100.92	0.83

BAF= Bioaccumulation Factor TF= Translocation Factor

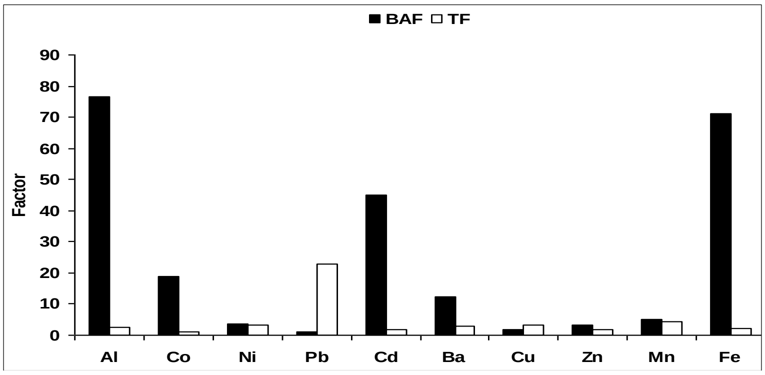

Fig 1: Average bioaccumulation (BAF) and translocation factor (TF) of elements in *E. alba*. BA: bioaccumulation factor, TF: translocation factor

As shown in Table (4) and figure (1), *E. alba* had bioaccumulation and translocation factors above one (BAF > 1 and TF > 1) in the studied locations for all considered metals, except for Ni and Fe (TF of Ni in locality-1 = 0.79 and BAF in locality-2 = 0.77; TF of Fe in locality-2 = 0.83; (Table 4). Fe followed the Al levels in the leaves and concentrations were higher than 1000 mg kg^{-1}. In general, BAF was higher for Fe, Al, Cd, and Co followed by Co, B, Ni, and Pb. In addition, TF of Fe in locality-2 = 0.83; therefore, *E. alba* has a good potential to be used in phytostabilization of Ni in non-polluted locations and for Fe in polluted locations. The leaf Fe concentration was above 1000 mg kg^{-1}. For the studied localities, in general, both maximal and minimal BAF factors were found for Al and Pb, respectively. However, the highest TF factor was detected for Pb while the lowest for Cd, respectively.

Table 5: Pearson correlation coefficient of elements in *E. alba* (roots, stems and leaves), soil *: $p < 0.05$, **: $p < 0.01$.

		Soil									
		Al	Co	Ni	Pb	Cd	Ba	Cu	Zn	Mn	Fe
Root	Al	-0.839*	-.899*	-.981**	-.953**	-0.759	-.985**	-.917*	-.921**	-.958**	-.898*
	Co	.969**	.923**	.861*	.929**	0.80	0.744	.945**	.964**	.931**	.962**
	Ni	-.996**	-.944**	-.910*	-.974**	-0.749	-.832*	-.994**	-.991**	-.963**	-.924**
	Pb	.953**	.936**	.978**	.999**	0.706	.922**	.993**	.990**	.993**	.953**
	Cd	-.957**	-.956**	-.952**	-.988**	-0.671	-.885*	-.989**	-.981**	-.982**	-.925**
	Ba	.921**	.900*	.983**	.989**	0.638	.938**	.976**	.970**	.982**	.933**
	Cu	-.985**	-.925**	-.932**	-.980**	-0.76	-.862*	-.993**	-.993**	-.970**	-.942**
	Zn	0.482	0.593	0.688	0.618	0.694	0.654	0.541	0.601	0.66	0.769
	Mn	.933**	.938**	.983**	.994**	0.729	.950**	.983**	.978**	.989**	.928**

	Fe	.937**	.941**	.973**	.986**	0.798	.912*	.970**	.984**	.991**	.985**
Stem	Al	-0.075	-0.048	0.129	0.065	-0.591	0.148	0.036	-0.02	0.049	-0.062
	Co	-.968**	-.973**	-.955**	-.991**	-0.799	-.884*	-.988**	-.996**	-.993**	-.971**
	Ni	.878*	.846*	.902*	.926**	0.497	.875*	.932**	.902*	.905*	0.796
	Pb	0.796	.850*	.970**	.925**	0.77	.975**	.877*	.893*	.935**	.910*
	Cd	-.984**	-.973**	-.878*	-.954**	-0.728	-0.781	-.976**	-.973**	-.949**	-.908*
	Ba	-.943**	-.954**	-.983**	-.997**	-0.767	-.929**	-.983**	-.990**	-.999**	-.970**
	Cu	-.948**	-.940**	-.981**	-.998**	-0.718	-.934**	-.991**	-.988**	-.993**	-.944**
	Zn	.901*	.902*	.932**	.945**	0.788	.846*	.924**	.951**	.957**	.997**
	Mn	-0.637	-0.688	-0.706	-0.689	-.876*	-0.661	-0.648	-0.706	-0.72	-.817*
	Fe	-0.703	-0.623	-0.738	-0.754	-0.126	-0.685	-0.763	-0.72	-0.727	-0.634
Leaves	Al	.948**	.891*	.960**	.984**	0.651	.907*	.985**	.977**	.971**	.926**
	Co	.960**	.934**	.965**	.994**	0.689	.910*	.995**	.988**	.985**	.932**
	Ni	.885*	.871*	.988**	.973**	0.721	.969**	.949**	.952**	.970**	.936**
	Pb	-0.514	-0.557	-0.799	-0.713	-0.187	-.855*	-0.659	-0.626	-0.702	-0.58
	Cd	-.911*	-.905*	-.990**	-.989**	-0.653	-.953**	-.972**	-.967**	-.984**	-.932**
	Ba	.913*	.942**	.993**	.992**	0.749	.950**	.968**	.974**	.996**	.963**
	Cu	.950**	.921**	.946**	.980**	0.612	.879*	.985**	.972**	.970**	.911*
	Zn	.920**	.893*	.957**	.974**	0.582	.912*	.972**	.955**	.962**	.890*
	Mn	-.909*	-0.769	-0.696	-0.795	-0.8	-0.594	-.843*	-.858*	-0.782	-.818*
	Fe	-.965**	-.945**	-.967**	-.995**	-0.77	-.917*	-.994**	-.994**	-.990**	-.949**

** Correlation is significant at the 0.01 level (2-tailed).* Correlation is significant at the 0.05 level (2-tailed

Pearson's correlation coefficients of all the considered metal levels in soil and each plant organ are presented in (Table 5). The Al content in soil revealed a significant, strong negative correlation with Al in roots ($r = -0.839$, $p < 0.05$), while a weak negative correlation was detected between soil Al and stem Al content ($r = -0.075$). A direct strong relationship was recorded for soil Al and leaf Al content ($r = 0.948$, $p < 0.01$). The Co content in soil showed significant strong positive correlations with roots ($r = 0.923$) and leaves ($r = 0.934$; $p < 0.01$).

The Ni content in soil revealed strong positive correlations with that in stems and leaves ($r = 0.902$, $r = 1\text{-}0.988$, $p < 0.01$), respectively; while, a strong negative correlation was found with roots ($r = -0.910$, $p < 0.05$). The Pb content in the soil revealed strong positive correlations with that in roots and stems ($r = 0.999$, $r = 0.925$, $P < 0.01$ for each), respectively. However, no significant correlation was detected with leaves.

The Ba content of soil showed strong positive correlations with that in roots and in soil ($r = 0.938$, $r = 0.950$, $p < 0.01$ for each). However, strong negative correlations were found with stems ($r = -0.929$, $p < 0.01$). The strongest significant correlations were found between Mn in soil and Ba in stems ($r = -0.999$, $p > 0.01$). The positive correlations indicate that plants absorbed elements from the soil via their roots. Moreover, this correlation indicates that *E. alba* reflects progressive effects on the environmental pollution of soils.

The Cu content in soil showed strong negative correlation with that in roots and stems ($r = -0.980$, $r = -0.991$, $p < 0.01$). However, a strong positive correlation was found in leaves ($r = 0.985$, $p < 0.01$). The Zn content in soil showed no significant correlation in roots. However, Zn recorded strong positive correlations with stems and leaves ($r = 0.951$, $r = 0.955$, $p < 0.01$). Mn content in soil exhibited a strong positive correlation with root ($r = 0.989$, $p < 0.01$); however, non-significant correlations in soil with stems and leaves. Fe content in soil exhibited a strong positive correlation with root ($r = 0.985$, $p < 0.01$), while no significant correlation was found in soil with stems. In addition, strong negative correlations were recorded between soil and leaves ($r = -0.949$, $p > 0.01$).

Most correlations between soil and plant organs for all tested elements resulted in positive, significant correlations, indicating that, elements concentrations in plants are dependent on with their concentrations in the soils. Strong positive correlations were recorded for all the studied elements. The highest positive correlations of tested elements with the highest BAF were as follows: Al-Co in soil–root ($r = 0.969$, $p > 0.01$), Al-Zn in soil-stem ($r = 0.901$, $P < 0.05$), Al-Co in soil-leaf ($r = 0.960$, $p < 0.05$), Fe-Fe in soil-root ($r = 0.985$, $p < 0.05$), Fe-Zn in soil-stem ($r = 0.997$, $P < 0.05$), Fe-B in soil-leaf ($r = 0.963$, $p < 0.05$), Cd-Fe in soil-root ($r = 0.798$), Cd-Pb in soil-stem ($r = 0.788$) and Cd-B in soil- leaf ($r = 0.749$).

Discussion

Contamination of most agricultural soils in the world is due to their relative subjection to heavy metals (Elawa, 2015) [17]. The present study revealed the presence of high concentrations of a variety of heavy metals in the contaminated soil (Locality- 2) compared with that in the non-contaminated one (Locality- 1). These results are in accordance with Al Naggar *et al.* (2014) [3] in agricultural soils in Egypt. However, the concentrations of the tested elements in soils of non- contaminated locality-1 and contaminated locality-2 were below the Environmental Quality Standards set by the United States Environmental Protection Agency, USEPA (2000). Regarding the lower concentrations of heavy metals at Locality -2 of the contaminated soil compared with the safe limits, these lower concentrations may be due to their leaching downwards to deeper soil layers and also may be due to the removal of heavy metals by the repeated cultivation of the soil (Chauhan and Chauhan 2014).

Singh and Agrawal (2007) have revealed that increasing availability of heavy metals in the soil depends mainly on soil pH. The soil of locality-1 had an alkaline pH value, which reduced metal availability due to sobbing cations of soil colloids. Moreover, there is an inverse relationship between elements availability for plant and soil pH. Generally as the pH level of soil increase their availability decrease (Pérez-De-Los-Reyes *et al*., 2013). The heavy metals at the contaminated locality-2 however, showed higher levels than those at the non-contaminated locality-1, which has a lower pH value. Nanda and Abraham (2013) reported a similar finding, which proved that metal availability increasing with a decreasing pH value due to a reverse trend between solubility and pH levels. In this concern, Rodriguez *et al*., (2008) revealed that the pH is a remarkable factor that affects the cation mobility and regulates the solubility of heavy metals in soil.

The uptake of different minerals from the soil is based on the pH that affects the uptake of elements including heavy metals. The behavior observed for the considered elements was higher in less alkaline soil (locality -2) than alkaline soil (locality -1) (Bravo *et al*. 2017). In addition, soil pH has great influence on the availability of mineral elements and their toxicity for plants (Likar *et al*. 2015; Kabata-Pendias and Mukherjee 2007).

Average dry biomass values of the entire plant samples of *E. alba* exhibited moderate and low values at locality-1 and locality-2, respectively. *E. alba* had higher biomass production in the non-contaminated locality-1 with lower levels of the tested elements, compared to the polluted locality-2 (39.3±0. 6 and 30.2±3.7 g DM m^{-2}) $^{**}P < 0.001$) respectively. This finding is supported by Hadi *et al*. (2014) and Nagajyoti *et al*. (2010) [46] who reported that plants undergo heavy metal stress is subjected to a decrease of their biomass production. The obtained moderate biomass value in locality-1 is supported by Chaney *et al*. (1997) [12] who reported that hyper accumulation of heavy metals by plants is the more effective factor in phytoremediation even it was associated with a sharp decrease in plant biomass. The harvested parts of plants are not easy to handle and hence, the process of phytoremediation would not be economical. In addition, Mohammad *et al*. (2008) have suggested that the study of new hyperaccumulators by recent biotechnology will be of great value. In this concern Vymazal, (2011a, 2011 b) [68, 69] reported that, for selecting the best plants for phytoextraction, these plants must be characterized by rapid growth rate, high plant biomass, ability to grow in other different areas, easy to be harvested and accumulate heavy metals in their parts, has a tolerance capacity to survive under high pollutant levels and high pollutant removal capacity. But, no species has been reported, so far, that includes all these properties. But, a fast-growing and non-accumulator species can be engineered to attain some of the above-mentioned properties (Clements *et al*. 2002) [15].

According to the standards proposed by Allen (1989) [4], heavy metals in soil samples of the locality- 1 were found to be within permissible limits for all the studied heavy metals. However, values of the locality- 2 exceeded the permissible limits for Cd, while Al, Co, Ni, Pb, Bo, Zn, Mn, and Fe were found to be within the normal range. Manganese is an essential element for plants and its contents in the soils of locality- 2 (7.51 mg kg^{-1}) have been reported higher than other considered heavy metals, except for Fe and Cu (MC Grath and Smith 1990). As compared to the polluted locality-2, values of all considered heavy metals were lower at locality-1, which may be due to the lower soil pH. Soil pH has been reported to correlate negatively with metal solubility and hence their uptake from soil (Hough *et al*. 2003).

Although the micronutrients Zn and Mn have a similar ionic potential (charge/size ratio) show relatively the same concentration pattern both in locality-1 and locality-2 soils.

The different organs of *E. alba* showed higher amounts of the tested elements than the corresponding soil in non-contaminated locality-1, which matches the results reported by Kim *et al*. (2003) [35].

Fe values of locality- 2 soil samples were significant at $P > 0.05$ and had the highest values (1162.6 mg kg^{-1}) among heavy metals. These findings were in accordance with Kabata-Pendias and Pendias (1984) [30] who reported that Fe is not only easily soluble but that plants may take up a very large amount of it.

The Cd value in soil samples in locality-1 recorded the lowest value (0.01 mg kg^{-1}) compared to Co and Pb (0.03 and 1.09 mg kg^{-1}), respectively.

- The overall means of heavy metals concentrations in *E. alba* parts (root, stems and leaves) to be affected by the concentrations of heavy metals in the corresponding soils of both non-contaminated (locality 1) and contaminated (locality 2) (except for Al and Ni).
- *E. alba* accumulated noticeable levels of elements in all its different organs, compared to the corresponding soil, indicating its efficiency in reducing pollutants from the corresponding soil.

Comparing the interaction between the levels of the studied elements in plant organs and the studied localities revealed that the tested elements were transferred from soil to leaves. *E. alba* recorded significantly higher concentrations of Al, Cu, Mn, and Fe in its leaves compared with other studied elements and in Locality- 2 compared with Locality -1. Fe and Mn concentrations in plant organs were higher in locality-2 than in locality-1. Our results are in good accordance with Turnau *et al*. (2010) [65], who reported that many plant species that grow at polluted sites contained more Fe in their leaves than those growing at non-contaminated sites. Fe of the leaves at locality-1 recorded the highest values among all tested plant organs at the studied locations. However, the lowest value was recorded for the content of stem Co at locality-2. Khan *et al*. (2006) [33]reported Fe to be among the highest values of heavy metals in leaves. Maximal values of Fe (1162.80 and 1323.80 mg kg^{-1}) were recorded in the leaves at locality-1 and locality-2, respectively. Similar findings were reported by Iqbal and Hamayun (2010).

In the present study, the Mn value was below the Fe value for each plant organ (roots, stems or leaves) of *E. alba*. This finding was in accordance with previous reports (Thien 2005; Ain Nihla 2006) [64, 2] that found the level of Mn uptake to be lower compared to Fe in plant organs. Moreover, Kabata-Pendias (2011) [28] reported that Mn and Fe values are interrelated in metabolic functions and that Fe values are more important for plant tissues than Mn for plant health. The highest content of Mn was recorded in the leaves of *E. alba*, followed by the roots in both the studied localities, which has been confirmed by KabataPendias and Pendias, (1993).

Mn exhibited higher values in plant organs than Zn. This result is not in consistent with the values reported that Mn is preferentially linked to cell wall more than Zn and the latter element is more easily relocated to other parts (Kabata-Pendias and Pendias 1993).

Comparing the interaction of the tested elements in plant roots in locality-1 (unpolluted) to locality-2 (polluted) revealed that the root Cd in locality-1 (0.82 mg kg^{-1}) was higher than Co and Pb in the root samples in the same location. This proved the ability of plants to accumulate heavy metals, despite their lower values in the soil. This finding is supported by Mc Grath *et al*. (2001), who reported that metal can be accumulated in plants for both high and low concentrations in soil.

In the present study, the interaction between plant organs and the studied localities revealed that the concentration of Ni in root tissues in non-contaminated locality-1 was higher than its concentration in other plant organs. This indicated an increase of plant availability of this metal in the soil with its limited mobility in other plant organs, which agrees with previous reports (Outridge and Noller 1991; Fitzgerald *et al*.2003) [49]. In addition, numerous studies reported that heavy metals are largely retained in below-ground tissues (Bonanno 2011). However, differentiations in heavy metal levels in various plant parts have been reported to compartmentalize and translocate through the vascular system of plants (kim *et al*. 2003) [35].

In this study, the interaction between plant parts and the studied localities revealed that Pb concentration was higher in leaves in the studied locations than in roots and stems, which may be due to exposure to dust pollution. This suggestion is in accordance with previous publications (Lesage *et al*.2007; Vymaza *et al* 2007). In contrast, Kabata-Pendias and Pendias, (2001) [28] recorded that the restricted translocation to the shoots was apparent for Pb.

Table (4) reveals the ability of *E. alba* to accumulate and transport the tested elements, determined by estimating their bioaccumulation factor (BAF) and translocation factor (TF). BAF is defined as the ratio of metal concentration in the roots in comparison with that of the surrounding soil, while TF is the ratio of metal concentrations in shoots to that in roots (Malik *et al*.2011) [38]. Hyper accumulator plants should have BAF > 1 or TF > 1, as well as a total accumulation above 1000 mg kg^{-1} for Cu, Co, Cr, or Pb; or above 10000 mg kg^{-1} for Fe, Mn, or Zn (Kabata-Pendias 2011) [29].

The potential for phytostabilization of a plant means that the immobilization of metals at the roots requires the plant to have a BAF value above one and a TF value below one (BAF > 1 and TF < 1) (Yoon *et al*. 2006) [73]. Furthermore, TF above one (> 1) represents the translocation of metals effectively from the roots to the shoots. Plants with both bioaccumulation and translocation factors above one i.e. plants that are able to tolerate the accumulation of heavy metals in plant shoots, provided to have a good potential to be used in phytoextraction for soil cleaning (Zhang *et al*. 2002) [74].

The BAF of the investigated heavy metals (except Pb in the non-contaminated locality-1 and Ni in the contaminated locality - 2) were greater than1, and this may indicate that *E. alba* has a high accumulation ability to these heavy metals (Galal and Shehata 2015). On the other hand, the TF of the investigated heavy metals exceeded 1 in the non-contaminated locality -1 and contaminated locality -2 (except Ni in non-contaminated locality-1 and Fe in the contaminated locality 2). Therefore, these heavy metals are considered to be suitable elements for phytostabilization, which reduces metal mobility and their leaching into groundwater (Galal and Shehata 2015).

For the phytostabilization of Ni and Fe, *E. alba* had bioaccumulation and translocation factors above one, both in unpolluted locality-1 and in the polluted locality-2 and for all considered metals except for Ni and Fe (TF of Ni in locality-1 = 0.79 and TF of Fe in locality-2 = 0.83). Therefore, *E. alba* has a good potential for phytostabilization of Ni in non-polluted locations as well as of Fe in polluted locations (yoon *et al*. 2006) [73]. For Ni in contaminated locality-2, TF (5.45) and the BAF (0.77) indicate that when Ni is present within the roots, it is easily translocated to the shoots [9] (Reeve and Baker 2000) [55]. The leaf Fe of the non-polluted locality-1 is higher than 1000 mg kg^{-1}, categorizing *E. alba* as a Fe-hyper-accumulator (Yoon *et al*. 2006) [73]. However, Sumanta *et al*. (2010) reported that *Eclipta sp.*, grown at a contaminated site showed more Fe accumulation in stems and leaves compared with roots. In addition uptake and translocation of Pb indicated that Pb attained mobility within the plant under specific conditions (Meers *et al*. 2005) [43]. Pb is considered to be the least bioavailable metal, indicating higher Pb accumulation in the roots due to its restricted mobility; therefore, its content can be controlled by phytostabilization. However, its higher values in leaves may be due to exposure to air dust pollution. One advantage of this strategy over phytoextraction is that elimination of the metal-laden plant material is not required (Susarla *et al*. 2002).

In the present study, shoots attained the maximum accumulation of Cd in *E. alba*, a similar finding was found in *E. alba* among various terrestrial plants was reported by Anwar *et al*. (2011) who reported that *E. alba* has the capacity to act as a scavenger of heavy metals (Cd, Cr, Cu, Pb, and Zn) (Fytianos *et al*. 2001).

Ni also exhibited high translocation in shoots compared to roots in polluted locality-2, while it recorded high accumulation in roots in locality-1. Moreover, BAF had its highest value for Fe (100.92) and its lowest value for Ni, while the highest TF was observed for Ni (5.45) and the lowest for Fe (0.83). Deficiency of Fe content may be due to Ni phytotoxicity, competition on membrane transporters. In this regard, competition between Ni and Fe in biochemical and physiological processes was reported to affect the uptake of Ni by Fe transporters in the roots (Pandey and Sharma 2002). In addition, metal interactions between the elements resulted in different values of translocation factors. These interactions may have the influence on the uptake and subsequent translocation of a certain element, despite its low availability in the soil (Meers *et al*. 2005) [43]. Hence, such interactions could affect the uptake of metals from soil and their subsequent distribution throughout the plant (Weis *et al*. 2004). The present study reported higher bioavailability of Ba and Cd metals in locality-1. Amusan *et al*. (2005) [7] reported that bioaccumulation factor (e.g. soil-plant transfer ratios) may be affected by other factors than total soil element concentration. (Chambers and Sidle 1991) [11] reported that metal levels in plants differ greatly when related to soil metal.

E. alba shows promising ability to be used for phytoextraction of all the considered heavy metals, except for Ni in the studied locations. Moreover, it can be used as the hyper accumulator of Al in polluted locations and as Al accumulator in non-polluted locations. In the present study, *E. alba* had Fe concentrations above 1000 mg kg^{-1} in its shoots in locality-1 and higher concentrations in roots than in shoots in locality-2. The Al concentration was above

1000 mg kg^{-1} in shoots at locality-1 and locality-2; therefore, the species was identified as a hyper accumulator for Fe in locality-1 and for Al in locality-1 and locality-2, and as Fe accumulator in locality-1.These results are in accordance with (Kakar *et al*.2011) [31].

Most heavy metals attained lower values in stems of *E. alba* compared to roots and leaves, which is in accordance with Planquart *et al*. (1999) [53], who reported that stems function as a transferring organ; therefore, it attained the minimum concentrations of most heavy metals. Consequently, *E. alba* with strongly correlated metal concentrations with those found in the soil can be considered an indicator plant for metal availability (Alyemenia and Almohisen 2014) [6]. On average, all considered heavy metals had BF and TF values above unity with Ni as the only exception.

Pb and Cd exhibited strong negative correlations in soil with plant organs of *E. alba*. These results were in accordance with those of Demirezen and Aksoy (2004). They reported that weak correlations for metal concentrations between plants and soils indicate that the uptake of metals by plants may depend on factors other than their content levels in the surrounding environment. However, this may be affected by other heavy metal sources, such as air pollution resulted from traffic emissions (Wong *et al*.2003; Onder and Dursun 2006) [48].

Cd in soils showed non-significant correlations for all the considered heavy metal in roots, stems, and leaves. However, it showed the highest negative correlation ($r = -0.876$, $p > 0.05$) with Mn in stems. (Pichte *et al*.2000) [52] reported that in plant tissue Cd correlated poorly with soil Cd concentration. The present results found negative and non-significant correlations for soil and plant organs for all metals at the investigated locations. Correlations were not significant for Zn, Cr, and Pb. Positive correlations for Al, Co, and Cu in soil and leaves, Co, Mn, and Fe in soil and roots, Ni the in soil, stems, and leaves, pH in the soil, roots, and leaves, Ba in the soil, roots, and leaves, and Zn in soil, stems and leaves.

The use of the Pearson Correlation Coefficient showed that most correlations of heavy metals between soil and plant organs were strongly positively correlated a result that has been supported by occurred studies (Fatoki 2003 ; Addo 2012) [18]. In addition, the positive correlations indicate that absorbing elements by plant roots and not by their leaves e.g. from air dust. Moreover, *E. alba* can be considered a potential indicator of heavy metal availability. This finding was supported by Alyemeni and Almohisen (2014) [6] who reported that plants having heavy metal concentrations that correlated strongly with those in the soil are considered good potential indicators of heavy metal availability (Alyemenia and Almohisen 2014) [6]. In addition, positive correlations between soil and plant organs indicated that this species reflects the cumulative effects of environmental cleaning from soil pollution, thus suggesting their potential use in biomonitoring most of the examined heavy metals (Bonanno 2011) [8].

Al and Ba in water exhibited strong and significant negative correlations for most of the considered elements in plant leaves.

Strong positive correlations were recorded for all studied elements. The highest positive correlations were recorded for the element Al, Fe, and Cd that had the highest BAF and also the highest r-values between soil and plant organs. This allows the conclusion that the highest BAF, besides a significant positive correlation between the tested elements, especially Al, Fe, and Cd in soil and plant organs suggests *E. alba* as a powerful phytoremediator.

In the present study, results showed the factors that affect metal of this plant from the studied locations into *E. alba*. The detected metal removal ability of *E. alba* allows utilization of this plant for phytostabilization, phytoextraction, and phytoexclusion of heavy metals techniques. In addition, full consideration of suitable conditions for phytoremediation purposes, plant-soil interactions, and the levels of elements contamination of the soil should be considered (Addo *et al*.2012) [1].

However, the concentrations of the tested elements in soils of non- contaminated locality-1 and contaminated locality-2 were below the Environmental Quality Standards set by the United States Environmental Protection Agency, USEPA (2000) [67]. A significant positive correlation was found between plant variables with soil indicated that *E. alba* maybe useful plant for absorbing these elements from soil, in case of their concentrations fall within the normal range.

Conclusions

E. alba hyper accumulates the metals and produces low to moderate biomass, thus suggesting the plant arable for pollutant removal without causing harmful damage to the ecosystem. The BAFs for all considered elements in *E. alba* were higher than unity, except for Pb in the non-contaminated location and for Ni in the contaminated location. The measured high BAFs of Al, Fe, Cd, and Co in locality-1 and of Fe, Al, Co, and Ba in locality-2 indicate a high potential of this plant species to accumulate the considered elements in its organs.

It can be concluded that *Eclipta alba* having phytoremediation potential characteristics can be exploited for heavy metals direction and decontamination of contaminated soils.

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