



Bioremediation of potentially toxic elements of sewage sludge using sunflower (*Helianthus annuus* L.) in greenhouse and field conditions

Masih Bayat · Ali Faramarzi · Jalil Ajalli · Mehrdad Abdi · Hassan Nourafcan

Received: 13 January 2021 / Accepted: 9 June 2021 / Published online: 10 August 2021
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract The bioremediation of sewage sludge, containing potentially toxic elements (heavy metals), by the hyperaccumulator sunflower (*Helianthus annuus* L.), was determined in greenhouse (G) and field (F) conditions in Isfahan, Iran. The soil pots, mixed with dried sewage sludge at 0, 15, 30, 45, and 60 mg/kg, were planted with sunflower seedlings and kept in the greenhouse (G) and in the field (F). Different soil physicochemical and plant biochemical properties including heavy metal uptake of nickel (Ni), chromium (Cr), lead (Pb), and cadmium (Cd) were determined. In contrast with the soil pH, soil salinity, organic matter, nitrogen, and not soil CaCO₃, were significantly enhanced by increasing sewage sludge. Sewage sludge was significant on plant uptake of Ni (2.27–4.25 mg/kg), Cr (3.27–4.75 mg/kg), Cd (13.85–15.27 mg/kg), and total chlorophyll (1.69–1.99 mg/g) in the greenhouse, and plant uptake of Ni (1.75–2.75 mg/kg) and Cd (1.37–2.25 mg/kg), and chlorophyll b (0.06–0.26 mg/g), total chlorophyll (0.57–1.16 mg/g), and carotenoids (1.10–1.61 mg/g) in the field. Although Pb was not significantly affected by sewage sludge, it showed the highest

bioaccumulation factor of 0.96 at 15 mg/kg. Interestingly, the heavy metals were all positively and significantly correlated with each other and with plant carotenoids, similar to the positive and significant correlations between Pb with chlorophyll a and b. Accordingly, the increased levels of carotenoids, acting as antioxidant, may be an indicator of oxidative stress. Sunflower plants can be used as an efficient method for the bioremediation of the soils polluted with sewage sludge including Ni, Cr, and Cd.

Keywords Antioxidants · Cadmium · Carotenoids · Chromium · Correlation · Hyperaccumulators · Lead · Nickel

Introduction

Anthropogenic activities are one of the most important sources of environmental contamination. The accumulation of potentially toxic elements (heavy metals) in the soil by the use of sewage sludge results in the pollution of the environment with health, economic, and environmental consequences (Khelifi et al., 2019). Different methods have been used to bioremediate soils, which are contaminated with heavy metals including the use of hyperaccumulator plants. The other important aspect of bioremediating the environments polluted with heavy metals is the recycling of sewage sludge in the areas with water deficiency such

M. Bayat · A. Faramarzi (✉) · J. Ajalli · M. Abdi
Department of Agronomy and Plant Breeding, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran
e-mail: Aliifaramarzii52@gmail.com

H. Nourafcan
Department of Horticulture, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran

as semi-arid and arid areas (Ignatowicz, 2017; Kacprzak et al., 2017).

Heavy metals (53) are categorized based on their density (> 5 g/cm³). Among the heavy metals, nickel (Ni) is essential for plant growth; however, cadmium (Cd)/ lead (Pb) and chromium (Cr) are not essential for plant growth, and if absorbed by plant, the symptoms of toxicity may appear. The negative effects of heavy metals on plant growth are by affecting plant growth and physiology including the enzymatic activities, which eventually decrease plant growth. For example, the activity of membrane proteins including H⁺-ATPases is altered by heavy metals affecting the functionality and permeability of cell membrane. Heavy metals can also negatively affect plant growth due to oxidative stress resulting in the production of reactive oxygen species (Berni et al., 2019; Miransari, 2011).

Although there has been research on the use of hyperaccumulators to bioremediate polluted environments, more research is essential to find the most efficient methods, which may effectively bioremediate the accumulation of heavy metals resulted by sewage sludge, while environmentally and economically recommendable. The hyperaccumulator plants are able to accumulate high level of heavy metals while their growth is not affected. There are genes in such plants, regulating the accumulation of heavy metals in plant tissues, without affecting plant growth and the appearance of toxicity symptoms (Behera, 2014; Liu et al., 2016).

Sunflower (*Helianthus annuus* L.) is one of the most important industrial crop plants, which may be used as a hyperaccumulator for the bioremediation of polluted environments (Dhiman et al., 2017; Marathe & Ravichandran, 2019). Although there has been research on the use of sunflower as a hyperaccumulator, more research is required to find how sunflower plants may bioremediate the heavy metals of sewage sludge.

With respect to the above-mentioned details, and because there are not much data on the use of sunflower as an hyperaccumulator plant for the bioremediation of soils polluted with sewage sludge containing heavy metals, the present research was conducted. The objective was to determine the phytoextraction potential of sunflower plants for the removal of Ni, Cd, Pb, and Cr from the soil polluted

with different levels of sewage sludge in the greenhouse and field conditions.

Materials and methods

The experimental treatment

The dried sewage sludge used in the present research was obtained from the industrial sewage sludge in Isfahan, Iran, and was mixed with the pot (height and diameter of 25 and 20 cm, respectively) soils under greenhouse and field conditions at control, 15, 30, 45, and 60 mg/kg in 2018. The soil used for the experiment was obtained from the Research Field of Isfahan University and was planted with sunflower plants (Golshid genotype) (Fig. 1).

Planting and harvest

The sunflower seeds were first planted in seedling trays, and the seedlings were then planted in the pots. The pots were irrigated to the moisture level of 70% (of field capacity) to avoid water deficiency in the pots. If there was drainage, the drained water was reused to irrigate the pots. The pots were also kept under field conditions and were treated according to the agronomical practices in the region including weeding, controlling pests, and irrigation until the harvest. At harvest, the sunflower plants were collected and washed with distilled water for 48 h and were dried in oven at 85 °C. The samples were milled and kept in plastic bottles for chemical analyses. Soil samples were also collected from the pots and were sieved using a 2-mm mesh.

Analysis

The chemical used for the analyses was all purchased from Merck (Germany), with the purity of 99.9%. The samples were analysed by qualified technicians using highly precise machines.

Soil physicochemical properties

The physicochemical properties of the soil samples including pH and salinity (EC) (ratio of 1:1), organic matter (Walkey & Black, 1934), and soil total nitrogen



Fig. 1 Different stages of the experiment

by the Kjeldahl method (Boltz & Howell, 1978) were determined (Table 1) (Miransari et al., 2008).

Plant uptake of heavy metals

Pleat leaf samples were collected one week before harvest and analysed for heavy metal uptake of Ni, Cr,

Pb, and Cd using atomic absorption spectrophotometer (PerklinElmer model 3030). Accordingly, the sample extracts were prepared by the wet oxidation method using nitric, perchloric, and sulphuric acid at the ratio of 1, 4, and 40, respectively (Greman et al., 2001).

Table 1 Analysis of variance indicating the effect of sewage sludge on the heavy metal uptake and biochemical properties of sunflower in greenhouse and field conditions

Parameters	Greenhouse	Field
Ni	0.0048*	< .0001**
Cr	0.0062*	0.1092
Pb	0.9536	0.3825
Cd	0.0018*	0.0006**
Cha	0.8649	0.0586
Chb	0.6561	< .0001**
Cht	< .0001**	0.0168*
Car	0.2970	0.0042*

*: Significant at $P \leq 0.05$; **: Significant at $P \leq 0.01$

Ni, nickel; Cr, chromium; Pb, lead; Cd, cadmium; P, phosphorus; K, potassium; Cha, chlorophyll a; Chb, chlorophyll b; Cht, total chlorophyll; Car, carotenoid; NO₃, nitrate

Plant pigment contents

The plant samples were also analysed for pigment contents including chlorophyll a (Cha), b (Chb), total (Cht), and carotenoids (Car) on the basis of milligram per gram fresh weight (Li et al., 2009) using the following formulas in which Abs₆₄₅ and Abs₆₆₃ are the absorbance at the wavelengths of 645 and 663 nm, and V and W are acetone volume (ml) and leaf fresh weight (g), respectively.

$$\text{Cha (mg g}^{-1}\text{)} = \left[\frac{(12.7 * \text{Abs}_{663}) - (2.6 * \text{Abs}_{645})}{V/W * 1000} \right]$$

$$\text{Chb (mg g}^{-1}\text{)} = \left[\frac{(22.9 * \text{Abs}_{645}) - (4.68 * \text{Abs}_{663})}{V/W * 1000} \right]$$

$$\text{Cht (mg g}^{-1}\text{)} = \text{Chl.a} + \text{Chl.b}$$

Bioaccumulation factor

The bioaccumulation factor for the aerial part was calculated using the following formula:

Bioaccumulation factor = (heavy metal concentration in the aerial part)/heavy metal concentration in the soil.

Statistical analysis

Data were subjected to analysis of variance using SAS. Means were compared by least significant difference (LSD) at $P \leq 0.05$. The graphs were plotted using SAS Proc Plot.

Results

Analysis of variance

Analysis of variance indicated the experimental treatments significantly affected the measured parameters (Table 1).

Physicochemical properties of the soil

The physicochemical properties of the greenhouse and field soil, affected by different levels of sewage sludge, are presented in Table 1. According to the results, the pH values of the greenhouse and field soils were in the range of 7.30–7.74 and 7.91–8.08, respectively (Fig. 2). The pH values were not significantly affected by different levels of sewage sludge. However, soil salinity was significantly enhanced by increasing sewage sludge levels, ranging from 1.13 to 3.70 dS/m (greenhouse) and 0.68 to 2.58 dS/m (field) (Table 2).

The use of sewage sludge significantly increased soil organic levels, which was in the range of 0.64–3.77 and 0.68–2.58% in the greenhouse and field conditions, respectively (Table 1). With increasing the level of sewage sludge, N percentage increased in the greenhouse ranging from 0.06 to 0.22% and in the field ranging from 0.03 to 0.14%. The percentage of CaCO₃ was not a function of sewage sludge levels ranging from 37.31 to 44.44% in the greenhouse and 36.63 to 46.03% in the field (Table 2).

Heavy metal uptake

Increased levels of sewage sludge increased Ni concentration ranging from 2.27 to 4.25 mg/kg in the greenhouse and 1.75 to 2.75 mg/kg in the field. Similarly, Cr also followed a similar trend as the least Cr concentration was resulted by the control level (3.27 mg/kg) and the highest Cr concentration (4.75 mg/kg) was resulted by the highest level of

Fig. 2 Different levels of sewage sludge affecting the measured parameters including: **a** Ni, **b** Cr, **c** Cd, and **d** total chlorophyll in the greenhouse

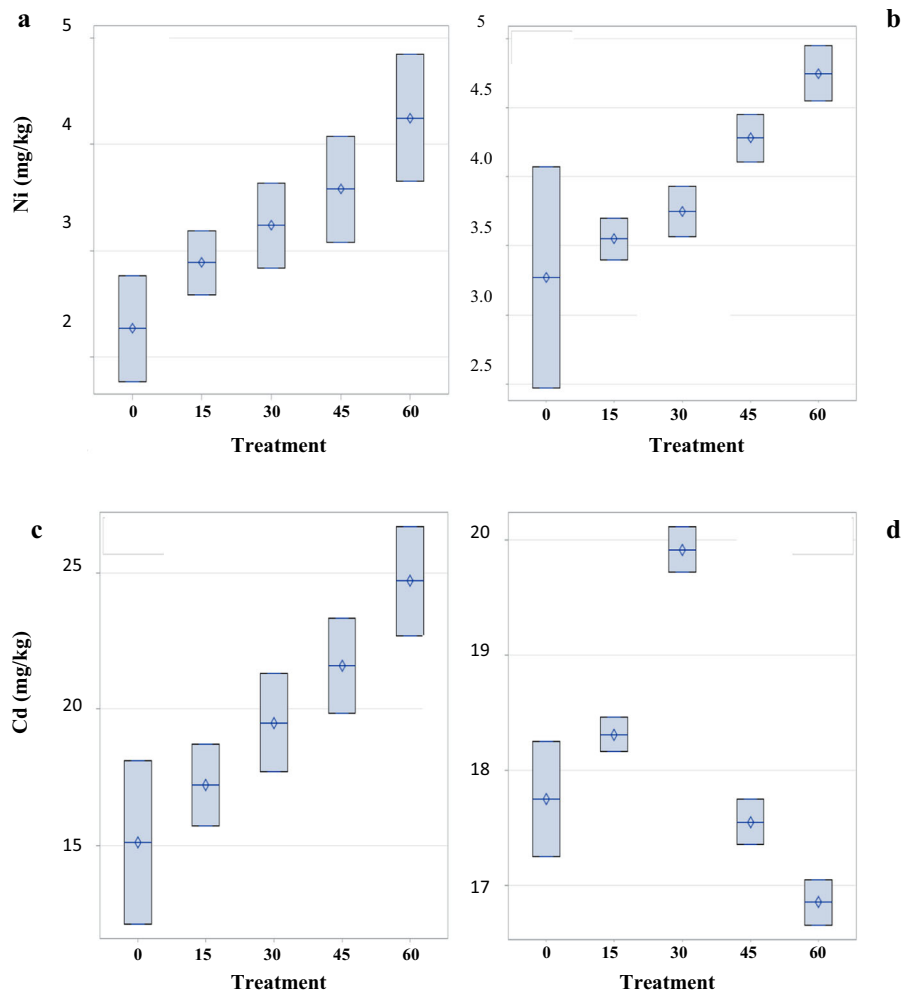


Table 2 Physicochemical properties of the greenhouse and field pot soils treated with different levels of sewage sludge

	Sewage sludge (mg/kg)	pH	Salinity (dS/m)	Organic matter (%)	N (%)	CaCO ₃ (%)
Greenhouse	Control	7.74	1.13 ^d	0.64 ^e	0.06 ^e	37.31 ^b
	15	7.52	2.62 ^c	1.25 ^d	0.09 ^d	41.41 ^a
	30	7.50	2.64 ^c	2.53 ^c	0.16 ^c	44.31 ^a
	45	7.40	3.06 ^b	3.04 ^b	0.18 ^b	43.06 ^a
	60	7.30	3.70 ^a	3.77 ^a	0.22 ^a	42.59 ^a
Field	Control	8.08	0.76 ^c	0.68 ^d	0.03 ^d	38.33 ^b
	15	8.06	1.14 ^b	1.45 ^c	0.07 ^c	44.43 ^a
	30	8.04	1.25 ^b	1.98 ^b	0.10 ^b	46.03 ^a
	45	7.97	1.29 ^b	2.17 ^b	0.11 ^b	37.33 ^b
	60	7.91	1.66 ^a	2.58 ^a	0.14 ^a	36.63 ^b

Mean values followed by the same letters are not significantly different at $P \leq 0.05$ using LSD

sewage sludge (60 mg/kg) in the greenhouse and ranging from 2.75 to 3.80 mg/kg in the field. However, in the case of Pb, the effects of sewage sludge were not significant on plant Pb concentration ranging from 13.85 (30 mg/kg sewage sludge) to 15.27 mg/kg (60 mg/kg sewage sludge) in the greenhouse and ranging from 8.45 (control treatment) to 11.23 mg/kg (30 mg/kg) in the field. Similar to Ni and Cr, the increased levels of sewage sludge enhanced plant uptake of Cd ranging from 1.51 (control) to 2.47 mg/kg (60 mg/kg sewage sludge) in the greenhouse and ranging from 1.37 to 2.25 mg/kg in the field (Table 3, Fig. 1).

Plant pigment contents

There was not a clear trend of sewage sludge on plant pigment contents including Cha, Chb, Cht, and Car (Fig. 3). Plant Cha content was in the range of 1.24 mg/g (control treatment) and 1.43 mg/g (30 mg/kg) in the greenhouse and in the range of 0.44 mg/g (control) to 0.89 mg/g (45 mg/kg) in the field. Chb was in the range of 0.39 mg/g (60 mg/kg) and 0.57 mg/g (30 mg/kg) in the greenhouse and in the range of 0.06 (60 mg/kg) and 0.26 (45 mg/kg) in the field. Cht ranged from 1.69 mg/g (60 mg/g) to 1.99 mg/g (30 mg/kg) in the greenhouse and 0.57 mg/g (60 mg/kg) to 1.16 mg/g (45 mg/kg) in the field. Plant carotenoid content was in the range of 2.26 mg/g (control treatment) to 2.67 mg/g (45 mg/kg) in the greenhouse and in the range of 1.10 mg/g (60 mg/kg) to 1.61 mg/g (control treatment) in the field (Table 3).

Bioaccumulation factor

The BCF values for the aerial parts decreased with increasing the heavy metal concentration, in the greenhouse and in the field. The corresponding values for Ni in greenhouse ranged from 0.07 to 0.19, for Cr from 0.08 to 0.24, for Pb from 0.25 to 0.96, and for Cd from 0.04 to 0.011. In the field, the values were in the range of 0.05–0.12 for Ni, 0.06–0.23 for Cr, 0.18–0.70 for Pb, and 0.0375–0.10 for Cd (Table 4).

Coefficients of correlation

The coefficients of correlation are presented in Table 5. Accordingly, the heavy metals were all positively and significantly correlated with each other and with Car.

There were also positive and significant correlations between Pb with Cha and Chb. The correlations between Cha with Chb and Car were positive and significant.

Discussion

The present research is about the use of sunflower plants for the bioremediation of the soils polluted with sewage sludge containing Ni, Cr, Pb, and Cd. The results indicated the positive effects of sunflower on the bioremediation of such polluted soils. There were significant differences between different rates of sewage sludge on the removal of Ni, Cr, and Cd by plant, and for Pb, there was a high uptake at 15 mg/kg from the polluted soil. Saleem et al. (2018) tested the use of plant growth promoting rhizobacteria (PGPR) on the removal of Pb from a polluted soil by sunflower and found the PGPR are able to enhance the phytoextracting ability of sunflower for the removal of Pb. Interestingly, according to the correlation coefficients, all the measured heavy metals were significantly and positively correlated with Car, which indicates under the oxidative stress of heavy metals, the concentration of plant Car, acting as an antioxidant, increases and can be used as an oxidative stress indicator.

Sunflower is a suitable crop plant for the bioremediation of soils polluted with sewage sludge containing heavy metals, which is due to the following reasons: (1) production of high biomass, (2) increased activity of antioxidants, (3) localization of heavy metals to the non-active parts of the plant, and (4) production of osmolytes (Zamani et al., 2020; Zehra et al., 2020). The results of the present research are in line with the above-mentioned details including the high biomass of sunflower (Zhao et al., 2019) and the increased production of antioxidants, specifically Car, which can alleviate the oxidative stress of heavy metals on the growth and physiology of sunflower.

Soil physicochemical properties

The results indicated soil pH was not a function of sewage sludge levels, which is similar to the results by Paneque et al. (2016) who found the use of sewage sludge at the rate of 1.5–15 t/ha did not affect soil pH. It is because more time is required to alter soil pH as it is a function of soil buffer capacity determined by

Table 3 Plant heavy metal (Ni, Cr, Pb, Cd) uptake and pigment (chlorophyll a, b, total, and carotenoid) contents affected by different levels of sewage sludge

Treatment	Ni (mg/kg)	Cr	Pb	Cd Greenhouse	Cha	Chb (mg/g)	Cht	Car
0	2.27 ± 0.27	3.27 ± 0.23	14.25 ± 1.38	1.51 ± 0.12	1.24 ± 0.13	0.54 ± 0.10	1.78 ± 0.02	2.26 ± 0.14
15	2.89 ± 0.27	3.55 ± 0.23	14.46 ± 1.38	1.72 ± 0.12	1.38 ± 0.13	0.45 ± 0.10	1.83 ± 0.02	2.38 ± 0.14
30	3.24 ± 0.27	3.75 ± 0.23	13.85 ± 1.38	1.95 ± 0.12	1.43 ± 0.13	0.57 ± 0.10	1.99 ± 0.02	2.54 ± 0.14
45	3.58 ± 0.27	4.28 ± 0.23	14.89 ± 1.38	2.16 ± 0.12	1.33 ± 0.13	0.43 ± 0.10	1.76 ± 0.02	2.67 ± 0.14
60	4.25 ± 0.27	4.75 ± 0.23	15.27 ± 1.38	2.47 ± 0.12	1.30 ± 0.13	0.39 ± 0.10	1.69 ± 0.02	2.57 ± 0.14
	Field							
0	1.75 ± 0.1c	2.75 ± 0.24	8.45 ± 1.0	1.37 ± 0.1d	0.44 ± 0.10	0.15 ± 0.01b	0.60 ± 0.11b	1.61 ± 0.10a
15	1.89 ± 0.1bc	3.47 ± 0.24	10.2 ± 1.0	1.57 ± 0.1 cd	0.59 ± 0.10	0.10 ± 0.01c	0.68 ± 0.11b	1.51 ± 0.10a
30	2.18 ± 0.1b	3.51 ± 0.24	11.23 ± 1.0	1.78 ± 0.1bc	0.61 ± 0.10	0.15 ± 0.01b	0.75 ± 0.11b	1.19 ± 0.10b
45	2.56 ± 0.1a	3.53 ± 0.24	10.08 ± 1.0	1.97 ± 0.1ab	0.89 ± 0.10	0.26 ± 0.01a	1.16 ± 0.11a	1.70 ± 0.10a
60	2.75 ± 0.1a	3.80 ± 0.24	10.53 ± 1.0	2.25 ± 0.1a	0.51 ± 0.10	0.06 ± 0.01d	0.57 ± 0.11b	1.10 ± 0.10b

Mean values followed by the same letters are not significantly different at $P \leq 0.05$ using LSD
The values have been presented with the standard error

soil CEC, moistures, organic matter, etc. However, interestingly soil salinity was increased by enhancing sewage sludge levels, which is due to the presence of different minerals such as Na^+ , Cl^- , Ca^{2+} , and Mg^{2+} affecting the salinity of soil solution and hence soil salinity (Elloumi et al., 2016; Mohamed et al., 2018).

Although due to the presence of heavy metals in sewage sludge, its usability may not be high for the improvement in soil, its organic matter can profoundly affect different soil properties. For example, increased levels of nutrients and CEC, and the improved structure of the soil by the organic matter of sewage sludge can be considered as the positive effects of recycling sewage sludge. The results also indicated due to the increased percentage of soil N by sewage sludge, it can be used as an organic fertilizer to improve the fertility of soil (Elloumi et al., 2016; Koutroubas et al., 2020; Mohamed et al., 2018). Yue et al. (2017) found that the production of biochar from municipal sewage sludge increased organic and black carbon, soil total N, and available P and K resulting in the increased growth of turf grass. Although the use of biochar significantly increased soil heavy metals, most of such metals were not of high bioavailability due to their presence in the residual fraction of the soil.

Heavy metal uptake

With increasing the level of sewage sludge, the plant uptake of Ni, Cr, and Cd, and not Pb, significantly increased in the greenhouse and in the field indicating sunflower is a suitable hyper accumulator for the removal of Ni, Cr, and Cd from the polluted soil. The results of the present research are similar to the results of Elloumi et al. (2016), who found the use of sewage sludge increased sunflower uptake of Ni and Cr.

Plant pigment contents

Plant pigment contents, which control different plant activities including the process of photosynthesis (Cha, Chb and Cht) and plant response under stress (Car), were not directly correlated with the levels of sewage sludge. However, the stress of heavy metals degrades plant pigment contents affecting plant physiological activities including the process of photosynthesis. Such effects are functions of sunflower genotype, growth stage, climate, and environmental conditions. Among such pigments, just the increased

Fig. 3 Different levels of sewage sludge affecting the measured parameters including: **a** Ni, **b** Cd, **c** Chb, **d** Cht, and **e** Car in the field

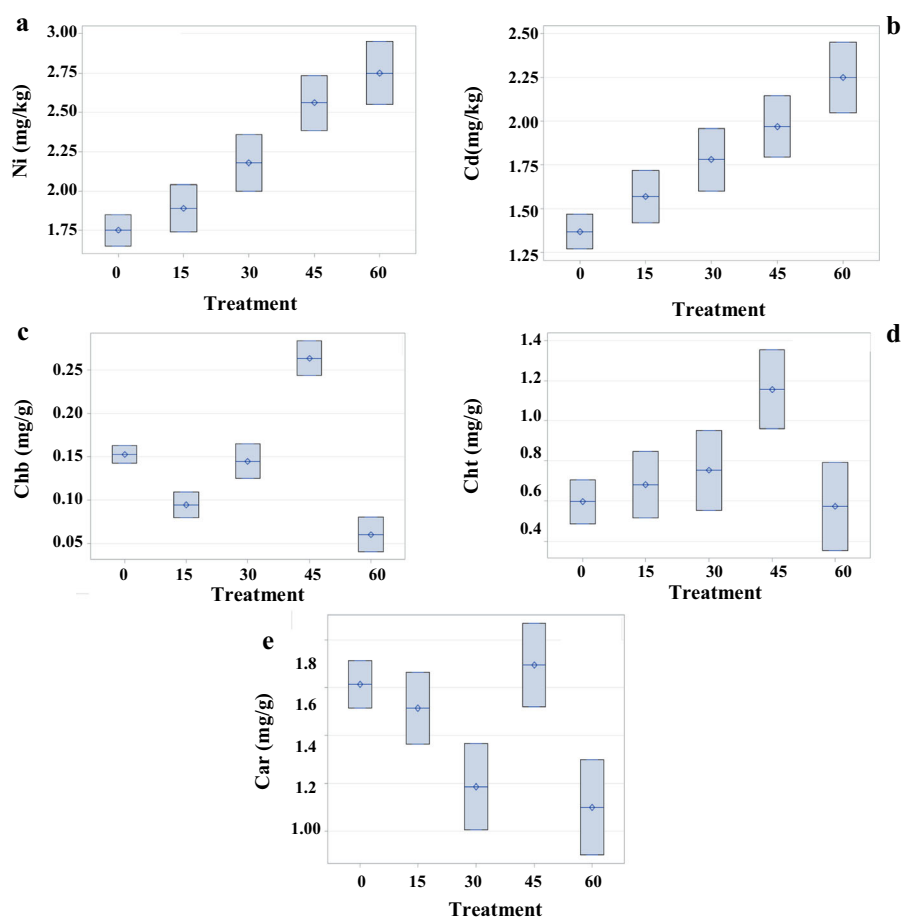


Table 4 Bioaccumulation (BCF) factors for the aerial parts (\pm corresponding standard deviation values) in the greenhouse and field

Heavy metal concentration	Ni	Cr	Pb	Cd
mg/kg	Greenhouse			
15	0.19 \pm 0.02	0.24 \pm 0.01	0.96 \pm 0.01	0.11 \pm 0.01
30	0.111 \pm 0.01	0.13 \pm 0.006	0.46 \pm 0.06	0.07 \pm 0.006
45	0.08 \pm 0.01	0.10 \pm 0.004	0.33 \pm 0.03	0.05 \pm 0.004
60	0.07 \pm 0.01	0.08 \pm 0.003	0.25 \pm 0.03	0.04 \pm 0.003
	Field			
15	0.12 \pm 0.01	0.23 \pm 0.02	0.70 \pm 0.10	0.10 \pm 0.01
30	0.07 \pm 0.006	0.12 \pm 0.01	0.37 \pm 0.06	0.06 \pm 0.009
45	0.06 \pm 0.004	0.08 \pm 0.01	0.22 \pm 0.04	0.04 \pm 0.004
60	0.05 \pm 0.003	0.06 \pm 0.01	0.18 \pm 0.3	0.0375 \pm 0.003

The values are the means of three replicates

levels of Car can alleviate the stress by scavenging reactive oxygen species under the oxidative stress (Ishtiyag et al., 2018).

Bioaccumulation factor

One of the interesting results of the present research is the high BCF value of sunflower for Pb, although there were not significant differences between different rates of sewage sludge on plant Pb uptake. In the

Table 5 Coefficient of correlations among the plant measured parameters

	Ni	Cr	Pb	Cd	Cha	Chb	Cht	Car
Ni	1.00000	0.92839**	0.60423*	0.98714**	0.50252	0.15875	−0.19373	0.80474**
Cr	0.92839**	1.00000	0.64788**	0.96579	0.44952	−0.00210	−0.31715	0.80750**
Pb	0.60423*	0.64788**	1.00000	0.59204*	0.87759**	0.60428*	−0.00491	0.84012**
Cd	0.98714**	0.96579**	0.59204**	1.00000	0.45742	0.09132	−0.23634	0.80835**
Cha	0.50252	0.44952	0.87759**	0.45742	1.00000	0.75887**	0.45307	0.82533**
Chb	0.15875	−0.00210	0.60428*	0.09132	0.75887**	1.00000	0.50552	0.46982
Cht	−0.19373	−0.31715	−0.00491	−0.23634	0.45307	0.50552	1.00000	0.13281
Car	0.80474**	0.80750**	0.84012**	0.80835**	0.82533**	0.46982	0.13281	1.00000

Ni, nickel; Cr, chromium; Pb, lead; Cd, cadmium; P, phosphorus; K, potassium; Cha, chlorophyll a; Chb, chlorophyll b; Cht, total chlorophyll; Car, carotenoid; NO₃[−], nitrate. ***Significant at P = 0.05 and 0.01, respectively

greenhouse, the plant was able to absorb 96% of the soil Pb at 15 mg/kg, and in the field, it was equal to 70%. Such results indicate sunflower is a hyperaccumulator for Pb and can absorb high amounts of Pb in polluted environments. Raza altaf et al. (2020) also indicated sunflower is able to accumulate high amounts of Pb and its accumulating potential increases in the presence of plant growth promoting rhizobacteria. In another interesting research, Marathe (2020) indicated sunflower is able to absorb about 70% of Pb from leachate. Mousavi et al. (2018) also found similar results.

Coefficients of correlations

The heavy metals in the sewage sludge were all positively and significantly correlated indicating the concentration of sewage sludge determines the concentration of Ni, Cr, Pb, and Cd. Interestingly, all the measured heavy metals were positively and significantly correlated with Car, acting as an antioxidant. This indicates one of the plant responses to the stress of heavy metals is the increased production of Car, which can scavenge reactive oxygen species (Sharma et al., 2016). Accordingly, Car can be used as an indicator for the detection of heavy metals in sunflower (Paul et al., 2016).

Conclusion

The present research investigated the effects of sunflower (*Helianthus annuus*) plants on the

bioremediation of heavy metals including Ni, Cr, Pb, and Cd resulted by sewage sludge. The investigation of soil polluted with sewage sludge indicated soil pH and CaCO₃ were not affected by sewage sludge; however, soil salinity, organic matter, and soil nitrogen were significantly increased by the use of sewage sludge. Although there were significant differences between different rates of sewage sludge on the uptake of all measured heavy metals, the plant indicated to be a hyperaccumulator for Pb (high BCF for plant aerial part), as it absorbed (at 15 mg/kg) about 96 and 70% of Pb in the greenhouse and in the field, respectively. Plant pigment contents were not affected by sewage sludge in a clear trend. However, the significant and positive correlation of all heavy metals with plant carotenoids indicated the important role of such pigments, especially plant carotenoids, as antioxidants, in the alleviation of heavy metal stress in the soils polluted with sewage sludge. Due to its unique properties including high biomass production, production of antioxidants, localization of heavy metals to non-active parts of the plant, and production of osmolytes, sunflower plants can be efficiently used for the removal of Ni, Cr, and Cd from the soils polluted with sewage sludge. The recycling of sewage sludge by mixing with the field soil under sunflower cultivation is a suitable method for the treatment of sewage sludge, from health, economic, and environmental perspectives.

Acknowledgements The authors would like to thank very much the international publisher, AbtinBerkeh Scientific Ltd.

Company (<https://AbtinBerkeh.com>), Isfahan, Iran, for editing the manuscript and revisiting it according to the journal format.

Author contribution All authors contributed equally to the manuscript.

Funding There was not any funding for the research.

Data availability The authors selected not to share data.

Declarations

Conflict of interest The authors declare they do not have any conflict of interest.

References

- Behera, K. K. (2014). Phytoremediation, transgenic plants and microbes. In *Sustainable agriculture reviews* (pp. 65–85). Cham: Springer.
- Berni, R., Luyckx, M., Xu, X., Legay, S., Sergeant, K., Hausman, J. F., Lutts, S., Cai, G., & Guerriero, G. (2019). Reactive oxygen species and heavy metal stress in plants: Impact on the cell wall and secondary metabolism. *Environmental and Experimental Botany*, *161*, 98–106.
- Boltz, D. F., & Howell, J. A. (1978). Colorimetric determination of nonmetals. Boltz and J.A. Howell, eds., 2nd ed. In D. F. Boltz, & J. A. Howell (Eds.), *Colorimetric determination of nonmetals. Chemical analysis*, 2nd edn (Vol. 8, pp. 197–251). New York, Chichester Brisbane: John Wiley & Sons.
- Dhiman, S. S., Zhao, X., Li, J., Kim, D., Kalia, V. C., Kim, I. W., Kim, J. Y. & Lee, J. K. (2017). Metal accumulation by sunflower (*Helianthus annuus* L.) and the efficacy of its biomass in enzymatic saccharification. *PLoS ONE*, *12*, e0175845.
- Elloumi, N., Jerbi, B., Zouari, M., Abdallah, F. B., Ayadi, H., & Kallel, M. (2016). Effects of sewage sludge fertilizer on heavy metal accumulation and consequent responses of sunflower (*Helianthus annuus*). *Environmental Science and Pollution Research*, *23*, 20168–20177.
- Greman, H., Velikonja-Bolta, S., Vodnik, D., Kos, B., & Lestan, D. (2001). EDTA enhanced heavy metal phytoextraction: Metal accumulation, leaching and toxicity. *Plant and Soil*, *235*, 105–114.
- Holleman, A. F. (2019). *Lehrbuch der anorganischen Chemie*. Walter de Gruyter GmbH & Co KG.
- Ignatowicz, K. (2017). The impact of sewage sludge treatment on the content of selected heavy metals and their fractions. *Environmental Research*, *156*, 19–22.
- Ishtiyag, S., Kumar, H., Varun, M., Kumar, B., & Paul, M. S. (2018). Heavy metal toxicity and antioxidative response in plants: An overview. In *Plants under metal and metalloids stress* (pp. 77–106). Singapore: Springer.
- Kacprzak, M., Neczaj, E., Fijałkowski, K., Grobelak, A., Grosse, A., Worwag, M., Rorat, A., Brattebo, H., Almås, Å., & Singh, B. R. (2017). Sewage sludge disposal strategies for sustainable development. *Environmental Research*, *156*, 39–46.
- Khelifi, F., Melki, A., Hamed, Y., Adamo, P., & Caporale, A. G. (2019). Environmental and human health risk assessment of potentially toxic elements in soil, sediments, and ore-processing wastes from a mining area of southwestern Tunisia. *Environmental Geochemistry and Health*, *42*, 4125–4139.
- Koutroubas, S. D., Antoniadis, V., Damalas, C. A., & Fotiadis, S. (2020). Sunflower growth and yield response to sewage sludge application under contrasting water availability conditions. *Industrial Crops and Products*, *154*, 112670.
- Li, G., Wan, Sh., Zhou, J., Yang, Zh., & Qin, P. (2009). Leaf chlorophyll fluorescence, hyperspectral reflectance, pigments content, malondialdehyde and proline accumulation responses of castor bean (*Ricinus communis* L.) seedlings to salt stress levels. *Industrial Crops and Product*, *31*, 13–19.
- Liu, X., Chen, J., Wang, G. H., Wang, W. H., Shen, Z. J., Luo, M. R., Gao, G. F., Simon, M., Ghoto, K., & Zheng, H. L. (2016). Hydrogen sulfide alleviates zinc toxicity by reducing zinc uptake and regulating genes expression of antioxidative enzymes and metallothioneins in roots of the cadmium/zinc hyperaccumulator *Solanum nigrum* L. *Plant and Soil*, *400*, 177–192.
- Marathe, S., & Ravichandran, N. (2019). Potential of sunflower to extract heavy metals from leachate. *International Journal of Geosciences*, *10*, 1115.
- Miransari, M. (2011). Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. *Biotechnology Advances*, *29*, 645–653.
- Miransari, M., Bahrami, H. A., Rejali, F., & Malakouti, M. J. (2008). Using arbuscular mycorrhiza to alleviate the stress of soil compaction on wheat (*Triticum aestivum* L.) growth. *Soil Biology and Biochemistry*, *40*, 1197–1206.
- Mohamed, B., Mounia, K., Aziz, A., Ahmed, H., Rachid, B., & Lotfi, A. (2018). Sewage sludge used as organic manure in Moroccan sunflower culture: Effects on certain soil properties, growth and yield components. *Science of the Total Environment*, *627*, 681–688.
- Mousavi, S. M., Moteszarehadeh, B., Hosseini, H. M., Alikhani, H., & Zolfaghari, A. A. (2018). Root-induced changes of Zn and Pb dynamics in the rhizosphere of sunflower with different plant growth promoting treatments in a heavily contaminated soil. *Ecotoxicology and Environmental Safety*, *147*, 206–216.
- Paneque, M., José, M., Franco-Navarro, J. D., Colmenero-Flores, J. M., & Knicker, H. (2016). Effect of biochar amendment on morphology, productivity and water relations of sunflower plants under non-irrigation conditions. *CATENA*, *147*, 280–287.
- Paul, S. A. K. T. I. S., Wong, L. S., & Ong, G. H. (2016). The responses of carotenoids in cyanobacteria *Anabaena cylindrica* as potential bio-indicator for heavy metals detection. *Pollution Research*, *35*, 259–262.
- Raza Altaf, A., Teng, H., Saleem, M., Raza Ahmad, H., Adil, M., & Shahzad, K. (2020). Associative interplay of *Pseudomonas gessardii* BLP141 and pressmud ameliorated growth, physiology, yield, and Pb-toxicity in sunflower. *Bioremediation Journal* in press.

- Saleem, M., Asghar, H. N., Zahir, Z. A., & Shahid, M. (2018). Impact of lead tolerant plant growth promoting rhizobacteria on growth, physiology, antioxidant activities, yield and lead content in sunflower in lead contaminated soil. *Chemosphere*, *195*, 606–614.
- Sharma, A., Kumar, V., Singh, R., Thukral, A. K., & Bhardwaj, R. (2016). Effect of seed pre-soaking with 24-epibrassinolide on growth and photosynthetic parameters of *Brassica juncea* L. in imidacloprid soil. *Ecotoxicology and Environmental Safety*, *133*, 195–201.
- Walkey, A., & Black, T. A. (1934). An examination of the Dugtjaraff method for determining soil organic matter and proposed modification of the chronic and titration method. *Soil Science*, *37*, 23–38.
- Yue, Y., Yao, Y., Lin, Q., Li, G., & Zhao, X. (2017). The change of heavy metals fractions during hydrochar decomposition in soils amended with different municipal sewage sludge hydrochars. *Journal of Soils and Sediments*, *17*, 763–770.
- Zamani, S., Naderi, M. R., Soleymani, A., & Nasiri, B. M. (2020). Sunflower (*Helianthus annuus* L.) biochemical properties and seed components affected by potassium fertilization under drought conditions. *Ecotoxicology and Environmental Safety*, *190*, 110017.
- Zehra, A., Sahito, Z. A., Tong, W., Tang, L., Hamid, Y., Wang, Q., Cao, X., Khan, M. B., Hussain, B., Jatoi, S. A., & He, Z. (2020). Identification of high cadmium-accumulating oil-seed sunflower (*Helianthus annuus*) cultivars for phytoremediation of an Oxisol and an Inceptisol. *Ecotoxicology and Environmental Safety*, *187*, 109857.
- Zhao, X., Joo, J. C., Lee, J. K., & Kim, J. Y. (2019). Mathematical estimation of heavy metal accumulations in *Helianthus annuus* L. with a sigmoid heavy metal uptake model. *Chemosphere*, *220*, 965–973.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.