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Phytoremediation of some heavy metals in contaminated soil



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Abstract

Background: Several cultivated areas are irrigated with low-quality waters from the drainage system due to the shortage of suitable source of water for agricultural activities. Most of these drainage waters are contaminated with heavy metals which are concentrated in surface layer of the soil and translocated to plant and food chains. The region of Sahl El Husseiniya, Alsharqia government, is mainly irrigated with wastewater of Bahr El-Bagar drain.

Objective: Two types of hyperaccumulator plants represented by kenaf (*Hibiscus cannabinus L.*) and flax (*Linum usitatissimum L.*) were cultivated successively in the study area. Humic acid and gibberellin were used in this experiment as foliar sprayings to enhance the plant ability to absorb heavy metal ions from the soil. In addition, three soil additives represented by sulfur, vermiculite, and compost were also applied.

Results: Sulfur was the soil additive of the most pronounced effect on the uptake of Cr, Co, Cd, and Mn by the hyperaccumulator plants, while humic acid was of more favorable effect as a foliar treatment on Co and Cr uptake by flax and Cd and Mn uptake by kenaf. However, the foliar application of gibberellin enhanced plant growth and was of the best effect on both Co and Cr uptake by kenaf-cultivated soils and both Cd and Mn in flax-cultivated soils. In general, heavy metals were more concentrated in roots than in shoots. Comparing the efficiency of the two crops in cleaning soils, results implied that kenaf was of more favorable effect on the removal of Cr, Co, and Cd, while flax was of higher superiority in the removal of Mn. The efficiency of kenaf on removal of the studied metal ions followed the descending order of Cr > Co > Mn > Cd where their removal percentage values reached 50.71, 38.27, 33.98, and 14.43%, respectively. Flax phytoremediation efficiency followed the descending order of Mn > Cr > Co > Cd, where their removal percentage values reached 54.36, 36.95, 28.72, and 11.37%, respectively. Double season phytoremediation efficiency followed the order of Cr \geq Mn > Co > Cd achieving 66.87, 65.63, 54.66, and 23.40%, respectively.

Keywords: Phytoremediation, Bahr El-Bagar drain, Soil contamination, Kenaf, Flax, Humic acid, Gibberellin, Sulfur

Introduction

Heavy metal contamination is one of the major abiotic stresses that cause environmental pollution in recent decades (Osman et al. 2017). Although heavy metals are naturally occurring in the soil, geologic and anthropogenic activities increase the concentration of them to amounts that may be harmful for both plants and animals due to their potential toxicity which disturbs their physiology and development (Bettaieb and Arbaoui 2018). Metals are taken up by plant roots and translocated to shoot system and then pose a potential threat

to human health as they enter the food chain (Arbaoui et al. 2014).

The reuse of drainage water without appropriate treatment may cause adverse effects on soils and crops. Bahr El-Baqar drain is one of the most polluted drains in Egypt. It is located in the eastern part of the Nile Delta and receives untreated wastewater starting from east of Cairo and pours into El-Manzala Lake. Nearly 58% of the total wastewater of Bahr El-Baqar drain comes from agricultural drainage, 2% from industrial wastes, and 40% from domestic and commercial drainage that led to contamination of soils, which irrigated by this water with a lot of heavy metals, e.g., lead, cadmium, nickel, and mercury (Abdel-Fattah and Helmy 2015).

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Phytoremediation (phytoextraction) is a promising technology for cleaning the polluted sites by using plants to extract the heavy metals from the contaminated soil and accumulate them in roots, stems, and branches. Edible crops are not recommended for such a process for the fear of introducing these potentially toxic metal ions into the food chain; thus, fiber crop would be the best suited candidate for use in phytoremediation (Arbaoui et al. 2014; Osman et al. 2017). The efficiency of phytoremediation differs depending on plant morphological, physiological, and anatomical characteristics that affect the mechanisms of ion uptake. Logically, sequential cycles of planting and harvesting of the hyperccumulator plants will finally reduce the concentration of toxic metals in soils to an acceptable level for other uses (Osman et al. 2017).

Kenaf (*Hibiscus cannabinus* L.) is an annual herbaceous plant belonging to the family Malvaceae. It is characterized by rapid growth, high biomass, and tolerance to different environmental conditions. Its fibers are used for manufacturing of biomaterials. Its pulp is used for the paper industry and textile. Consequently, no risk to human health and the environment is expected as it does not enter the food chain (Arbaoui et al. 2016; Cartoga et al. 2005).

Flax (*Linum usitatissimum* L.) of the Linaceae family is an annual dicotyledonous plant and one of the oldest fibrous plants in the world (5,000 BCE). At that time, flax was generally used for fiber production, while nowadays, the main production is focused on oil. It is believed that the origin center of cultivated flax is the Middle East. This plant showed a metal tolerance dependent on species (Szalata et al. 2014).

The importance of sulfur (S) in agriculture has been known for more than a century. However, the continual use of concentrated nitrogen and phosphorus fertilizer formulations that are lacking S, greater export of S from soil in high crop yields, reduced use of S-containing pesticides, reduced S input through rainwater, and reduction of emissions of SO₂ from fossil fuel burning to the atmosphere have led to an increase of S deficiency in soils (Lucheta and Lambais 2012).

Vermiculite is a naturally occurring sheet hydrous phyllosilicate. Its size usually ranges from 1 mm to 1 cm diameter. Its chemical formula is: $(Mg,Fe^{+2},Fe^{+3})_3$ [(Al, Si)₄O₁₀](OH)₂·4H₂O. Vermiculite is a 2:1 clay, meaning it has two tetrahedral sheets for every one octahedral sheet. It is a limited expansion clay with a medium shrink-swell capacity. It has a high cation exchange capacity (CEC) at 100–150 meq/100 g. Vermiculite clays are weathered micas in which the potassium ions between the molecular sheets are replaced by magnesium and iron ions (Folorunso 2015).

Compost is a soil amendment produced through the metabolism of an organic substrate by aerobic microbes under controlled conditions. Composting is an ancient agricultural technology going back to biblical times but still has important applications in modern agriculture. Compost provides a primary source of nutrients for the crop in organic cropping systems, while it provides a supplementary nitrogen source that compliments fertilizer nitrogen to provide a more sustainable farming system in conventional cropping systems (Seyedbagheri 2010).

Gibberellins are numerous groups of plant regulator hormones (Falkowska et al. 2011). They play a central role in the regulation of plant growth and development (Khan et al. 2009).

Humic acids are considered as the main fractions of humic substances and the most active components of soil and compost organic matter. In the recent years, they have been demonstrated to exert several direct and indirect biological effects on plants, including physiological, morphological, biochemical, and genetic effects (Ferrara and Brunetti 2008).

This work aimed to (1) study the effect of some soil additives (vermiculite, sulfur, and compost) and foliar spraying (humic acid and gibberellins) on heavy metal accumulation through consecutive cultivation of kenaf followed by flax plants in contaminated soil of Sahl El Husseiniya region, (2) study the translocation of heavy metals from soil to root and shoot systems of the two cultivated plants in addition to the growth criteria to evaluate the kenaf and flax potentialities for phytoremediation of the contaminated sites, and (3) study the distributions and chemical fractions of heavy metals in the studied soils under the wastewater irrigation conditions in the area of study.

Materials and methods

Area of study

It is the Sahl El Husseiniya region, Alsharqia government, which is irrigated with the wastewater of Bahr El-Baqar drain. The selected area is located between latitudes 30° 50′ 59.5″ N and longitudes 31° 59′ 00″ E.

Kenaf and flax cultivation

Seeds of the summer crop kenaf (*Hibiscus cannabinus* L.) cultivar Giza 3 and the winter crop flax (*Linum usitatissimum*) cultivar Sakha 1 were obtained from Agricultural Research Centre, Giza, Egypt. The cultivation land was divided into 12 sections each of 4×5 m area, plants seeds were placed in soil at 30 cm depth and left to grow till the end of the season, and then, samples were collected at harvesting stage. Plants were irrigated with the wastewater of Bahr El-Baqar drain. The experiment statistical design was split plot design for each cultivation season. Kenaf seeds were germinated in June, and the fully grown plants were harvested in October 2017; afterwards, soil was cleaned, plowed, and smoothed prior

to germination of flax seeds in November 2017, and the fully grown plants were harvested in April 2018.

Treatments via foliar spraying and soil additives

Kenaf and flax shoot systems were sprayed twice, 30 days after cultivation and at the flowering stage, with humic acid (40 mg/l) or gibberellin (200 mg/l) solutions. Humic acid salt (Sigma-Aldrich) was dissolved initially in alkaline medium (pH was adjusted to 9 with NaOH), and then, pH was readjusted to 7 with HCl. Gibberellin solution was prepared from 80% A_3 basis stock solution (Sigma-Aldrich).

Vermiculite (Sigma-Aldrich), elemental sulfur (Sigma-Aldrich), and vegetarian compost (produced in Desert Research Center) were added once initially as soil additives during the cultivation of both of kenaf and flax. The addition dosage was 2 ton/feddan for vermiculite, elemental S, and compost.

Field measurements and sample collection

Yielded plants for each treatment were counted and weighed in the field per square meter. Heights of ten plants were measured. Plant samples were collected carefully as a whole, cleaned thoroughly from soil and dirt, washed with distilled water, separated into shoot and root, and preserved in paper bags. Each plant sample was represented by three replicates. Soil samples were collected at a depth of $0-30\,\mathrm{cm}$, and each soil sample was represented by three replicates. Water sample was preserved in the field via addition of few drops of HNO₃ for heavy metal analysis.

Sample preparation

Water sample was filtered prior to analysis. Soil samples were air dried, crushed gently, and sieved through a 2-mm sieve prior to analysis. A part of each soil sample (1 g) was digested according to Shumo et al. (2014) using a mixture of concentrated HNO $_3$ and H $_2$ O $_2$ for the determination of total heavy metal content. Plant samples were oven dried at 55 °C and grinded to fine particles. A part of each plant sample (1 g) was digested as mentioned before according to Shumo et al. (2014).

Sample analyses

Heavy metals in water, soil, and plant samples were analyzed using Inductively Coupled Argon Plasma, iCAP 6500 Duo, Thermo Scientific, England. One thousand milligrams per liter of multi-element certified standard solution, Merck, Germany, was used as the stock solution for instrument standardization.

The chemical fractionation of heavy metals (Cr, Co, Cd, and Mn) in the studied soils was conducted by using a technique recommended by Tessier et al. (1979) after modification by Kashem and Singh (2001). Accordingly,

the heavy metals were separated into six different fractions, namely water-soluble (F1), exchangeable (F2), carbonate-bound (F3), iron and manganese oxide-bound (F4), organic matter-bound (F5), and residual (F6).

Statistical analysis

All assays were made in triplicate. Data were subjected to an analysis of variance (ANOVA) using the Duncan's test with statistical significance at P < 0.05 (Waller and Duncan 1969). Means having the same alphabetical letters in the same column are not significant at significance probability value (P) = 0.05.

Calculations

- Remediated metal fraction (mg/kg) = Initial metal concentration in soil before planting – final metal concentration in soil after planting
- Planting season remediation (%) = (remediated metal fraction/initial metal concentration in soil before planting) × 100
- Double season phytoremediation (%) = [(Initial metal concentration in soil its corresponding concentration in soil after the second planting season)/Initial metal concentration in soil] × 100
- Bioconcentration factor "BCF" (root or shoot) =
 Concentration of metal in plant parts (root or
 shoot)/concentration of metal in soil (Nizam et al.
 2016)

Results

Heavy metal contents in soil and plant parts and remediation percentages

Cobalt

Concerning the kenaf first planting season (Table 1), Co was accumulated in shoot samples in concentrations ranging from 0.02 to 1.77 with a mean of 0.54 mg/kg, while the root samples recorded concentrations ranging from 1.94 to 5.27 with a mean of 3.27 mg/kg; subsequently, the Co concentration in the whole plant samples ranged from 2.09 to 7.04 with a mean of 3.80 mg/kg. Concentrations of Co in the soil samples after kenaf planting were remarkably reduced to the range of 13.18–16.91 with a mean of 14.72 mg/kg compared with 21.35 mg/kg as an initial concentration. The Coremediated fraction ranged from 4.44 to 8.17 with a mean of 6.64 mg/kg representing remediation percentage ranging from 20.80 to 38.27 with a mean of 31.09%.

Sulfur soil addition accompanied with gibberellin as foliar spraying was of the highest significant effect on the accumulation of Co in the shoot (1.77 mg/kg), root (5.27 mg/kg), and whole plant sample (7.04 mg/kg). Such a finding occurred in the lowest significant Co soil content (13.18 mg/kg). This treatment achieved the highest

Table 1 Total content of Co in soil and plant parts in milligrams per kilogram (kenaf first planting season)

Treatment		First planting season (kenaf)										
Soil additive	Foliar spraying	Shoot	Root	Whole plant	Initial soil	Soil after first planting	Remediated fraction	Remediation (%)	BCF shoot	BCF root	BCF whole plant	
Sulfur	Humic acid	1.15 ^c	4.86 ^b	6.01 ^b	21.35	13.58 ^h	7.78 ^e	36.44 ^e	0.085 ^c	0.358 ^b	0.443 ^b	
Sulfur	Gibberellin	1.77 ^a	5.27 ^a	7.04 ^a		13.18 ^l	8.17 ^a	38.27 ^a	0.134 ^a	0.400 ^a	0.534 ^a	
Sulfur	Control	1.64 ^b	4.06 ^d	5.70 ^c		13.95 ^g	7.41 ^f	34.71 ^f	0.118 ^b	0.291 ^d	0.409 ^c	
Compost	Humic acid	0.17 ^f	2.21 ⁱ	2.38 ^j		15.50 ^d	5.86 ⁱ	27.45 ⁱ	0.011 ^f	0.143 ⁱ	0.154 ⁱ	
Compost	Gibberellin	0.13 ^g	2.13 ^j	2.26 ^k		15.09 ^e	6.27 ^h	29.37 ^h	0.009 ^g	0.141 ^j	0.150 ^j	
Compost	Control	0.13 ^g	1.97 ^k	2.09 ^l		14.92 ^f	6.43 ^g	30.12 ⁹	0.009 ^g	0.132 ^k	0.140 ^k	
Control	Humic acid	0.02 ⁱ	4.07 ^c	4.09 ^d		13.35 ^j	8.00 ^c	37.47 ^c	0.001 ^j	0.305 ^c	0.306 ^d	
Control	Gibberellin	0.04 ^h	2.94 ^h	2.98 ^h		13.25 ^k	8.10 ^b	37.94 ^b	0.003 ^h	0.222 ^f	0.225 ^f	
Control	Control	0.04 ^h	3.18 ^f	3.22 ^f		13.55 ⁱ	7.80 ^d	36.53 ^d	0.003 ^h	0.235 ^e	0.238 ^e	
Vermiculite	Humic acid	0.04 ^h	3.05 ^g	3.09 ⁹		16.52 ^c	4.84 ^j	22.67 ^j	0.002 ⁱ	0.185 ^h	0.187 ⁹	
Vermiculite	Gibberellin	0.77 ^d	1.94 ^l	2.71 ⁱ		16.91 ^a	4.44 ^l	20.80 ^l	0.046 ^d	0.115 ^l	0.160 ^h	
Vermiculite	Control	0.52 ^e	3.5 ^e	4.02 ^e		16.81 ^b	4.54 ^k	21.26 ^k	0.031 ^e	0.208 ^g	0.239 ^e	
Mean		0.54	3.27	3.80		14.72	6.64	31.09	0.04	0.23	0.27	
Minimum		0.02	1.94	2.09		13.18	4.44	20.80	0.001	0.115	0.140	
Maximum		1.77	5.27	7.04		16.91	8.17	38.27	0.134	0.400	0.534	

significant Co remediation percentage, i.e., 38.27% (soil-remediated fraction was 8.17 mg/kg). Also, this treatment showed the highest significant BCF calculated values for the shoot, root, and whole plant samples (0.134, 0.400, and 0.534, respectively).

The kenaf roots' remediation efficiencies of Co were significantly higher than the corresponding ones of shoots, where the calculated BCF values of shoot samples ranged from 0.001 to 0.134 with a mean of 0.04 in comparison with a range of 0.115–0.400 with a mean of 0.23 in root samples. The whole plant BCF values ranged from 0.140 to 0.534 with a mean of 0.27.

The results of flax which was planted in the second planting season successive to kenaf are represented in Table 2. Co was accumulated in shoots with concentrations ranging from 0.13 to 0.81with a mean of 0.38 mg/kg, while the roots recorded concentrations ranging from 0.14 to 1.96 with a mean of 0.76 mg/kg; subsequently, the whole plant concentrations ranged from 0.34 to 2.77 with a mean of 1.14 mg/kg.

Concentrations of Co in the soil samples after flax planting were remarkably reduced to the range of 9.68–13.40 with a mean of 11.55 mg/kg. The soil-remediated Co fraction ranged from 1.77 to 3.90 with a mean of 3.16 mg/kg representing remediation percentage ranging from 13.36 to 28.72 with a mean of 21.41%. The double season phytoremediation using kenaf followed by flax achieved remediation percentage for Co which ranged from 37.24 to 54.66 with a mean of 45.88%.

In contrast to kenaf, sulfur soil addition accompanied with humic acid as foliar spraying in case of flax was the treatment of the highest significant accumulation of Co in the shoot (0.81 mg/kg), root (1.96 mg/kg), and whole plant (2.77 mg/kg). This occurred in the lowest significant Co soil content (9.68 mg/kg). This treatment achieved the highest significant remediation percentage, i.e., 28.72% (soil-remediated fraction was 3.90 mg/kg). Also, it achieved the highest significant BCF calculated values for the shoot, root, and whole plant samples (0.084, 0.202, and 0.286, respectively). Sulfur soil addition accompanied with humic acid as foliar spraying showed the highest significant double season phytoremediation percentage that reached 54.66%.

In agreement with kenaf, flax roots' remediation capacities of Co were higher than the corresponding ones of shoots, where the calculated BCF values of shoot samples ranged from 0.010 to 0.084 with a mean of 0.03 in comparison with a range of 0.012–0.202 with a mean of 0.07 in root samples. The whole plant BCF values ranged from 0.034 to 0.286 with a mean of 0.11. Flax BCF values were markedly lower than the corresponding ones of kenaf which means that kenaf is more efficient than flax in Co phytoremediation.

Chromium

Concerning with kenaf first planting season (Table 3), Cr was accumulated in shoot samples with concentrations ranging from 1.25 to 7.54 with a mean of 3.57 mg/kg, while the root samples recorded concentrations ranging

Table 2 Total content of Co in soil and plant parts in milligrams per kilogram (flax second planting season)

Treatment		Second planting season (flax)										
Soil additive	Foliar spraying	Shoot	Root	Whole plant	Soil after second planting	Remediated fraction	Remediation (%)	Double season phytoremediation (%)	BCF shoot	BCF root	BCF whole plant	
Sulfur	Humic acid	0.81 ^a	1.96ª	2.77 ^a	9.68 ^l	3.90 ^a	28.72 ^a	54.66 ^a	0.084 ^a	0.202 ^a	0.286 ^a	
Sulfur	Gibberellin	0.68 ^b	1.73 ^b	2.41 ^b	10.73 ^j	2.45 ⁱ	18.59 ⁱ	49.74 ^c	0.063 ^b	0.161 ^b	0.225 ^b	
Sulfur	Control	0.62 ^c	1.02 ^d	1.64 ^c	10.25 ^k	3.70 ^d	26.52 ^b	51.99 ^b	0.060 ^c	0.100 ^d	0.160 ^c	
Compost	Humic acid	0.20 ^h	1.22 ^c	1.42 ^d	11.74 ^d	3.76 ^b	24.26 ^d	45.01 ⁱ	0.017 ^g	0.104 ^c	0.121 ^d	
Compost	Gibberellin	0.47 ^d	0.14 ^l	0.61 ^h	11.62 ^e	3.47 ^f	23.00 ^e	45.57 ^h	0.040 ^d	0.012 ^l	0.052 ^g	
Compost	Control	0.17 ^j	0.34 ^h	0.51 ⁱ	11.18 ^h	3.74 ^c	25.07 ^c	47.63 ^e	0.015 ^h	0.030 ^h	0.046 ^h	
Control	Humic acid	0.45 ^e	0.90 ^e	1.35 ^e	11.03 ⁱ	2.32 ^h	17.38 ^j	48.34 ^d	0.041 ^d	0.082 ^e	0.122 ^d	
Control	Gibberellin	0.42 ^f	0.60 ^f	1.02 ^f	11.48 ^f	1.77 ^j	13.36 ^l	46.23 ⁹	0.037 ^e	0.052 ^f	0.089 ^e	
Control	Control	0.30 ^g	0.49 ^g	0.79 ⁹	11.25 ^g	2.31 ^h	16.97 ^k	47.31 ^f	0.027 ^f	0.044 ^g	0.070 ^f	
Vermiculite	Humic acid	0.16 ^k	0.29 ⁱ	0.45 ^j	13.21 ^b	3.31 ^g	20.04 ^h	38.13 ^k	0.012 ^j	0.022 ⁱ	0.034 ⁱ	
Vermiculite	Gibberellin	0.13 ^l	0.21 ^k	0.34 ^l	13.40 ^a	3.51 ^e	20.76 ⁹	37.24 ^l	0.010 ^k	0.016 ^k	0.025 ^k	
Vermiculite	Control	0.18 ⁱ	0.23 ^j	0.41 ^k	13.08 ^c	3.73 ^c	22.19 ^f	38.74 ^j	0.014 ⁱ	0.018 ^j	0.031 ^j	
Mean		0.38	0.76	1.14	11.55	3.16	21.41	45.88	0.03	0.07	0.11	
Minimum		0.13	0.14	0.34	9.68	1.77	13.36	37.24	0.010	0.012	0.034	
Maximum		0.81	1.96	2.77	13.40	3.90	28.72	54.66	0.084	0.202	0.286	

from 16.61 to 28.66 with a mean of 20.51 mg/kg; subsequently, the whole plant sample concentrations ranged from 17.86 to 36.20 with a mean of 24.08 mg/kg. Concentrations of Cr in the soil samples after kenaf planting were remarkably reduced to the range of 34.00–47.50 with a mean of 43.21 mg/kg compared with 68.98 mg/kg as an

initial concentration. The soil-remediated Cr fractions ranged from 21.49 to 34.98 with a mean of 25.78 mg/kg representing remediation percentage ranging from 31.15 to 50.71 with a mean of 37.37 mg/kg.

Sulfur soil addition accompanied with gibberellin as foliar spraying was the treatment of the highest significant

Table 3 Total content of Cr in soil and plant parts in milligrams per kilogram (kenaf first planting season)

Treatment		First pl	First planting season (kenaf)										
Soil additive	Foliar spraying	Shoot	Root	Whole plant	Initial soil	Soil after first planting	Remediated fraction	Remediation (%)	BCF shoot	BCF root	BCF whole plant		
Sulfur	Humic acid	6.06 ^b	24.83 ^b	30.89 ^b	68.98	36.24 ^k	32.75 ^b	47.48 ^b	0.167 ^b	0.685 ^b	0.852 ^b		
Sulfur	Gibberellin	7.54 ^a	28.66 ^a	36.2 ^a		34.00 ^l	34.98 ^a	50.71 ^a	0.222 ^a	0.843 ^a	1.065 ^a		
Sulfur	Control	5.62 ^c	23.42 ^c	29.04 ^c		38.11 ^j	30.88 ^c	44.77 ^c	0.147 ^c	0.615 ^c	0.762 ^c		
Compost	Humic acid	3.82 ^e	18.41 ⁱ	22.23 ^f		45.43 ⁹	23.56 ^f	34.15 ^f	0.084 ^e	0.405 ^g	0.489 ^f		
Compost	Gibberellin	3.09 ^f	18.61 ^g	21.70 ^g		45.62 ^e	23.37 ^h	33.88 ^h	0.068 ^f	0.408 ^g	0.476 ^g		
Compost	Control	1.25 ^l	16.61 ^l	17.86 ^l		47.50 ^a	21.49 ^l	31.15 ^l	0.026 ^k	0.350 ^k	0.376 ^k		
Control	Humic acid	4.61 ^d	22.35 ^d	26.96 ^d		41.99 ⁱ	26.99 ^d	39.13 ^d	0.110 ^d	0.532 ^d	0.642 ^d		
Control	Gibberellin	2.14 ⁱ	21.18 ^e	23.32 ^e		43.84 ^h	25.15 ^e	36.46 ^e	0.049 ^h	0.483 ^e	0.532 ^e		
Control	Control	1.93 ^j	18.93 ^f	20.86 ⁱ		45.54 ^f	23.45 ^g	34.00 ^g	0.042 ⁱ	0.416 ^f	0.458 ^h		
Vermiculite	Humic acid	2.87 ^g	18.46 ^h	21.33 ^h		46.71 ^c	22.28 ^j	32.30 ^j	0.061 ^g	0.395 ^h	0.457 ^h		
Vermiculite	Gibberellin	2.25 ^h	17.03 ^k	19.28 ^j		47.09 ^b	21.90 ^k	31.75 ^k	0.048 ^h	0.362 ^j	0.409 ^j		
Vermiculite	Control	1.67 ^k	17.59 ^j	19.26 ^k		46.46 ^d	22.53 ^l	32.66 ⁱ	0.036 ^j	0.379 ⁱ	0.415 ⁱ		
Mean		3.57	20.51	24.08		43.21	25.78	37.37	0.09	0.49	0.58		
Minimum		1.25	16.61	17.86		34.00	21.49	31.15	0.026	0.350	0.38		
Maximum		7.54	28.66	36.20		47.50	34.98	50.71	0.222	0.843	1.06		

effect on the shoot accumulation of Cr (7.54 mg/kg), root (28.66 mg/kg), and whole plant samples (36.2 mg/kg). This was related to the lowest significant Cr soil content of Cr (34.00 mg/kg). This treatment achieved also the highest significant remediation percentage, i.e., 50.71% (soil-remediated fraction was 34.98 mg/kg). Moreover, this treatment showed the highest significant BCF calculated values for the shoot, root, and whole plant samples (0.222, 0.843, and 1.065, respectively).

The kenaf roots' remediation efficiencies of Cr were significantly higher than the corresponding ones of shoots, where the calculated BCF values of shoot samples ranged from 0.026 to 0.222 with a mean of 0.09 in comparison with a range of 0.350–0.843 with a mean of 0.49 in root samples. The whole plant BCF values ranged from 0.38 to 1.06 with a mean of 0.58.

The results of flax (second planting season) are represented in Table 4. Cr was accumulated in shoots with concentrations ranging from 1.76 to 4.99 with a mean of 3.06 mg/kg, while the roots recorded Cr concentrations ranging from 3.93 to 8.21 with a mean of 5.81 mg/kg; subsequently, the whole plant Cr concentrations ranged from 7.10 to 13.19 with a mean of 8.86 mg/kg. Concentrations of Cr in the soil samples after flax planting were remarkably reduced to the range of 22.85–37.56 with a mean of 32.31 mg/kg.

The soil-remediated Cr fractions ranged from 8.90 to 13.39 with a mean of 10.90 mg/kg representing remediation percentage ranging from 19.16 to 36.95 with a mean of 25.53%.

The double season phytoremediation using kenaf followed by flax achieved remediation percentage for Cr which ranged from 45.55 to 66.87 with a mean of 53.16%.

In contrast to kenaf, sulfur soil addition accompanied with humic acid as foliar spraying in case of flax was the treatment which recorded the highest significant accumulation of Cr in the shoot (4.99 mg/kg), root (8.21 mg/kg), and whole plant (13.19 mg/kg). This was related to the lowest significant Cr soil content (22.85 mg/kg). This treatment achieved the highest significant remediation percentage, i.e., 36.95% (soil-remediated fraction was 13.39 mg/kg), as well as the highest significant BCF calculated values for the shoot, root, and whole plant (0.218, 0.359, and 0.577, respectively). Sulfur soil addition accompanied with humic acid as foliar spraying showed the highest significant double season phytoremediation percentage that reached 66.87%.

In agreement with kenaf, flax roots' remediation capacities of Cr were higher than the corresponding ones of shoots, where the calculated BCF values of shoot samples ranged from 0.047 to 0.218 with a mean of 0.10 in comparison with a range of 0.111–0.359 with a mean of 0.19 for root samples. The whole plant BCF values

Table 4 Total content of Cr in soil and plant parts in milligrams per kilogram (flax second planting season)

Treatment		Second planting season (flax)										
Soil additive	Foliar spraying	Shoot	Root	Whole plant	Soil after second planting	Remediated fraction	Remediation (%)	Double season phytoremediation (%)	BCF shoot	BCF root	BCF whole plant	
Sulfur	Humic acid	4.99 ^a	8.21 ^a	13.19 ^a	22.85 ^l	13.39 ^a	36.95 ^a	66.87 ^a	0.218 ^a	0.359 ^a	0.577 ^a	
Sulfur	Gibberellin	3.16 ^e	6.21 ^d	9.36 ^c	23.85 ^k	10.16 ^h	29.85 ^b	65.42 ^b	0.132 ^b	0.260 ^b	0.392 ^b	
Sulfur	Control	3.09 ^f	5.60 ^h	8.68 ⁹	28.70 ^j	9.41 ^k	24.69 ^f	58.39 ^c	0.108 ^d	0.195 ^d	0.302 ^c	
Compost	Humic acid	2.54 ⁱ	6.55 ^b	9.09 ^e	32.52 ^h	12.91 ^b	28.42 ^c	52.86 ^e	0.078 ^f	0.201 ^c	0.280 ^d	
Compost	Gibberellin	3.08 ^f	4.84 ^k	7.91 ⁱ	34.21 ^f	11.41 ^d	25.01 ^e	50.41 ^g	0.090 ^e	0.141 ^j	0.231 ^g	
Compost	Control	3.18 ^d	3.93 ^l	7.10 ^l	35.50 ^e	11.99 ^c	25.26 ^d	48.54 ^h	0.090 ^e	0.111	0.200 ^k	
Control	Humic acid	3.46 ^c	6.13 ^e	9.58 ^b	31.87 ⁱ	10.13 ⁱ	24.10 ⁱ	53.80 ^d	0.109 ^d	0.192 ^e	0.301 ^c	
Control	Gibberellin	3.98 ^b	5.18 ⁱ	9.15 ^d	33.15 ^g	10.69 ⁹	24.38 ^g	51.94 ^f	0.120 ^c	0.156 ⁱ	0.276 ^e	
Control	Control	2.80 ^g	4.87 ^j	7.66 ^k	35.87 ^c	9.67 ^j	21.23 ^h	48.00 ^j	0.078 ^f	0.136 ^k	0.214 ⁱ	
Vermiculite	Humic acid	2.58 ^h	6.33 ^c	8.90 ^f	35.89 ^b	10.82 ^f	23.16 ^j	47.97 ^k	0.072 ^g	0.176 ^f	0.248 ^f	
Vermiculite	Gibberellin	2.12 ^j	5.89 ^g	8.00 ^h	35.74 ^d	11.35 ^e	24.10 ⁱ	48.19 ⁱ	0.059 ^h	0.165 ^g	0.224 ^h	
Vermiculite	Control	1.76 ^k	6.00 ^f	7.75 ^j	37.56 ^a	8.90 ^l	19.16 ^k	45.55 ¹	0.047 ⁱ	0.160 ^h	0.206 ^j	
Mean		3.06	5.81	8.86	32.31	10.90	25.53	53.16	0.10	0.19	0.29	
Minimum		1.76	3.93	7.10	22.85	8.90	19.16	45.55	0.047	0.111	0.200	
Maximum		4.99	8.21	13.19	37.56	13.39	36.95	66.87	0.218	0.359	0.577	

ranged from 0.200 to 0.577 with a mean of 0.29. Flax BCF values (root and whole plant) were markedly lower than in case of kenaf. This means that kenaf was more efficient than flax in Cr phytoremediation.

Cadmium

Concerning kenaf first planting season (Table 5), Cd was accumulated in shoot samples with concentrations ranging from 0.16 to 0.36 with a mean of 0.26 mg/kg, while the root samples recorded Cd concentrations ranging from 0.16 to 0.87 with a mean of 0.50 mg/kg; subsequently, the whole plant sample Cd concentrations ranged from 0.32 to 1.23 with a mean of 0.76 mg/kg. Concentrations of Cd in the soil samples after kenaf planting were remarkably reduced to the range of 10.97–11.73 with a mean of 11.38 mg/kg compared with 12.82 mg/kg as an initial concentration. The soil-remediated Cd fraction ranged from 1.09 to 1.85 with a mean of 1.44 mg/kg representing remediation percentage which ranged from 8.50 to 14.43 with a mean of 11.26%.

Sulfur soil addition accompanied with humic acid as foliar spraying was the treatment that resulted in the highest significant accumulation of Cd in the shoot (0.36 mg/kg), root (0.87 mg/kg), and whole plant samples (1.23 mg/kg). Also, it was related to the lowest significant Cd soil content (10.97 mg/kg) that revealed that it was the best treatment in case of Cd. This treatment achieved the highest significant remediation percentage, i.e., 14.43% (soil-remediated fraction was 1.85 mg/kg). Also, this treatment showed the highest significant BCF

calculated values for the shoot, root, and whole plant samples (0.033, 0.079, and 0.112, respectively).

The kenaf roots' remediation efficiencies of Cd were significantly higher than those of shoots, where the calculated BCF values of shoot samples ranged from 0.014 to 0.033 with a mean of 0.023 in comparison with a range of 0.014–0.079 with a mean of 0.045 for root samples. The whole plant BCF values ranged from 0.027to 0.112 with a mean of 0.067.

The results of flax (second planting season) are represented in Table 6. Cd accumulated in shoots with concentrations ranging from 0.10 to 0.28 with a mean of 0.17 mg/kg, while the roots recorded Cd concentrations ranging from 0.12 to 0.33 with a mean of 0.23 mg/kg; subsequently, the whole plant concentrations ranged from 0.22 to 0.61 with a mean of 0.40 mg/kg.

Concentrations of Cd in the soil samples after flax planting were remarkably reduced to the range of 9.82–10.52 with a mean of 10.23 mg/kg. The soil-remediated Cd fractions ranged from 1.06 to 1.26 with a mean of 1.14 mg/kg representing remediation percentage ranging from 9.40 to 11.37 with a mean of 10.05%. The double season phytoremediation using kenaf followed by flax achieved remediation percentage for Cd which ranged from 17.94 to 23.40 with a mean of 20.17%.

In contrast to kenaf, sulfur soil addition accompanied with gibberellin as foliar spraying in case of flax was the treatment which resulted in the highest significant accumulation of Cd in the shoot (0.28 mg/kg), root (0.33 mg/kg), and whole plant (0.61 mg/kg). This occurred in the

Table 5 Total content of Cd in soil and plant parts in milligrams per kilogram (kenaf first planting season)

Treatment		First pl	anting	season (ke	enaf)						
Soil additive	Foliar spraying	Shoot	Root	Whole plant	Initial soil	Soil after first planting	Remediated fraction	Remediation (%)	BCF shoot	BCF root	BCF whole plant
Sulfur	Humic acid	0.36 ^a	0.87 ^a	1.23 ^a	12.82	10.97 ^k	1.85ª	14.43 ^a	0.033 ^a	0.079 ^a	0.112 ^a
Sulfur	Gibberellin	0.33 ^b	0.85 ^b	1.18 ^b		11.08 ^j	1.74 ^b	13.57 ^b	0.030 ^b	0.077 ^b	0.106 ^b
Sulfur	Control	0.32 ^c	0.72 ^c	1.04 ^c		11.12 ⁱ	1.70 ^c	13.26 ^c	0.029 ^c	0.065 ^c	0.094 ^c
Compost	Humic acid	0.25 ^g	0.35 ⁱ	0.61 ^g		11.45 ^f	1.37 ^f	10.69 ^f	0.022 ^g	0.031 ⁱ	0.053 ^g
Compost	Gibberellin	0.23 ^h	0.35 ⁱ	0.58 ⁱ		11.48 ^e	1.34 ⁹	10.45 ⁹	0.020 ^h	0.030 ^j	0.051 ^h
Compost	Control	0.19 ⁱ	0.30 ^j	0.48 ^j		11.50 ^d	1.32 ^h	10.30 ^h	0.017 ⁱ	0.026 ^k	0.042 ⁱ
Control	Humic acid	0.27 ^e	0.53 ^f	0.80 ^e		11.28 ^h	1.54 ^d	12.01 ^d	0.024 ^e	0.047 ^f	0.071 ^e
Control	Gibberellin	0.28 ^d	0.54 ^e	0.82 ^d		11.29 ⁹	1.53 ^e	11.93 ^e	0.025 ^d	0.048 ^e	0.073 ^d
Control	Control	0.26 ^f	0.56 ^d	0.82 ^d		11.28 ^h	1.54 ^d	12.01 ^d	0.023 ^f	0.050 ^d	0.073 ^d
Vermiculite	Humic acid	0.23 ^h	0.37 ^h	0.59 ^h		11.64 ^c	1.18 ⁱ	9.20 ⁱ	0.020 ^h	0.032 ^h	0.051 ^h
Vermiculite	Gibberellin	0.23 ^h	0.43 ^g	0.65 ^f		11.70 ^b	1.12 ^j	8.74 ^j	0.020 ^h	0.037 ⁹	0.056 ^f
Vermiculite	Control	0.16 ^j	0.16 ^k	0.32 ^k		11.73 ^a	1.09 ^k	8.50 ^k	0.014 ^j	0.014 ^l	0.027 ^j
Mean		0.26	0.50	0.76		11.38	1.44	11.26	0.023	0.045	0.067
Minimum		0.16	0.16	0.32		10.97	1.09	8.50	0.014	0.014	0.027
Maximum		0.36	0.87	1.23		11.73	1.85	14.43	0.033	0.079	0.112

Table 6 Total content of Cd in soil and plant parts in milligrams per kilogram (flax second planting season)

Treatment		Second	Second planting season (flax)											
Soil additive	Foliar spraying	Shoot	Root	Whole plant	Soil after second planting	Remediated fraction	Remediation (%)	Double season phytoremediation (%)	BCF shoot	BCF root	BCF whole plant			
Sulfur	Humic acid	0.25 ^b	0.32 ^b	0.57 ^b	9.87 ^j	1.10 ^h	10.03 ^f	23.01 ^b	0.025 ^b	0.032 ^b	0.058 ^b			
Sulfur	Gibberellin	0.28 ^a	0.33 ^a	0.61 ^a	9.82 ^k	1.26 ^a	11.37 ^a	23.40 ^a	0.029 ^a	0.034 ^a	0.062 ^a			
Sulfur	Control	0.24 ^c	0.29 ^c	0.53 ^c	9.89 ⁱ	1.23 ^b	11.06 ^b	22.85 ^c	0.024 ^c	0.029 ^c	0.054 ^c			
Compost	Humic acid	0.14 ^g	0.23 ^f	0.37 ^g	10.37 ^e	1.08 ⁱ	9.43 ^k	19.11 ^h	0.014 ^g	0.022 ^e	0.036 ^f			
Compost	Gibberellin	0.14 ^g	0.23 ^f	0.37 ⁹	10.38 ^d	1.10 ^h	9.58 ^j	19.03 ⁹	0.013 ^g	0.022 ^e	0.036 ^f			
Compost	Control	0.13 ^h	0.20 ^h	0.33 ^h	10.39 ^c	1.11 ⁹	9.65 ^h	18.95 ⁱ	0.013 ^g	0.019 ^f	0.032 ^g			
Control	Humic acid	0.18 ^e	0.26 ^d	0.44 ^e	10.14 ^h	1.14 ^e	10.11 ^e	20.90 ^d	0.018 ^e	0.026 ^d	0.043 ^d			
Control	Gibberellin	0.19 ^d	0.25 ^e	0.45 ^d	10.18 ⁹	1.11 ⁹	9.83 ^g	20.59 ^e	0.019 ^d	0.025 ^d	0.044 ^d			
Control	Control	0.17 ^f	0.22 ^g	0.39 ^f	10.22 ^f	1.06 ^j	9.40 ^l	20.28 ^f	0.017 ^f	0.022 ^e	0.038 ^e			
Vermiculite	Humic acid	0.11 ⁱ	0.14 ⁱ	0.25 ⁱ	10.52 ^a	1.12 ^f	9.62 ⁱ	17.94 ^k	0.010 ^h	0.013 ^g	0.024 ^h			
Vermiculite	Gibberellin	0.10 ^j	0.14 ⁱ	0.24 ^j	10.51 ^b	1.19 ^d	10.17 ^d	18.02 ^j	0.010 ^h	0.013 ^g	0.023 ^h			
Vermiculite	Control	0.10 ^j	0.12 ^j	0.22 ^k	10.52 ^a	1.21 ^c	10.32 ^c	17.94 ^k	0.010 ^h	0.011 ^h	0.021 ⁱ			
Mean		0.17	0.23	0.40	10.23	1.14	10.05	20.17	0.017	0.022	0.039			
Minimum		0.10	0.12	0.22	9.82	1.06	9.40	17.94	0.010	0.011	0.021			
Maximum		0.28	0.33	0.61	10.52	1.26	11.37	23.40	0.029	0.034	0.062			

soil of the lowest significant Cd content (9.82 mg/kg). This treatment achieved the highest significant remediation of Cd, i.e., 11.37% (soil-remediated fraction was 1.26 mg/kg), as well as the highest significant BCF calculated values for the shoot, root, and whole plant samples (0.029, 0.034, and 0.062, respectively). Sulfur soil addition accompanied with gibberellin as foliar spraying showed the highest significant double season phytoremediation percentage that reached 23.40%.

In agreement with kenaf, flax roots' remediation capacities of Cd were higher than the corresponding ones of shoots, where the calculated BCF values of shoot samples ranged from 0.010 to 0.029 with a mean of 0.017 in comparison with a range of 0.011–0.034 with a mean of 0.022 in root samples. The whole plant BCF values ranged from 0.021 to 0.062 with a mean of 0.039. Flax BCF values were markedly lower than the corresponding BCF values of kenaf. This means that kenaf is more efficient than flax in Cd phytoremediation.

Manganese

Concerning growing kenaf in the first planting season (Table 7), Mn was accumulated in shoot samples with concentrations ranging from 30.07 to 37.56 with a mean of 33.15 mg/kg, while the root samples recorded Mn concentrations ranging from 65.63 to 133.61 with a

mean of 97.82 mg/kg; subsequently, concentration of Mn in the whole plant samples ranged from 100.96 to 171.17 with a mean of 130.97 mg/kg. Concentrations of Mn in the soil samples after kenaf planting were remarkably reduced to the range of 514.31–679.06 with a mean of 588.06 mg/kg compared with 779.06 mg/kg as an initial concentration. The soil-remediated Mn fraction ranged from 100.00 to 264.75 with a mean of 191.00 mg/kg representing remediation percentage ranging from 12.84 to 33.98 with a mean of 24.52%.

Sulfur soil addition accompanied with humic acid as foliar spraying was the treatment that resulted in the highest significant accumulation of Mn in the shoot (37.56 mg/kg), root (133.61 mg/kg), and whole plant samples (171.17 mg/kg). These results occurred in the soil which is characterized by the lowest significant Mn content (514.31 mg/kg). This treatment achieved the highest Mn significant remediation percentage, i.e., 33.98% (soil-remediated fraction was 264.75 mg/kg). Also, this treatment showed the highest significant BCF calculated values for the shoot, root, and whole plant samples (0.073, 0.260, and 0.333, respectively).

The kenaf roots' remediation efficiencies of Mn were significantly higher than those of shoots, where the calculated BCF values of shoot samples ranged from 0.050 to 0.073 with a mean of 0.057 in comparison with a range of 0.097–0.260

Table 7 Total content of Mn in soil and plant parts in milligrams per kilogram (kenaf first planting season)

Treatment		First pla	First planting season (kenaf)										
Soil additive	Foliar spraying	Shoot	Root	Whole plant	Initial soil	Soil after first planting	Remediated fraction	Remediation (%)	BCF shoot	BCF root	BCF whole plant		
Sulfur	Humic acid	37.56 ^a	133.61 ^a	171.17 ^a	779.06	514.31 ¹	264.75 ^a	33.98 ^a	0.073 ^a	0.260 ^a	0.333 ^a		
Sulfur	Gibberellin	31.36 ^j	126.49 ^b	157.85 ^c		586.71 ^f	192.35 ⁹	24.69 ^f	0.053 ^f	0.216 ^c	0.269 ^d		
Sulfur	Control	32.71 ^g	121.39 ^e	154.10 ^e		592.16 ^e	186.90 ^h	23.99 ^g	0.055 ^e	0.205 ^d	0.260 ^e		
Compost	Humic acid	34.56 ^d	78.99 ⁹	113.55 ^g		540.46 ⁱ	238.60 ^d	30.63 ^d	0.064 ^b	0.146 ^e	0.210 ^f		
Compost	Gibberellin	31.70 ⁱ	76.92 ^h	108.62 ^h		546.06 ^h	233.00 ^e	29.91 ^e	0.058 ^d	0.141 ^f	0.199 ⁹		
Compost	Control	32.95 ^f	71.65 ⁱ	104.60 ^j		654.86 ^b	124.20 ^k	15.94 ^j	0.050 ^h	0.109 ^g	0.160 ^h		
Control	Humic acid	34.87 ^c	69.69 ^j	104.56 ⁱ		651.21 ^c	127.85 ^j	16.41 ⁱ	0.054 ^e	0.107 ^h	0.161 ^h		
Control	Gibberellin	35.33 ^b	65.63 ^l	100.96 ^l		679.06 ^a	100.00 ^l	12.84 ^k	0.052 ^g	0.097 ⁹	0.149 ⁱ		
Control	Control	34.48 ^e	68.56 ^k	103.04 ^k		646.16 ^d	132.90 ⁱ	17.06 ^h	0.053 ^f	0.106 ^h	0.159 ^h		
Vermiculite	Humic acid	31.99 ^h	123.19 ^d	155.18 ^d		536.81 ^j	242.25 ^c	31.10 ^c	0.060 ^c	0.229 ^b	0.289 ^b		
Vermiculite	Gibberellin	30.27 ^k	125.39 ^c	155.66 ^b		584.51 ^g	194.55 ^f	24.97 ^f	0.052 ^g	0.215 ^c	0.266 ^d		
Vermiculite	Control	30.07 ^l	112.29 ^f	142.36 ^f		524.41 ^k	254.65 ^b	32.69 ^b	0.057 ^d	0.214 ^c	0.271 ^c		
Mean		33.15	97.82	130.97		588.06	191.00	24.52	0.057	0.170	0.227		
Minimum		30.07	65.63	100.96		514.31	100.00	12.84	0.050	0.097	0.149		
Maximum		37.56	133.61	171.17		679.06	264.75	33.98	0.073	0.260	0.333		

with a mean of 0.170 in root samples. The whole plant BCF values ranged from 0.149 to 0.333 with a mean of 0.227.

The results of flax (second planting season) are represented in Table 8. Mn was accumulated in shoots with concentrations ranging from 13.63 to 39.11 with a mean of 26.82 mg/kg, while the roots recorded concentrations ranging from 26.99 to 114.81 with a mean of 51.51 mg/kg; subsequently, the whole plant concentrations of Mn ranged from 40.62 to 153.92 with a mean of 78.32 mg/kg.

Concentrations of Mn in the soil samples after flax planting were remarkably reduced to the range of 267.75–435.31 with a mean of 352.14 mg/kg. The soil-remediated Mn fractions ranged from 149.21 to 318.96 with a mean of 236.77 mg/kg representing remediation percentage ranging from 25.53 to 54.36 with a mean of 39.96%. The double season phytoremediation using kenaf followed by flax achieved remediation percentage for Mn which ranged from 44.12 to 65.63 with a mean of 54.80%.

In contrast to kenaf, sulfur soil addition accompanied with gibberellin as foliar spraying in case of flax was the treatment that resulted in the highest significant content of Mn in the shoot (39.11 mg/kg), root (114.81 mg/kg), and whole plant (153.92 mg/kg). These values were related to the soil of the lowest significant Mn content (267.75 mg/kg). This treatment achieved the highest significant Mn remediation percentage, i.e., 54.36% (soil-remediated fraction was 318.96 mg/kg), as well as the highest significant BCF calculated values for the shoot, root, and whole plant samples (0.146, 0.429, and 0.575,

respectively). Sulfur soil addition accompanied with gibberellin as foliar spraying showed the highest significant double season phytoremediation percentage of Mn which was 65.63%.

In agreement with kenaf, flax roots' remediation capacities of Mn were higher than the corresponding values of shoots, where the calculated BCF values of shoot samples ranged from 0.041to 0.146 with a mean of 0.078 in comparison with a range of 0.074–0.429 with a mean of 0.152 in root samples. The whole plant BCF values ranged from 0.121 to 0.575 with a mean of 0.230. In contrast to Co, Cr, and Cd results, flax BCF values were markedly higher than in case of kenaf. This means that flax is more efficient than kenaf in Mn phytoremediation.

Overview of metals under study

The studied metal concentrations in initial soil sample followed the descending order of Mn > Cr > Co > Cd, where their concentrations were 779.06, 68.98, 21.35, and 12.82 mg/kg, respectively (Tables 1, 2, 3, 4, 5, 6, 7 and 8).

Kenaf phytoremediation capacities of the studied metal ions followed the descending order of Cr > Co > Mn > Cd where their values reached 50.71, 38.27, 33.98, and 14.43%, respectively. Also, BCF values followed the same descending order where they reached 1.065, 0.534, 0.333, and 0.112, respectively. Flax phytoremediation capacities followed the descending order of Mn > Cr > Co > Cd, where their values reached 54.36, 36.95, 28.72, and 11.37%, respectively. Meanwhile BCF values followed the order of $Cr \ge Mn > Co > Cd$ where their values reached

Table 8 Total content of Mn in soil and plant parts in milligrams per kilogram (flax second planting season)

Treatment		Second	d planting	season ((flax)						
Soil additive	Foliar spraying	Shoot	Root	Whole plant	Soil after second planting	Remediated fraction	Remediation (%)	Double season phytoremediation (%)	BCF shoot	BCF root	BCF whole plant
Sulfur	Humic acid	16.31 ^k	58.51 ^c	74.81 ^f	341.61 ⁹	182.81 ⁱ	33.58 ^k	56.15 ^e	0.048 ^h	0.171 ^c	0.219 ^d
Sulfur	Gibberellin	39.11 ^a	114.81 ^a	153.92 ^a	267.75 ^l	318.96 ^a	54.36 ^a	65.63 ^a	0.146 ^a	0.429 ^a	0.575 ^a
Sulfur	Control	33.48 ^b	45.88 ^f	79.35 ^e	367.81 ^e	224.36 ^f	37.89 ⁱ	52.79 ⁹	0.091 ^c	0.125 ^e	0.216 ^e
Compost	Humic acid	30.88 ^d	49.17 ^e	80.04 ^d	315.51 ^j	224.96 ^f	41.62 ^e	59.50 ^c	0.098 ^b	0.156 ^d	0.254 ^c
Compost	Gibberellin	13.63 ^l	26.99 ^l	40.62 ^l	336.11 ^h	209.96 ⁹	38.45 ^h	56.86 ^e	0.041 ⁱ	0.080 ⁱ	0.121 ¹
Compost	Control	21.59 ^j	30.56 ^k	52.14 ^k	410.91 ^b	243.96 ^e	37.25 ^j	47.26 ^j	0.053 ^g	0.074 ^j	0.127 ^k
Control	Humic acid	26.71 ^g	98.01 ^b	124.71 ^b	390.91 ^c	260.31 ^d	39.97 ^f	49.82 ^h	0.068 ^f	0.251 ^b	0.319 ^b
Control	Gibberellin	32.82 ^c	30.98 ^j	63.79 ⁹	370.11 ^d	308.95 ^b	45.50 ^b	52.49 ^g	0.089 ^d	0.084 ⁱ	0.172 ^j
Control	Control	23.38 ⁱ	40.15 ^g	63.52 ^h	354.41 ^f	291.76 ^c	45.15 ^c	54.51 ^f	0.066 ^f	0.113 ^g	0.179 ⁱ
Vermiculite	Humic acid	25.28 ^h	32.49 ^h	57.77 ^j	312.91 ^k	223.91 ^f	41.71 ^d	59.83 ^b	0.081 ^e	0.104 ^h	0.185 ^h
Vermiculite	Gibberellin	29.49 ^e	58.33 ^d	87.81 ^c	435.31 ^a	149.21 ^j	25.53 ^l	44.12 ⁱ	0.068 ^f	0.134 ^f	0.202 ^f
Vermiculite	Control	29.16 ^f	32.22 ⁱ	61.37 ⁱ	322.3 ⁱ	202.11 ^h	38.54 ⁹	58.63 ^d	0.090 ^c	0.100 ^h	0.190 ⁹
Mean		26.82	51.51	78.32	352.14	236.77	39.96	54.80	0.078	0.152	0.230
Minimum		13.63	26.99	40.62	267.75	149.21	25.53	44.12	0.041	0.074	0.121
Maximum		39.11	114.81	153.92	435.31	318.96	54.36	65.63	0.146	0.429	0.575

0.577, 0.575, 0.286, and 0.062, respectively. Hence, it can be noticed that kenaf is a more efficient accumulator than flax in case of Cr, Co, and Cd, while flax is more efficient in case of Mn only.

Regarding double season phytoremediation percentage, phytoremediation capacities followed the descending order of $Cr \ge Mn > Co > Cd$, where their values reached 66.87, 65.63, 54.66, and 23.40%, respectively. Sulfur accompanied with humic acid showed the highest significant accumulation percentage for Co and Cr that reached 54.66 and 66.87%, respectively. On the other hand, sulfur with gibberellin achieved the highest significant percentages for Cd and Mn that reached 23.40 and 65.63, respectively (Table 1, 2, 3, 4, 5, 6, 7 and 8).

The main effect of soil additives showed no specific significant trend at the level of the two growing seasons and the four studied metals. However, in most cases, the additives' efficiencies followed the order of sulfur > control > compost > vermiculite; this is according to the concentration of metals in soils after planting of each phytoremediator plant.

Relationship between uptake of heavy metals by hyperaccumulated plants and pollutant distribution in remediated soils as affected by application treatment Sequential fractionation techniques are being used increasingly to provide more useful assessments of soil heavy metal contamination than is possible with single extractions or total metal concentrations alone. In addition, heavy metal fractionation is a fairly widely used technique for understanding the mechanisms of heavy metal distribution in soils and to help assess bioavailability of trace metals in soils. Such assessment assumed that the metal bioavailability decreases with each successive extraction step in the procedure. Therefore, metals in water-soluble and exchangeable fractions would be readily bioavailable forms to growing plants, whereas the metals in residual form would be more hardily available form.

In addition, it is well known that residence time directly relates to the bioavailability of heavy metals in soils. Generally, bioavailability of heavy metals decreases with increasing residence time (McLaughlin 2001). Such time effect is attributed to the reactions between metal ions and soils, which mainly include complexation, adsorption, and precipitation of metal ions in the soil particle surface or diffusion into the mesopores and micropores of soil. However, the source of pollution of soil system becomes a more important factor in bioavailability of heavy metals in soil system since the distributions of such pollutants will vary according to its source. This part investigates the effect of remediation effect of the additives on distribution of the investigated heavy metals or in other meaning the potentially toxic metals (PTEs). The results of this work may be considered as a guide for selecting the suitable remediation materials that should be applied according to conditions of pollution in soil or soils under study.

Data in Table 9 represent Co⁺² distribution in the studied soil as affected by the remediation added materials. The readily available form represented by the sum of water-soluble and exchangeable forms ranged between 1.80 and 1.02 mg kg⁻¹ in both the first and second cultivation seasons. This means the readily available form represents the lowest fraction among the studied ones. It is worthy to mention here that the highest values of the readily available fraction were detected by the sulfur-treated soil. Similar trends were observed for the other studied metal ions with numerical values which ranged between 4.65 and 2.44, 1.54 and 0.75, and 71.06 and 32.23 mg kg⁻¹ for Cr, Cd, and Mn, respectively. The application of sulfur aims at enhancing phytoremediation in contaminated soils by decreasing soil pH which increases the bioavailability of PTEs in polluted soils; more details about the reasons of this result will be documented in the "Discussion" section. Also, data showed that in almost all cases regardless of the type of pollutant, the highest values of the water-soluble (WS) form were achieved due to added sulfur while the lowest ones were achieved owing to the application of vermiculite. In addition, as a general result, regardless of the PTM form, its values after the second cultivation season were lower than the corresponding ones after the first one.

The control treatment (uncultivated soil) was of the highest contents of all the fractions of the studied metal ions. Concentrations of the moderately available forms (MAF) were high owing to sulfur application and ranged between 3.78 and 3.35, 12.07 and 8.27, 2.95 and 2.74, and 196.46 and 113.27 mg kg⁻¹ for Co, Cr, Cd, and Mn, respectively.

Application of vermiculite to the contaminated soils decreased MAF to the lowest values compared to the contaminant reductions occurred owing to the other additives. For example, after the first cultivation season, the MAF value for Mn was $104.88~\rm mg~kg^{-1}$ which was decreased to $64.46~\rm mg~kg^{-1}$ in the second cultivation season. The corresponding MAF value achieved due to application of the compost was $159.88~\rm in$ the first season which was decreased to $91.95~\rm mg~kg^{-1}$ in the second one. The behavior of the other studied metal ions did not vary widely from that of Mn.

Application of clay and modified clay minerals in contaminated soils is usually used to minimize the hazards of inorganic pollutants on cultivated plants or even human health (Saber et al. 2012). Usually, such remediation materials react with heavy metals to form complexes

Table 9 Effect of applied treatments on PTE distribution (mg kg⁻¹) in the first and second planting seasons

Treatment	First plantin	ng season			Second pla	nting season		
	Total	RAF	MAF	HAF	Total	RAF	MAF	HAF
Со								
Control	13.55	1.36	3.39	8.80	11.25	1.13	2.81	7.31
Compost	14.92	1.49	3.29	9.70	11.18	1.12	2.46	6.71
Sulfur	13.95	1.80	3.78	8.37	10.25	1.33	3.35	5.57
Vermiculite	16.81	1.28	3.20	12.33	13.16	1.02	2.43	9.71
Cr								
Control	44.10	4.21	11.22	28.67	29.64	2.46	7.91	19.27
Compost	46.49	4.55	10.76	31.18	26.82	2.68	8.05	16.09
Sulfur	37.75	4.65	12.07	20.03	22.48	3.00	8.27	11.21
Vermiculite	46.46	3.73	8.65	34.08	37.44	2.44	7.49	27.53
Cd								
Control	11.22	1.12	2.81	7.29	10.20	1.02	2.55	6.63
Compost	10.53	1.26	2.85	6.42	8.92	0.89	2.68	5.35
Sulfur	11.41	1.54	2.95	6.92	9.83	1.83	2.74	5.26
Vermiculite	11.73	1.17	2.73	7.83	10.47	0.75	2.09	7.63
Mn								
Control	646.16	64.62	161.54	420.00	354.41	35.44	88.60	230.37
Compost	654.86	65.48	159.88	392.92	410.91	41.09	91.95	246.55
Sulfur	592.16	71.06	196.46	324.64	367.80	47.14	113.27	207.39
Vermiculite	524.42	52.44	104.88	367.10	322.30	32.23	64.46	225.61

RAF readily available form, MAF moderately available forms, HAF hardly available form

and compounds in hardly available form (HAF). In this work, application of vermiculite increased the HAF of all the PTM. Results in the same table show that the hardly available form represented by the sum of both organic and residual fraction recorded the highest values for the different studied PTM s compared to the other studied forms. Data in Table 9 show that numerically, the HAF ranged between 5.26 and 420 mg kg⁻¹ for all pollutants, and the highest values were achieved due to application of vermiculite, while the lowest ones were achieved due to application of sulfur in both the first and second cultivation seasons.

After the second cultivation season, concentrations of the studied PTEs reached the lowest values and safe levels for most of the PTMs. The PTM concentrations in the soils decreased depending on mechanisms which differed according to type of the soil additives. Complexation seems to be the responsible mechanism upon application of vermiculite (reference), *chelation* upon application of compost, enhancing uptake by reducing soil pH upon application of sulfur and phytoremediation (extraction of the metal ions) as represented by metal ion uptake by flax and kenaf plants. Also, it could be deduced that cultivation of flax as a hyperaccumulator plant seems preferable to be used in phytoremediation of most studied PTMs.

Growth criteria of kenaf and flax in their growing seasons

It is clear from Table 10 that the highest heights values for both kenaf and flax plants were achieved due to sulfur application as a soil additive together with gibberellin application as foliar spraying. The average height values were 4.11 and 1.31 m whereas the average weight values per plant were 3.35 and 0.12 kg for kenaf and flax plants, respectively. Application of sulfur with humic acid resulted in the second highest values for both height and weight of kenaf and flax plants where the average height values reached 4.06 and 1.28 m and the average weight per plant values reached 2.95 and 0.09 kg for kenaf and flax, respectively.

Criteria of the irrigation wastewater

Irrigation water is a main source of pollution that affects soil, plants, animals, and subsequently humans. As shown in Table 11, concentrations of Co, Cr, Cd, and Mn are all higher than the permissible limits. The studied metal concentrations in water sample followed the order of Mn > Cr > Co > Cd, where their concentrations were 1.1165, 0.487, 0.066, and 0.025 mg/l, respectively.

Discussion

The selected planting site seems to be more contaminated with Co than some other sites of Sahl El Husseinia as shown by Badawy et al. (2013) who indicated that 11 soil sampled from Sahl El Husseiniya that had been irrigated with Bahr El-Baqar drain water recorded Co concentrations which ranged from 4.00 to 16.33 with a mean of 6.97 mg/kg in comparison with 21.35 mg/kg in the current study (Table 1). Also, Mn concentrations in the current study were found to be higher than the corresponding ones reported in the same study, wherein Mn concentrations ranged from 68.58 to 817.60 mg/kg

Table 10 Growth criteria of kenaf and flax planting seasons

Treatment		First planting season	(kenaf)	Second planting season (flax)			
Soil addition	Foliar spraying	Average height (m)	Average weight per plant (kg)	Average height (m)	Average weight per plant (kg)		
Sulfur	Humic acid	4.059 ^b	2.95 ^b	1.280 ^b	0.09 ^b		
	Gibberellin	4.110 ^a	3.35 ^a	1.317 ^a	0.12 ^a		
	Control	4.045 ^c	2.15 ^c	1.275 ^c	0.07 ^c		
Compost	Humic acid	4.015 ^d	1.89 ^e	1.250 ^e	0.05 ^e		
	Gibberellin	4.015 ^d	1.93 ^d	1.262 ^d	0.06 ^d		
	Control	3.915 ^e	1.16 ⁹	1.226 ^g	0.05 ^e		
Control	Humic acid	3.642 ^j	0.90 ^j	1.195 ⁱ	0.03 ^g		
	Gibberellin	3.700 ⁱ	0.97 ⁱ	1.200 ^h	0.04 ^f		
	Control	3.163 ^k	0.64 ^k	1.180 ^j	0.02 ^h		
Vermiculite	Humic acid	3.844 ^g	1.03 ^h	1.240 ^f	0.05 ^e		
	Gibberellin	3.990 ^f	1.18 ^f	1.250 ^e	0.05 ^e		
	Control	3.827 ^h	0.97 ⁱ	1.240 ^f	0.04 ^f		
Mean		3.863	1.593	1.280	0.056		
Minimum		3.163	0.636	1.250	0.024		
Maximum		4.110	3.354	1.317	0.120		

Table 11 Concentrations of heavy metals under study in irrigation wastewater of Bahr El-Baqar drain and their permissible limits in milligrams per liter

Element	Concentration	Permissible limit*
Со	0.066	0.05
Cr	0.487	0.1
Cd	0.025	0.01
Mn	1.1165	0.2

*According to Rowe and Abdel-Magid (1995) recommended limits for constituents in reclaimed water for irrigation

with a mean of 250.14 mg/kg in comparison with 779.06 mg/kg in the current study (Table 7). Meanwhile, Cr concentrations were higher than those shown by El-Aassar et al. (2018) who indicated that Cr concentrations in an area located in the vicinity of Bahr El-Baqr drain ranged from 15.28 to 49.78 mg/kg with a mean of 34.19 mg/kg in comparison with 68.98 mg/kg in the current study (Table 3). However, Cd concentrations obtained herein were in agreement with their corresponding ones that ranged from 11.75 to 13.25 mg/kg with a mean of 12.52 mg/kg in comparison with 12.82 mg/kg in the current study (Table 5).

Sulfur was the best significant soil additive for the four studied metals and also for the two phytoremediators. It is well known that sulfur-containing compounds play an important role in plants' defense against stresses (Gangwar et al. 2014). Yet, sulfur often is forgotten in soil fertility management discussions which tend to focus only on N, P, and K. Sulfur is a macronutrient element, essential for life, and is used by plants in amounts similar to those of P, and both are closely associated in the processes of protein and enzyme synthesis (McGrath et al. 2014). So, S will be consumed by plants and will not accumulate in the soil or harm the environment. Cui et al. (2004) concluded that sulfur can increase uptake of some metals by plants through enhancing metal mobility, as soil pH decreased with S application and the solubility of the Zn and Cd was significantly increased in all treatments with S application. Also, Skwierawska et al. (2012) showed that the addition of elemental sulfur led to an increase in the cadmium content at a soil depth of 0-40 cm.

Although there was no specific trend, the soil additive efficiency could be ordered as follows: sulfur > control > compost > vermiculite. Malandrino et al. (2011) revealed that addition of vermiculite to polluted soil reduced the uptake of metal pollutants by *Lactuca sativa* and *Spinacia oleracea* because the influence of vermiculite on metal availability is first of all related to the increase in pH brought about by the addition of this amendment. More adsorption sites become, therefore, available due to the presence of pH-dependent charge. Pinamonti et al. (1997) revealed that addition of some types of

compost to polluted soil increased metal contents in the soil during 6 years of monitoring. The application of such compost caused a redistribution of the heavy metals, especially the more mobile ones, among the various chemical forms present in the soil.

Both humic acid and gibberellin were shown to be effective through foliar treatment, even if their performance is different for metals under study for each plant. Humic acid was the favorable foliar treatment for Co and Cr in case of flax and for Cd and Mn in case of kenaf, whereas gibberellin was the preferable foliar treatment for Co and Cr in case of kenaf and for Cd and Mn in case of flax. On the other hand, gibberellin showed the highest significant growth criteria followed by humic acid.

Gibberellins are extensively involved in all phases of plant growth and development, from seed germination to senescence. They promote seed germination; stimulate stem elongation, leaf expansion, flowering, and pollen and seed development; delay ripening; and inhibit senescence. Moreover, gibberellins are also involved in plant adaptation to abiotic stresses (Falkowska et al. 2011). Therefore, gibberellin showed the highest significant growth criteria. Besides this, exogenous application of plant hormones has also been reported to enhance stress tolerance in plants affected by heavy metals (Gangwar et al. 2014). So, gibberellin is collaborated with sulfur to enhance metal uptake and accumulation.

Humic acids are technically not a fertilizer; they are an effective agent to be used as a complement to organic or synthetic fertilizers (Khaled and Fawy 2011). So, they are collaborated with sulfur to enhance metal uptake and accumulation. Bakry et al. (2014) stated that humic acids may stimulate root and shoot growth and improve plant resistance to environmental stress. Therefore, humic acid showed the second significant growth criteria. Moreover, humic substances facilitate translocation of Fe and P from roots to shoots through prevention of their immobilization. In addition, humic substances act indirectly as suppliers and regulators of plant nutrients similar to synthetic ion exchangers and directly through uptake of humic substances by plant root reference.

All accumulated metal concentrations were higher in roots than in shoots in both the studied plants. This finding is a luminous conclusion, as the shoot system of both plants is the economical part, where kenaf fibers are extracted from the stems and the oil and fabric industries are dependent on seeds and stems of flax. For instance, Arbaoui et al. (2014) recorded that Zn and Cd concentrations in plant tissues were in order of root > leaf > stem regardless of soil metal concentration. Plants that overaccumulate heavy metals in their root tissues, excluding or limiting translocation to aboveground tissues, can be regarded as efficient phytostabilizer to heavy metals in soils (Hosman et al. 2017) where

phytostabilization is defined as the process to reduce the mobility of contaminants in soil through adsorption onto roots, adsorption and accumulation by roots, or precipitation within the root zone (Gupta and Sandalio 2011).

Predominatingly, phytoremediation plants prefer the accumulation of some minerals more than others. Bioconcentration factor (BCF) indicates the efficiency of a plant to accumulate a metal into its tissues from the surrounding environment with respect to metal concentration in the soil (Nizam et al. 2016; Hosman et al. 2017). BCF values showed that kenaf is a more efficient accumulator than flax in case of Cr, Co, and Cd. According to Cartoga et al. (2005), kenaf showed high tolerance to Cr toxicity in terms of the height of the plant and in terms of the biomass productivity. Plants obtained from the contaminated pots accumulated higher Cr in comparison with the values obtained for the control. Also, Arbaoui et al. (2014) showed that concentration of Cd in flax tissues increased significantly with increasing Cd in soil, notably in roots where concentrations of Cd in roots and leaves were two times higher than those in soil. Flax was more efficient in case of Mn only. Flax is naturally rich in minerals including Mn. For instance, Goyal et al. (2014) reported that flax seeds contained 3 mg Mn/100 g. This may interpret its higher accumulation efficiency of Mn.

The results of water sample (Table 11) agree to some extent with those obtained by Badawy et al. (2013) whose results recorded less Co concentrations (0.0008-0.001 mg/l) than both of the permissible limit (0.05 mg/l) and our study result (0.066 mg/l). Meanwhile, Cr concentrations (0.01-0.23 mg/l (were mostly higher than the permissible limit (0.1 mg/l) but are less than those obtained in the current study (0.487 mg/l). Mn-recorded concentrations (0.01-2.88 mg/l) were higher than both of the permissible limit (0.2 mg/l) and our study result (1.116 mg/l). Bahr El-Baqar drain receives industrial, agricultural, and municipal wastewaters (Abdel-Fattah and Helmy 2015), and all are predicted to contain various heavy metals with various concentrations as time passes. Prolonged use of such drain wastewater for irrigation leads to accumulation of heavy metals in soils and subsequent deterioration of soil quality. This hypothesis has been confirmed in this study, where the studied metals' concentrations in water sample followed the same order of Mn > Cr > Co > Cd, the same as soil samples (Tables 1, 2, 3, 4, 5, 6, 7 and 8).

Conclusions and recommendations

Wastewaters of Bahr El-Baqar drain are contaminated with heavy metals; most of them have concentrations higher than the permissible limits and found, generally, in the following descending order: Mn > Cr > Co > Cd. This pollutant sequence was reflected on the wastewater-

irrigated soil content of metals that followed the same sequence. Therefore, there is an urgent need for soil remediation. Both flax and kenaf are effective phytoremediators where they accumulate heavy metals in noticeable contents especially inside their roots more than shoot tissues. Considering the ratio between accumulated metal concentrations in each plant tissues as compared with its concentration in the surrounding soil in each season individually (mean whole plants BCF of all treatments), kenaf seemed more efficient phytoremediator than flax in case of Cr, Co, and Cd, while flax was more efficient in case of Mn only. Double season phytoremediation capacity followed the order of $Cr \ge Mn > Co > Cd$. Sulfur was the best soil additive that increased metal mobility in the soil, thus facilitating the remediation by both plants. Foliar application of both humic acids or gibberellin assisted both plants to remediate metals efficiently, though gibberellin showed higher values of growth criteria than the humic acid did. Depending on the achieved remediation percentage, flax is recommended to be planted with sulfur and humic acid to remediate soils of high Co and Cr concentrations, whereas sulfur and gibberellin are recommended for soils of high Cd and Mn. Kenaf is recommended to be used with sulfur and humic acid to remediate soils of high Cd and Mn concentrations, whereas sulfur with gibberellin are recommended for soils of high Co and Cr.

Abbreviations

BCF: Bioconcentration factor; HAF: Hardly available form; MAF: Moderately available forms; RAF: Readily available form; WS: Water soluble

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Authors' contributions

SS helped in the field work of plant cultivation, sample analyses, and article writing and revision. RB (late) helped in the field work of plant cultivation. YA helped in the sample analyses, statistical analyses, and article writing and revision. SS and YA contributed equally in all the article steps and wrote the paper. All authors read and approved the final manuscript.

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