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Humic Acids Enhance Potentially Toxic Elements (PTEs) Phytoextraction from a Contaminated Soil by Basil Plants

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Abstract

Elevated levels of potentially toxic elements (PTEs) in soil possess a potential threat to human health. Probably, the use of hyper-accumulator plants can be an eco-friendly and cheap approach to attain more desirable levels of these contaminants in soil. The current study is a trial to increase the efficiency of basil (hyperaccumulator) plants to phytoextract some PTEs, namely Cd, Pb and Co from aclay soil artificially contaminated with these PTEsby the aid of elevated doses of humic acids (HAs 0-50mg kg⁻¹) derived from a mature compost. A greenhouse experiment was conducted in 2021to attain this aim and lasted for 60 days, following a complete randomized design. During this investigation, soil moisture was kept gravimetrically ataround 70% of water holding capacityto avoid the leach out of soluble metal ions or organic complexes from the soil. By the end of this experiment, plants were harvested and the results indicated significant reductions in root and shoot dry weights and their lengths owing to the application of humic acids at a rate of 20mg kg⁻¹ versus the control (0 mg HAs kg⁻¹). Increasing the dose of HAs application (up to 50mg kg⁻¹) led to concurrent significant increases in the abovementioned growth parameters; yet, their values were still below the control plants. Likewise, significant increases were noticeable for PTEs contents within roots and shoots of plants treated with 20mg HAs kg⁻¹ while increasing the dose of HAs application resulted in significant reductions in their contents versus the lower application dose of HAs. Root-to-shoot translocation factor was >1 for each of Cd and Co, while the corresponding values for Pb were less than one. Unfortunately, all BAF values were below one and this result indicates that basil plants cannot be considered hyperaccumulators for Cd and Co but can be used for successful facilitated phytoextraction of these two contaminants from soil. In conclusion, humic acids although enhanced phytoextraction of the PTEs under study by basil plants, yet they cannot beconsidered the optimum chelating agent that could facilitate the phytoextraction of soil contaminants under conditions of the soil under study.

Keywords: Facilitated phytoextraction; Humic acids; Potentially toxic elements; *Ocimum basilicum*; Translocation factor; Bioaccumulation factor

1. Introduction

Potentially toxic elements are a group of elements involving heavy metals and metalloids (Duffus and John, 2002), whose densities exceed 5 Mg m⁻³(Ungureanu et al., 2022) and their atomic numbers exceed 20 (Ali and Khan, 2018) beside of some trace elements which are toxic for plants upon their existence at relatively low concentrations (Elbltagyet al., 2021; Ungureanu et al., 2022).

PTEs are not biodegradable (El-Shazly et al., 2016; Hashim et al., 2017; ElShazly et al., 2019 a and b; Abedin et al., 2019; Abdelhafez et al., 2021), and therefore persist in soils (Naila et al., 2019; Li et al., 2020; Sun et al., 2020). Plants grown on such contaminated soils might suffer deeply (Elshony et al., 2019; Abd-El-Hady and Abdelaty, 2022) and, at the same time, many human diseases might occur due to exposure to the PTEs (Abdelhafez et al., 2015; Adimalla et al., 2020; Wang et al., 2020).

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Although copper, is an essential element for all plants, yet its high doses lead to liver and kidney damage (Osredkar and Sustar, 2011), and in severe cases, it causes death (Lech et al., 2007). High concentrations of Cd cause human emphysema, endstage renal failures, osteoporosis and cancers (Järup, 2002). Exposure to excessive concentrations of Pb damages the neurologic system, leading to loss of neurological function (Chen et al., 2018; Huang et al., 2018; Hamid et al., 2019). Generally, these contaminants are brought to soils via various anthropogenic activities e.g. the burning of fossil fuels, municipal waste (Naila et al., 2019), sewage (Ali et al., 2016; Ibrahim et al., 2016; Abbas and Bassouny, 2018; Farid et al., 2019; Gao et al., 2019; Bassouny et al., 2020; Bassouny and Abbas, 2020), pesticides (Abbas and Meharg, 2008), metal mines (Mohamed et al., 2018), smelting and mining...etc. (Naila et al., 2019). The application of inorganic (Abdelhafez et al., 2012) or organic fertilizers leads also to accumulation of some PTEs in soil (Abdelhafez et al., 2014 a and b; Abdelhafez et al., 2016; Shi et al., 2019). In this regard, Liu et al. (2020) found that the long term application of huge normsoforganic fertilizers from livestock and poultry manure caused excessive concentrations of Zn and Cu in soil. Also, Matte et al. (2017) confirmed that pig manure is a source of soil pollution with Cu.

Physiochemical remediation approaches such as fixation (chemical immobilization of metals), excavation, soil burial, acidic metal leaching (Salt et al., 1995) microfiltration, flocculation, ion exchange and reverse osmosis (Raskin et al., 1996) are applicable for the temporary control of metal contamination (Akshata et al., 2014). Alternatively, the use of hyper-accumulator plants may be an effective approach to lessen the concentrations of soil contaminants to attain more desirable levels (Peer et al., 2005; Sytar et al., 2021). These plants have high capability to absorb high levels of contaminants from soil such as basil (Dinu et al., 2020) and exhibited both biological accumulation factor (BAC)>1 and translocation factor (TF)>1 (Buscaroli et al., 2017). This technique is a cheap and an eco-friendly one (El-Shazly et al., 2016; Sytar et al., 2021).

Basil, known by the "king of the herbs" (Mishra and Chandra, 2022) can treat several diseases and disorders in man and animals (Dhama et al., 2021). This aromatic herbmaygrow and tolerate high levels of PTEs in soil (Mishra and Chandra, 2022), and is considered a good example of contaminant phytoextractors (Dinu et al., 2020).

In spite of that some other factors may lessen considerably the availability of soil contaminants; hence their extraction efficiency by accumulator plants was affected considerably (Gavrilescu, 2022). Some synthetic or natural chelates may be included to improve PTEs phytoextraction (Liang et al., 2019; Dolev et al., 2020). Probably, humic acids (HA) have more advantages versus the synthetic chelates because some synthetic chelates such as EDTA do not undergo degradation (Sauvage et al., 2021) hence increase the possibility of leaching soil contaminants to reach the underground water (Abbas and Abdelhafez, 2013). In this context, the addition of 2% HA generally reduced the extractability of the soluble and exchangeable forms of metals (Halim et al., 2003).

Humic acids were found to be among the physiochemical approaches that can control available contents of PTEs in soil (Rong et al., 2020). It is one of the three main components of organic residues humic acids (HAs), fulvic acids (FA), and humin(Farid et al., 2018 and 2021; Elshaboury and Sakara, 2021; Elramady et al., 2022). This fraction is not soluble in water under acidic conditions, but become soluble and extractable at higher pH values (Kleber and Lehmann, 2019). It contains mainly acidic groups such as carboxyl and phenolic OH functional groups (Song et al., 2019), which enables them to interact with metal ions to form both watersoluble (Wen et al., 2019) and water insoluble complexes (Dongxue et al., 2018; Pukalchik et al., 2018; Mosa et al., 2020).

These organic macromolecules, therefore, play important roles in the transport, bioavailability, and solubility of PTEs (Evangelou *et al.*, 2007). Guo *et al.* (2006) found that upon release of organic acids during the microbial decomposition of organic material by soil biota, these acids become capable to bind the potentially toxic metal or decomposed it and ultimately reduce the metal toxicity.

The current work represents a trial to increase the efficiency of basil plants (*Ocimum basilicum* L.) to phytoextract some potentially toxic elements from soil namely Cd, Pb and Co by aid of HAs derived from a compost to attain environmentally acceptable levels of the contaminants in soil.

To fulfill this aim, a pot experiment was designed for growing basil plants under increasing doses of HAs additives because irrigating these plants should not exceed the saturation percentage to avoid the leach out of soluble metal ions or organic complexes from soil. Specifically, we anticipate that application of humic acids (HAs) increased significantly plant growth as a source of nutrients (Hypothesis 1) and also increased the efficiency of the grown plant to take up more contaminants in soil via formation of organic complexes (Hypothesis 2). Accordingly, the values of both the biological accumulation and translocation factors increased noticeably for PTEs owing to HAs applications, especially with increasing the dose of application (Hypothesis 3).

2. Materials and Methods

Materials of study

Compost was obtained from the Training Center for Recycling Agricultural Residues at Moshtohor (TCRAR), Agricultural Research Center (Egypt). Its chemical properties are presented in Table 1.

TABLE	1.	Physical	and	chemical	properties	of	the
compost							

Property	Unit	Value
Density	Mg m ⁻³	0.66
Moisture content	%	23.00
рН (1:10)		7.70
EC (1:10)	dSm ⁻¹	3.42
$N-NH_4$	mg kg ⁻¹	47.00
N-NO ₃	mg kg ⁻¹	119.00
Total nitrogen	%	1.65
Organic matter	%	34.10
Organic carbon	%	19.78
Ash	%	65.90
C/N ratio		12:1
Total phosphorus	%	1.24
Total potassium	%	0.82
Total humic substances	%	16.00
Humic acids	%	9.50
Fulvic acids	%	6.50

Seedlings of basil (Ocimum basilicum) plant were brought from the Faculty of Agriculture, BenhaUniversity. A composite surface soil sample (0-30)cm depth) collected from was Moshtohorvillage, Toukhcity, Qalubia Governorate, Egypt. Coordinates of location of the sampled soil are 31 ° 22' 26" E and 30° 36' 02" N. These samples were air dried, crushed and sieved via a 2 mm sieve. Physical and chemical properties as well as available contents of the PTEs of the investigated soil are presented in Table 2.

TABLE	2.	Location	$\boldsymbol{o}\boldsymbol{f}$	the	investigated	soil	and	its
	ph	ysical and						

Property	Soil
Particle size distribution	
Coarse- sand	7.30 %
Fine –sand	25.60 %
Silt	25.60 %
Clay	41.50 %
Textural class	clay
pH (1:2.5) soil – water suspension	7.50
E.C soil paste extract (dS/m)	2.02
Organic matter (mg/kg)	12.3
Organic carbon (mg/kg)	7.1
C.E.C (cmmol _c /kg)	33.20
Soluble cations and anions, $mmol_c L^{-1}$	
Ca ⁺⁺	9.50
Mg^{++}	5.33
Na ⁺	4.98
K^+	1.42
$\text{CO}_3^{=}$	-
HCO ₃ ⁻	1.85
Cl	10.14
$SO_4^{=}$	9.24
Total contents of PTEs in soil (mg/kg)	
Cd	0.75
Pb	4.23
Со	21.5

Humic acids extraction and purification

Humic acids were extracted from mature compost according to the method described by Sanchez-Monedero et al. (2002) where a 40 g weight of the compost sample was treated with 800 mL of 1.0 N KOH, shaken under N2 gas atmosphere in sealed bottles for 12 hr, centrifuged for 15 min and the supernatant was removed. The pH was then adjusted to 2 by addition of 2 M H₂SO₄ (Yonebayashi and 1988). The formed precipitates, Hattori, conventionally known as humic acids (HAs), were allowed to coagulate for 24 hr at 4° C then separated from the soluble fulvic acids by centrifugation for 15 min. The precipitated humic acids were washed several times with 0.05 N H₂SO₄ until the filtrate became colorless then dissolved in a small amount of 0.5 N KOH solutions. To reduce their ash content, humic acids were treated with 0.5% HCI-HF solution for 36 hours (Sanchez-Monedero et al., 2002), then transferred to cellophane bags and dialysed against distilled water until the test for Cl in the water outside the bags was negative (Chen et al., 1978). Afterwards, humic acids were air-dried.

Elemental analysis(C, H, N, S and O %)

Elemental analysis for carbon, hydrogen, nitrogen and sulphur contents of the purified humic acids was performed by gas chromatography Hewlett-Packard in the Micro Analytical Center, Faculty of Science, Cairo University. Oxygen was calculated by subtracting total amount of carbon%, hydrogen %,

nitrogen % and sulphur % from 100 (Farid et al., 2018). The obtained results are presented in Table 3.

TABLE 3. Chemical composition of the used humic acids										
Character	C, %	Н, %	N, %	S, %	O, %	C/N	C/H	O/H	N/H	
						ratio	ratio	ratio	ratio	
Content	48.00	4.92	2.92	4.75	39.41	16.44	9.76	8.01	0.59	

Infrared Spectrophotometer

An infrared spectrum (IR) of the HAs was performed with Backman-IR 250 double beam grading spectrophotometer. Scanning was done from 500 to 4000 cm⁻¹. The dried sample was prepared for

infrared spectra using KBr pellets technique (Schnitzer and Khan, 1972) and the results are presented in Fig 1 and Table 4.

TABLE 4. Optical density of some function groups of humic acids extracted by (KOH) as obtained from infrared analysis

Optical density (cm ⁻¹)	3400	2920 - 2860	1725 - 1700	1570 -1515	1400 -1390	1300	1230	1128
	H-bonding of OH	C-H stretching	C = O	C = C stretching	O-H bending	C ≡N stretch	OH phenolic	C–H or O-H stretching
Humic acid	0.45	0.50	1.02	0.71	0.79	0.75	0.93	0.90



Fig 1. FTIR spectra of humic acids extracted from compost

Total acidity of humic acids was determined according to the method described by Dragunova (1958) in presence of NaOH and BaCl₂ then titration against 0.1 N HCl using phenol phthalein as an indicator. Carboxyl groups of humic acids were determined by calcium acetate Ca (CH₃COO)₂ method (Schnitzer and Gupta, 1965). Carboxyl groups were determined according to Dragunova (1958). Phenolic OH groups were determined by subtracting COOH group content from total acidity according to Kononova (1966). The obtained results are displayed in Table 5.

 TABLE 5. Functional groups and total acidity of humic acids (mmol /100g
 HA) extracted from compact

(compost.				
Functional groups	Total	Total	Total	carboxyl	
	acidity	Carboxyl	phenolic	/phenol	
		groups	groups	ratio	
value	755	245	380	0.645	

The greenhouse investigation

The soil under investigation was spread on plastic sheets, then artificially contaminated with Cd, Pb and Co to reach the rates of 10,10 and 100 mg kg⁻¹ soil Cadmium chloride (Sigma using Aldrich, purity99.99%), lead (II) nitrate (Merck, purity \geq 99%), and *Cobalt*(II) *chloride* (Merck, purity \geq 98%) salts, respectively. Afterwards, soil portions, equivalent to 10 kg, were uniformly packed inplastic pots whose dimensions were 30 diameter \times 25 cm depth. Different rates of the purified HAswere addedin triplicates to the soil, 10 days prior to seeding cultivation, to attain 0, 20, 40 and 50 mg kg⁻¹ in order to reach equilibrium. After this period, 4 seedlings of Basil (Ocimum basilicum) plant with 5 leaves were planted in each pot and then were arranged under the greenhouse conditions in a complete randomized design. All plants received the recommended doses of NPK and were watered every 3-4 days to attain 70% of the water holding capacity. In this concern, loss of soil moisture was assessed gravimetrically in other non-planted pots (having the same treatments), considering that the seedlings weight until the end of the experiment isnegligible. After 60 days of incubation, whole plants were removed gently from the soil to avoid root damage; then placed on plastic sieve, washed with tap water to remove stunted dirt then with deionized water. The plant materials were separated into leaves, shoots, and roots, dried at 70°C for 48 h and their dry weights were determined

Plant and soil analyses

Dried plant materials were ground, portions were digested in a mixture of H_2SO_4 and $HClO_4$ (1:1) according to Peterburgski (1968). Particle size

distribution was conducted to determine the soil texture as described by Klute (1986). Calcium carbonate content was determined using Collin's calcimeter as described by Page et al. (1982).

Electrical conductivity (EC, model ICM 71150) as well as soluble ions were determined in soil paste extract. Soil reaction (pH) was measured using a glass electrode pH-meter (Orion Expandable ion analyzer EA920) in 1:2.5 soil-water suspension. Soil organic matter was determined according to Walkly-Black method. Calcium and magnesium were determined by the versinate method (0.01M Na₂EDTA). Sodium and potassium were determined by flame photometer (ILAE 201 Fisher Scientific Company).Chloride was determined by titration with silver nitrate using potassium chromate as an Carbonate bicarbonate indicator. and were determined by titration with HCl in presence of phenol-phthalein and methyl orange indicators, respectively. The sulphate was calculated by difference between total soluble cations and soluble anions. Total contents of Cd, Co and Pb were extracted by fusing a soil sample with a mixture of hydrofluoric (HF), sulphuric (H₂SO₄) and perchloric (HClO₄) acids according to Page et al. (1982) thendetermined in the filtrate using the Inductivity Coupled Plasma (ICP) Spectrometry (Plasma 400).

Data processing

Data obtained herein were statistically analyzed using SPSS (ver 18) statistical software through analysis of variance (2 way-ANOVA) and Dunken's tests to compare among means. All graphs were plotted using Sigma Plot 10 software. Biological accumulation and translocation factors were calculated as follows:

Biological accumulation factor (BAF)=
Concentration of PTEs in areal plant parts (mg kg^{-1})
Concentration of PTEs in soil (mg kg $^{-1}$)
(Zhai et al., 2016)
Translocation factor
(TF)=

Concentrations	of PEs	in the areal	plant	parts	(mg	kg –	¹)
Concent	rations	of PTEs in a	roots (mg kg	-1)		
			(Me	squit	a et	al.,	2021)

3. Results and Discussion

Effect of HAs applications on plant growth parameters

Data presented in Fig 2 reveal that all the investigated plant growth parameters i.e. root and shoot dry weights and their lengths decreased significantly owing to application of HAs at a rate of

20mg kg⁻¹ versus the control. Such reductions might indicate that HA application increased the phytotoxicity of PTEs in soil; consequently these contaminants diminished plant growth. In this context, two scenarios could be presented to explain such reductions (1) HAs decreased soil pH, consequently increased PTEs availability and/or (2) HAs form soluble complexes with PTEs and increase their uptake by plants (Evangelou *et al.*, 2004; Chen and Zhu, 2006; Bandiera *et al.*, 2009). The strong capability of HAs bind with PTEs may; on the other hand, decrease Co (Watanabe et al., 2017), Pb (An et al., 2015) and Cd phytoavailability (Farouk et al., 2011; Ondrasek et al., 2018; Ozfidan-Konakci et al., 2018). The molecular structure and functional groups of HAs might account for either PTEs mobilization or immobilization (Bai et al., 2020).



Fig. 2. Effect of humic acids on some growth parameters of basil plant. Similar letters indicate no significant variations among treatments

Increasing the rate of HAs applications resulted in significant increases in all the above mentioned parameters. The increases occurred in the growth parameters due to the applied HAs can be probably attributed to the higher nutrient contents applied to the soil upon application of the HA (Dinçsoy and Sönmez, 2019). Moreover, HAs is a source of Cfor plant growth (Chen and Zhu, 2006).

The acidifying effect of the organic acids released due to decomposition of the applied HAs (Steffen *et al.*, 2002) (which is in direct proportional to quantity of HA applied rate) might account for the more available contents of the different nutrients in soil (Chen and Zhu, 2006; Justi *et al.*, 2019) and subsequent absorption of these elements leading to higher values of the growth parameters (Dehsheikh *et al.*, 2020). The dissolution of CO₂ exhaled by organic matter decomposers in soil water (Steffen *et al.*, 2002; Clemente and Bernal, 2006) and the formation

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of H_2CO_3 acid may be considered an additional reason for increasing availability and uptake of the nutritive elements by plants. These results did not justify the first hypothesis

Effect of HAs applications on concentrations and uptake of PTEs by basil plants

Data presented in Table 6 contribute to declaration of the previously mentioned results. These data illustrate a trend for the effect of the applied HAs rates on concentrations of Cd, Co and Pb within plant roots and shoots similar ,to a great extent, to that occurred due to the applied HAs rates on plant growth parameters. Application of HAs at a rate of 20mg kg⁻¹ significantly raised concentrations of each of Cd, Co and Pb within different plant parts versus the control plants, consequently the uptake of these PTEs increased considerably. Probably, the formation of soluble humic aid complexes with PTEs account for such increases. On the other hand, rising the rate of HAs application resulted in simultaneous reductions in concentrations of PTEs within plant roots and shoots. This result might signify the enhancement effect of humic acid on increasing plant

growth; hence concentrations of PTEs seemed to be diluted within plant tissues. In general, humic acids applications decreased the uptake of both Cd and Pb by plants; yet it increased Co uptake. This is because only a small fraction of Co is mobile in soil (Pourret et al., 2016). Thus, Co ions were chelated by carboxylic and phenolic groups in HA forming soluble complexes that decreased Co(II) sorption on clay minerals (Baek and Yang, 2004). On the other hand, coagulation of PTEs-humic complexes might account for reductions in Cd-uptake by plants (Sholkovitz and Copland, 1981) via complexation by electrostatic attraction and inter-molecular bridging with the carboxyl groups of humic acids (Ai et al., 2020). It is probably the case between Pb and HAs (Clemente and Bernal, 2006), because Pb exhibits higher mobility in soil than Co (Kierczak et al., 2008); in addition to its higher stability constant with the functional groups of HAs than Co (Pandey et al., 2000). The above results; therefore, support the second hypothesis

TABLE 6. Concentrations and uptake of Cd, Pb and Co by basil plants as affected by humic acids applications

Humic acids	Cd			Pb			Co		
application	Root	Shoot	Total-	Root	Shoot	Total-	Root	Shoot	Total-uptake
dose,mg kg ⁻¹	content	content	uptake	content	content	uptake	content	content	mg pot ⁻¹
	mg kg ⁻¹	mg kg ⁻¹	mg pot ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg pot ⁻¹	mg kg ⁻¹	mg kg ⁻¹	
0	0.14 ± 0.03^{b}	0.16 ± 0.08^{d}	1.72±0.08d	1.51 ± 0.13^{d}	0.71 ± 0.17^{d}	6.76±0.48d	$0.40\pm0.10^{\circ}$	2.75 ± 0.18^{d}	23.28 ± 1.94^{d}
20	$0.76{\pm}0.16^{a}$	4.36 ± 0.10^{a}	29.08±1.73a	$3.99{\pm}0.24^{a}$	$1.98{\pm}0.12^{a}$	20.79±1.82a	11.06 ± 0.22^{a}	$61.22{\pm}1.05^{a}$	$408.72 \pm 31.50^{\circ}$
40	$0.59{\pm}0.04^{a}$	2.90 ± 0.08^{b}	21.94±1.62b	2.75 ± 0.23^{b}	1.36 ± 0.15^{b}	13.72±0.98b	10.57 ± 0.38^{ab}	58.64±1.13 ^b	442.15±36.09 ^b
50	$0.26{\pm}0.08^{b}$	0.99±0.11°	8.57±0.62c	$2.11 \pm 0.14^{\circ}$	$1.03{\pm}0.07^{\circ}$	10.42±0.71c	9.93±0.77 ^b	55.96±0.77 ^c	$472.67{\pm}39.16^{a}$

• Similar letters within each column indicate no significant variations among treatments

Effect of humic acids applications on the biological accumulation and translocation factors of these contaminants by basil plants

Bioaccumulation and translocation factors are widely used by many researchers to notify the success of the used plant species in the phytoextraction process of contaminants from soils (Trotta et al., 2006; Rezapour et al., 2019; Sarhan et al., 2022). In this context, the values of these factors should exceed "one" for hyperaccumulator plants (Takarina and Pin, 2016). Results obtained herein (Figure 3) indicate that root-to-shoot translocation factor was >1 for each of Cd and Co while the corresponding values of Pb were less than one. This result indicates that basil plants translocate considerable concentrations of Cd and Co from roots to the areal plant parts while retains Pb within its root. May be this plant can be used for phytoextraction of these two contaminants from soil. The other parameter i.e. the bioaccumulation factor (BAF) is important to highlight the capability of plants to hyperaccumulate contaminants from soil (BAF>1) (Buscaroli et al., 2017). Unfortunately, all BAF values were below one and this result indicates that basil plants are not hyperaccumulators for Cd and Co but can be used for successful facilitated phytoextraction of these two contaminants from soil. The use of humic acids increased the transfer factor of Cd at low application levels while decreased that of Co. In case of Pb, this factor remained unchangeable in presence and absence of the humic acids. Concerning, BAF, HAs successfully increased its values for all PTEs under investigation; yet these values were still below "one". This result did not validate the third hypothesis.

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Fig 3. Biological accumulation factors and the translocation factors of Cd, Pb and Co by basil plants as affected by humic acids applications. Similar letters indicate no significant variations among treatments

Conclusions

Application of humic acids at a rate of 20mg kg⁻¹ led to significant increases in PTEs (Cd, Pb and Co) uptake by the grown basil plants while recorded significant reductions in plant growth parameters (dry weights and lengths of plant roots and shoots). Moreover, this amendement increased the rate of PTEs translocation to areal plant parts recording TF>1 for extraction of Cd and Co from soil. Increasing the rate of application of humic substances (>20mg kg⁻¹) lessened considerably the uptake of the investigated PTEs by plants; hence improved plant growth parameters. Although, HAs increased soil-to-root biological accumulation factor; yet the estimated values were still below "one". This result indicates that humic acid is not the optimum chelating agent that could facilitate the phytoextraction of soil contaminants.

4. Conflicts of interest

There are no conflicts to declare.

5. Formatting of funding sources

There is no fund

6. References

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