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ABSTRACT: Heavy metals are among the most critical environmental pollutants close to industrial areas. One example is the cultivated fields in the south of Alborz industrial city in Iran, which is irrigated by treated industrial wastewater. It is contaminated by heavy metals and irrigation with wastewater treatment plants effluent, which made it salty. In this study, the application of 2 amendments, biosolids and cow manure, in improving the heavy metal accumulation in the ornamental sunflower from these types of soils was investigated. A greenhouse experiment using a completely randomized design with 4 replications and applying cow manure and biosolids in 3 weight ratios (6%, 12%, 25%) was conducted to evaluate the efficiency of sunflower in removing Pb, Ni, and Zn from the soil. Adding the amendments increased the rate of germination by 50% to 176%. Although the simultaneous utilization of cow manure in high ratios with biosolids and cow manure with low biosolids decreased the sunflower survival, nonetheless, the simultaneous addition of these organic amendments could increase the survival rate in other treatments. Moreover, the plants' biomass was increased by adding modifiers such as cow manure and biosolids. The results showed that in treatments with 2 modifiers, the remediation factor of Pb, Zn, and Ni has increased 83.7 to 95.5, 78.4 to 87.5, and 74.9 to 94.9, respectively, in comparison to the control one. Therefore, we conclude that adding biosolids and cow manure simultaneously could improve the ornamental sunflower ability to accumulate heavy metals.

KEYWORDS: Biosolids, sunflower, amendments, phytoremediation, industrial zone

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Introduction

Soil contamination by heavy metals has become a global concern due to its impacts on human health, toxicity for plants, and long-term effects on soil fertility.[1] They enter into the soil through human activities such as mining, applying pesticides, and producing industrial wastewater.[2] Therefore, removing soil contaminants is a fundamental issue in remediating natural ecosystems.[3]

Conventional remediation techniques, such as physical and chemical processes, are very expensive, often destructive to the local ecosystem, and require large quantities of hazardous waste to be treated.[4] Recently, using plants for remediating soils contaminated by heavy metals has attracted considerable interest because it is inexpensive and easy to be applied, it can be carried out on-site, it is eco-friendly, and it is a natural way. It can also be applied using solar energy,[5] which could receive public approval. This method does not leave behind residual and toxic materials; preserves and maintains soil physical, chemical, and biological properties; and prevents heavy metal penetration into groundwater.[5]

In general, 2 methods are employed to remove metals from contaminated soils by plants. The first one uses hyper-accumulating plants able to accumulate vast quantities of metals.[6] They produce insignificant quantities of biomass, grow slowly, and take longer to reach maturity.[6] Plants tolerant to metals such as corn, sunflower, and mustard are used in recent years that accumulate relatively large amounts of metallic contaminants and also produce large quantities of biomass.[5] Considering factors such as the plant's growth speed, more biomass production, easy planting and harvesting, impossibility of their use as a food and the entrance of metals to the food chain,[7] resistance to the high salinity of the soil,[8] high ability in the compatibility with the environment, and high ability to absorb a wide range of heavy metals,[9] the ornamental sunflower has been attracted many attentions for phytoremediation.

The low capability of plant roots in taking up metallic contaminants is the limiting factor in using hyper-accumulating and non-hyper-accumulating plants tolerant to metals in the process of heavy metal removal. Therefore, methods such as the application of chelating and acidifying agents, employment of

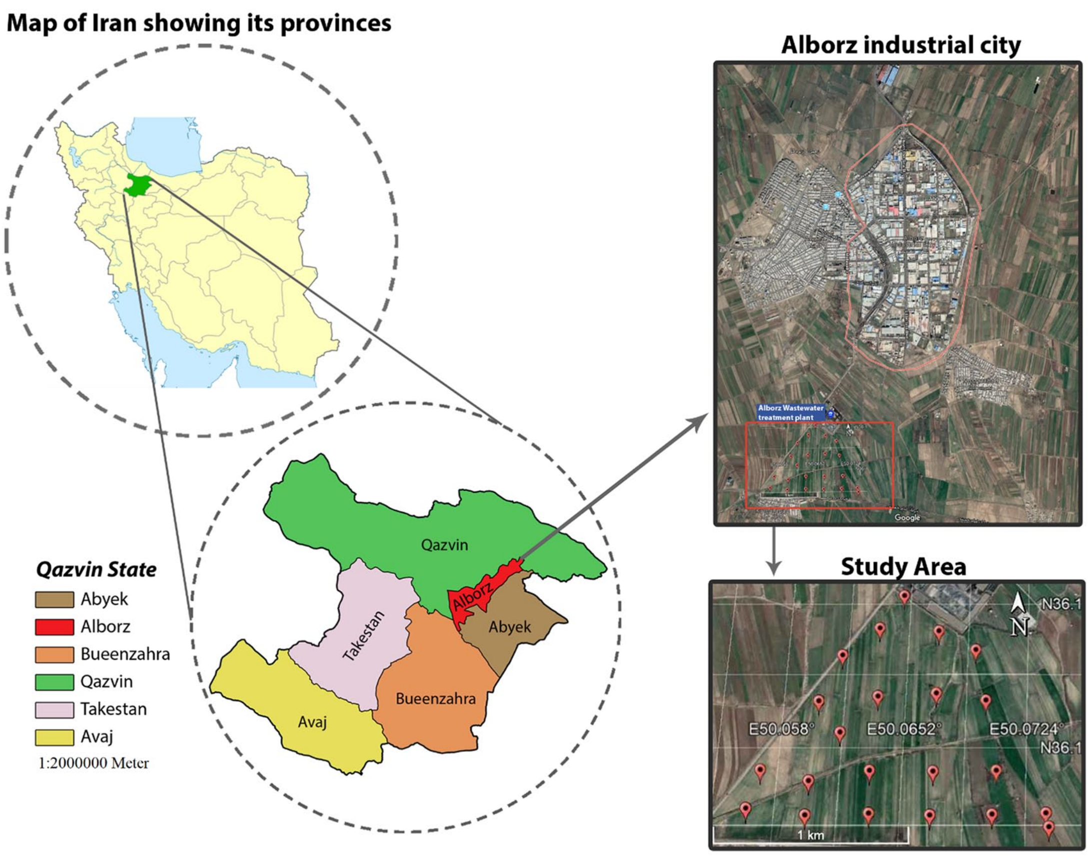

Figure 1. Location of study area and soil samples.

growth-regulating materials, biological methods, and utilization of organic fertilizers have been tested to increase phytoremediation efficiency. Although the utilization of some organic modifiers such as biochar reduces the mobility of heavy metals, the addition of modifiers such as compost and nitrogen fertilizers increases the availability and accumulation of heavy metals in plants.[10] They increase the biomass production and uptake of heavy metals by affecting the physiological and morphological characteristics of plants,[11] increasing soil fertility, and improving its physical properties.[5]

Also, application of organic fertilizers such as sewage sludge and manure substantially changes rhizosphere physical, chemical, and biological properties and increases corn and sunflower biomass and populations of microorganisms and their activity in soils.[12]

Phytoremediation could be useful in extremely contaminated regions.[13] However, it can be more successful in low to moderate areas due to the toxicity of heavy metals and the reduction of the plants' growth.[14]

In the south of the Alborz industrial city in Qazvin province, farmers have used the urban and industrial wastewater treatment plant effluent to cope with water scarcity and the limitations of groundwater use and the benefits of increasing soil fertility.[15] The findings of the study in this region confirmed the increase of soil heavy metals concentration as compared to the obtained soil samples from the irrigated region with well water.[15] Given that the products produced by these farms enter the food chain of the people of the region, it is necessary to refine soils of this area. Therefore, the main aim of this research was to investigate the amount of heavy metals removal in this area using an ornamental sunflower. Also, biosolid and cow manure modifiers were used to check the efficiency to improve soil salinity,[16] increase plant biomass,[17] improve soil physical and chemical properties,[18] increase microbial activity and growth,[19] and enhance sunflower ability to cope with salinity stress.

Materials and Methods

Study area

The sample area was located in the farmlands in the south of the Alborz wastewater treatment plant in Qazvin province, Iran (latitude 36º14′-36º15′N and longitude 50º05′-50º07′E; Figure 1). This plant purifies more than 500 units' wastewaters, such as textiles, tanneries, and chemicals. Industrial dust and irrigation with urban and industrial wastewater treatment plant effluent have contaminated the area with heavy metals and also increased soil salinity.

Soil sampling

Twenty-two soil samples were taken from 0 to 20 cm depth of topsoils in the study area in July 2017 (Figure 2). We considered

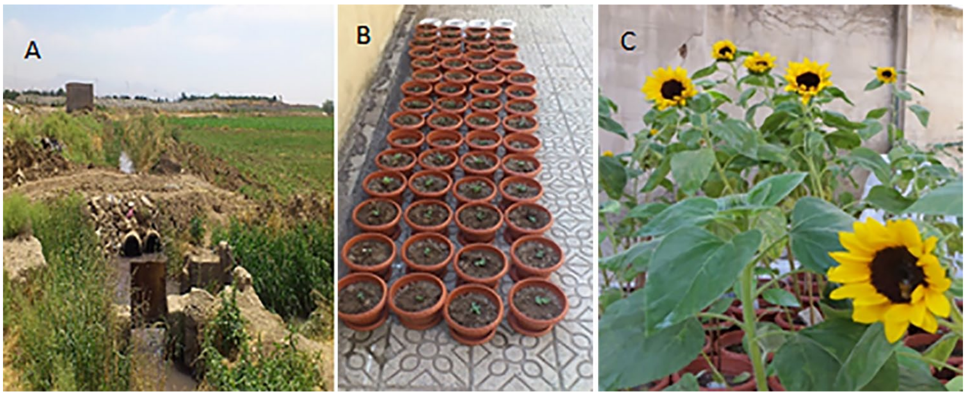

Figure 2. (A) The study area. (B) All different treatments at the beginning of the experimental time. (C) All different treatments at the end of the experimental time (photograph: Fatemeh Karimi, 2017).

Table 1. Allowable limit of heavy metal concentration in soil.[28,29]

ELEMENT	ALLOWABLE LIMIT OF HEAVY METAL CONCENTRATION IN SOIL (MG/G)						
	GERMANY	NETHERLANDS	SWEDEN	USA	IRELAND	IRAN (AGRICULTURAL LIMIT)	IRAN (ENVIRONMENTAL PROTECTION LIMIT)
Pb	70	40	40	15	50	75	300
Zn	150	100	100-150	140	150	500	200
Ni	50	15	30	21	30	110	50

this soil depth, attending the reasons exposed by Adesodun et al[20] Each soil sample was considered as a composite sample consisting of 4 sub-samples collected a few meters away from the designated point. The sub-samples thoroughly mixed to form a composite sample. Two samples were taken from every composite sample. Shortly after collection, one of the samples (2 kg) was transferred to the laboratory, and the other one (2 kg) was transferred to the greenhouse (latitude 36°26′N and longitude 50°00′E; Figure 2). In the greenhouse, all samples were mixed to homogenize for pot experiment.

Soil analysis

In the laboratory, samples were air-dried and passed through a 2 mm sieve. Then, some of the chemical and physical properties were determined. Soil texture was determined using the hydrometer method.[21] Percentages of sand, clay, silt, lime, and the pH and electrical conductivity were measured in the saturated extract.[22,23] Exchangeable phosphorous was calculated using the Olsen method[24] and exchangeable sodium and potassium were measured employing the extraction method with ammonium acetate and using the flame photometer readings.[25] Total nitrogen was determined using the Kjeldahl.[26] Organic carbon was measured employing the Walkely-Black method.[27]

After analyzing, concentrations of Ni, Pb, and Zn of the soil samples were compared to the allowable limit of heavy metals concentration in soil[28,29] (Table 1).

Pot experiment

A greenhouse experiment using a completely randomized design with 4 replications and employing fermented cow manure and urban wastewater treatment plant biosolids in 3 weight ratios (6%, 12%, 25%) was conducted to evaluate the efficiency of the sunflower in removing lead, nickel, and zinc from soil.

There were 16 series of treatment (Table 2), each treatment with 4 replications. In all, 64 pots were used (4 of them control ones). Improved seeds of a single-branch cultivar were bought from Alborz Seed Breeding Research Institute. Cow manure from a local livestock farm in Qazvin (latitude 36°27′N and longitude 50°04′E) and biosolids from the urban wastewater treatment plants (latitude 35°52′N and longitude 51°43′E) in the south of Tehran were prepared.

The seeds were surface-disinfected using 5% sodium hypochlorite, 96% ethanol, and distilled water.[12] The 4 seeds were planted in each 3 kg pot containing 2.5 kg of a mixture of cow manure, biosolids, and contaminated soil. In the greenhouse, there were 9.86 hours of sunshine on average. Also, the mean temperature (light/dark cycle) and the relative humidity were 32.14°C and 40%, respectively. During the growing period (from July 2017 to October 2017), the plants were irrigated using urban water (Table 3). The monthly water requirement was estimated based on the method for calculating the water needs of crop.[30]

Table 2. Design of treatments.

TREATMENTS	CODE NAME	REPLICATION
Cow manure (6%) + contaminated soil (96%)	T1	4
Cow manure (12%) + contaminated soil (88%)	T2	4
Cow manure (25%) + contaminated soil (75%)	T3	4
Biosolids (6%) + contaminated soil (96%)	T4	4
Biosolids (12%) + contaminated soil (88%)	T5	4
Biosolids (25%) + contaminated soil (75%)	T6	4
Cow manure (6%) + biosolids (6%) + contaminated soil (88%)	T7	4
Cow manure (6%) + biosolids (12%) + contaminated soil (82%)	T8	4
Cow manure (6%) + biosolids (25%) + contaminated soil (69%)	T9	4
Cow manure (12%) + biosolids (6%) + contaminated soil (82%)	T10	4
Cow manure (12%) + biosolids (12%) + contaminated soil (76%)	T11	4
Cow manure (12%) + biosolids (25%) + contaminated soil (63%)	T12	4
Cow manure (25%) + biosolids (6%) + contaminated soil (69%)	T13	4
Cow manure (25%) + biosolids (12%) + contaminated soil (63%)	T14	4
Cow manure (25%) + biosolids (25%) + contaminated soil (50%)	T15	4
Control	T16	4

Table 3. Some physical and chemical properties of irrigation water.

PROPERTY	UNIT	VALUE
pH		6.9 ± 0.01
EC	μS/cm	1,019 ± 4.5
TN	mg/L	5.2 ± 0.2
P	mg/L	ND
K	mg/L	ND
Na	mg/L	164.9 ± 0.02
Zn	ppm	0.2 ± 0.2
Ni	ppb	6.9 ± 0.02
Pb	ppb	6.7 ± 0.02

Abbreviations: EC, electrical conductivity; ND, not detected; TN, total nitrogen.

A dish was placed under each pot, and the drained water was poured back into the pot surface daily. The position of each treatment changed alternatively once a week. In the 4-leaf stage (after 3 weeks of the appearance), thinning was taken, and in each pot, 2 seedlings were kept. No other fertilizers or amendments were used. The cultivation period lasted about 3 months in 2017.

A total of 56 days after plant emergence, one pot was taken from the treatment of 4, 5, 7, 8, 9, 11, 12, and 116 days after planting, and the remaining pots were harvested. The stems were cut at soil level. The stems and roots and the remaining soil were labeled and separately placed in plastic bags and were transferred to the laboratory within 3 hours. In the laboratory, the stems and roots were washed several times with distilled water and separately placed in a laboratory oven at 70°C[31] to dry to constant weight. The dry sample (0.5 g) was digested for 32 minutes after adding 5 mL HNO_3 and 1 mL H_2O_2. Then, 50 mL distilled water was added to each sample, and the concentrations of the heavy metals in all the samples were analyzed using spectrometer (ICP-OES spectrometer; GBC, Australia).[32]

Germination and survival rates

Germination means the appearance of a stem or shoot on the top of the soil.[33] The germination rate expresses the number of seeds that germinate in a short period (14 days) of time.[34] The mentioned rate is given as follows:

$$GR(\%) = \frac{Gs}{Ts} \times 100 \quad (1)$$

where GR is the germination rate, Gs means the number of the germinated seeds after 14 days, and Ts is the total planted seeds.

Survival was defined as being green or viable until the end of the test period.[33] The survival rate is calculated as the following formula[35]:

$$SR(\%) = \frac{Rs}{Ts} \times 100 \quad (2)$$

where SR is the survival rate, Rs is the number of surviving seedlings, and Ts is the total seedlings.

Phytoremediation efficiency indices of the plants

In studies on heavy metal absorption, bioconcentration factor (BCF), translocation factor (TF), and remediation factor (RF) are crucial to understand. These indices were employed to evaluate plant ability in removing heavy metals from the environment. Bioconcentration factor was determined from the ratio of the concentrations of heavy metals in roots/shoots (mg/kg) to their concentrations in the soil (mg/kg). Translocation factor was calculated from the ratio of the concentrations of heavy metals in the shoot of the plants (mg/kg) to their concentrations in the roots (mg/kg). Remediation factor is defined as the ratio of an element accumulation in the shoots to that in soil.[36,37] The mentioned factors' formulas are as follows:

$$BCF = \frac{Cr}{Cp} \quad (3)$$

where BCF is the bioconcentration factor, Cr represents the heavy metal concentration in the plant roots in mg/kg, and Cp means the heavy metal concentration in the soil in mg/kg:

$$TF = \frac{Cs}{Cr} \quad (4)$$

where TF is the translocation factor, Cs means heavy metal concentration in the plant shoot in mg/kg, Cr is the heavy metal concentration in the plant roots in mg/kg:

$$RF(\%) = \frac{Cs \times Ms}{Cp \times Mp} \times 100 \quad (5)$$

where RF is the remediation factor, Cs represents heavy metal concentration in the plant shoot in mg/kg, Ms is the dry biomass weight of the shoot in kg, Cp is the heavy metal concentration in the soil in mg/kg, and Mp is the amount of the soil in the pot in kg.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA). Multiple means comparisons were carried out using Tukey and Dunnett test to compare different treatments to each other and the control. For the comparison of paired samples, Wilcoxon test was used. Differences at the $P < .05$ level were considered to be statistically significant. SPSS software package (IBM SPSS Statistics 17) was used to perform statistical analyses.

Table 4. Some physical and chemical properties of soil.

PROPERTY	UNIT	VALUE
pH		7.4 ± 0.2
EC	dS/m	4.3 ± 2.1
OC	%	1.5 ± 0.2
N	mg/kg	1,400 ± 131.5
P	mg/kg	97.4 ± 30.6
K	mg/kg	1,043.8 ± 96.2
Na	mg/kg	1,400 ± 61
Sand	%	56 ± 4.4
Silt	%	24 ± 2.8
Clay	%	20 ± 2.9
Zn	mg/kg	174 ± 84.2
Ni	mg/kg	51 ± 12.9
Pb	mg/kg	60 ± 27.9

Abbreviations: EC, electrical conductivity; OC, organic carbon.

Results

Chemical and physical properties of the contaminated soil sample and the amendments

The average values of the chemical and physical properties of cow manure, biosolids, and contaminated soil are presented in Tables 4 and 5. Comparison of the concentrations of Ni, Pb, and Zn of the soil samples with the allowable limit of heavy metals concentration in soil indicates that the content of Pb is equal to or greater than the standard amount of soil pollution with agricultural use in 50% of samples. In half of the samples, the content of Zn and Ni was equal to or greater than the standard amount of pollutants in the soil to protect the environment.[29] Also, the comparison of the average concentration of Ni and Zn with the allowed values of heavy metals concentration in the countries of Germany, Sweden, the United States, and the Netherlands demonstrated both elements are more, and for the Pb element other than the allowed value, in Germany, it exceeds the standards allowed by the other 3 countries.[28]

Germination and survival rate

The percentage of germination of various treatments is presented in Figure 3. Compared to the control group, the germination rate of all treatments has increased (Figure 3). Adding biosolids and cow manure could increase the germination rate of 50% to 176%. The survival of plants in various treatments is shown in Figure 3. The lack of application of the modifiers, the usage of cow manure alone, the simultaneous utilization of cow manure in high proportion with biosolids, and the simultaneous application of biosolids/cow manure with low biosolids (6%) diminished sunflower survival rate (Figure 3).

Plants biomass

In Table 6, the average dry biomass of the ornamental sunflower is presented in different treatments at the end of the test period. Based on the results, adding modifiers increased plant growth compared to the control plants ($P < .05$). The study of the growth of roots and shoots in treatments showed that the highest growth rate was observed in 14 and 15 treatments characterized by T14 and T15.

Table 5. Some physical and chemical properties of biosolids and cow manure.

PROPERTY	UNIT	VALUE	
		BIOSOLIDS	COW MANURE
pH		7.7 ± 0.9	8.2 ± 0.7
EC	dS/m	10.1 ± 0.7	13.9 ± 0.1
TKN	%	4.4 ± 0.6	1.05 ± 0.2
TP	%	0.09 ± 0.03	0.6 ± 0.1
K	%	0.01 ± 0.01	1.8 ± 0.02
Na	%	0.01 ± 0.02	0.5 ± 0.1
Zn	mg/kg	1,122 ± 10.3	302 ± 5.1
Ni	mg/kg	66 ± 3.4	19 ± 0.7
Pb	mg/kg	73 ± 4.4	10 ± 0.4

Abbreviations: EC, electrical conductivity; TKN, total Kjeldahl nitrogen; TP, total phosphorus.

Effects of the modifiers on the plant metal concentration

In Figure 4, the concentrations of Pb, Ni, and Zn in different treatment plants are presented at the end of the period. Simultaneous use of biosolids and cow manure modifiers compared to the separate use of cow manure and biosolids and the control plant increases the concentration of Pb, Ni, and Zn in the total treated plants (Figure 4). The effect of adding modifiers on the absorption of the Pb in different treatments was recorded in the following order:

$$15 > 12 > 14 > 11 > 9 > 13 > 8 > 10 > 6 > 7 > 5 > 4 > 3 > 2 > 1$$

Simultaneous use of 2 modifiers in ratios of 25 to 25 and 12 to 12 compared to other treatments had the most significant effect on increasing the concentration of Pb in the root and shoot of the ornamental sunflower ($P < .05$). The concentration of Pb in the roots of the plants of these 2 treatments was 104.81 and 102.30 mg/kg, respectively, and in the shoots of the plants of 2 treatments were 53.14 and 47.66 mg/kg, respectively. In Figure 4, the comparison of Pb concentration in the mid-term and the end of the period are presented.

The concentration of Ni element in plants at the end of the test period was recorded in the treatments in the following order: $15 > 14 > 9 > 12 > 11 > 13 > 7 > 10 > 8 > 4 > 6 > 5 > 4 > 1 > 2 > 3$. The highest amount of Ni absorption in the root was related to treatments of 9, 15, 11, 12, and 14 and in the shoot was related to the treatments of 15, 14, 6, 9, and 13, respectively. The maximum concentration of Ni in the roots of those treatments increased to 34.89, 34.10, 33.92, 33.47, and

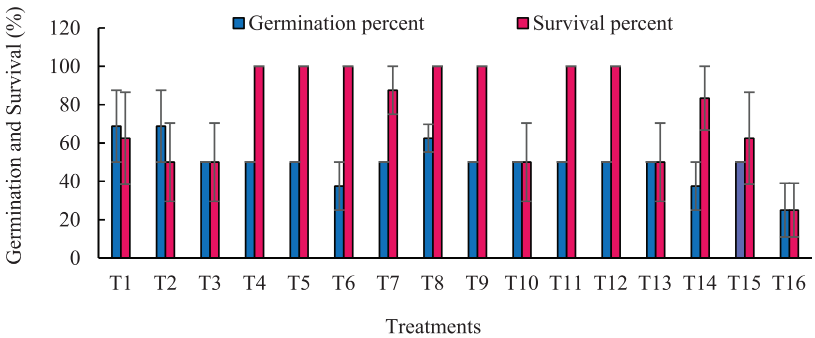

Figure 3. Germination and survival percentage of plants in different soil treatments. T1 indicates cow manure (6%) + contaminated soil (96%); T2, cow manure (12%) + contaminated soil (88%); T3, cow manure (25%) + contaminated soil (75%); T4, biosolids (6%) + contaminated soil (96%); T5, biosolids (12%) + contaminated soil (88%); T6, biosolids (25%) + contaminated soil (75%); T7, cow manure (6%) + biosolids (6%) + contaminated soil (88%); T8, cow manure (6%) + biosolids (12%) + contaminated soil (82%); T9, cow manure (6%) + biosolids (25%) + contaminated soil (69%); T10, cow manure (12%) + biosolids (6%) + contaminated soil (82%); T11, cow manure (12%) + biosolids (12%) + contaminated soil (76%); T12, cow manure (12%) + biosolids (25%) + contaminated soil (63%); T13, cow manure (25%) + biosolids (6%) + contaminated soil (69%); T14, cow manure (25%) + biosolids (12%) + contaminated soil (63%); T15, cow manure (25%) + biosolids (25%) + contaminated soil (50%); T16, control.

Table 6. Average biomass of plants (in grams).

TREATMENTS	SHOOT BIOMASS	ROOT BIOMASS
T1	34.8 ± 0.8d	6.8 ± 0.6ef
T2	35.0 ± 0.8d	6.9 ± 0.5ef
T3	39.2 ± 0.9d	7.0 ± 0.8e
T4	33.9 ± 0.7d	6.7 ± 0.75f
T5	36.0 ± 0.3d	6.7 ± 0.9ef
T6	37.6 ± 1.0d	6.8 ± 0.8ef
T7	43.9 ± 0.1 cd	7.9 ± 0.5d
T8	50.0 ± 0.1bcd	8.8 ± 0.8c
T9	55.6 ± 0.8bcd	9.0 ± 0.8b
T10	49.6 ± 0.4bcd	8.5 ± 0.45c
T11	56.6 ± 0.8abc	10.0 ± 0.7b
T12	59.2 ± 0.8abc	10.3 ± 0.5b
T13	58.7 ± 0.9ab	10.5 ± 0.4b
T14	64.7 ± 0.6a	11.8 ± 0.2a
T15	68.3 ± 1a	12.1 ± 0.2a
T16	19.0 ± 5.7e	4.1 ± 1.4g

Abbreviations: T1, cow manure (6%) + contaminated soil (96%); T2, cow manure (12%) + contaminated soil (88%); T3, cow manure (25%) + contaminated soil (75%); T4, biosolids (6%) + contaminated soil (96%); T5, biosolids (12%) + contaminated soil (88%); T6, biosolids (25%) + contaminated soil (75%); T7, cow manure (6%) + biosolids (6%) + contaminated soil (88%); T8, cow manure (6%) + biosolids (12%) + contaminated soil (82%); T9, cow manure (6%) + biosolids (25%) + contaminated soil (69%); T10, cow manure (12%) + biosolids (6%) + contaminated soil (82%); T11, cow manure (12%) + biosolids (12%) + contaminated soil (76%); T12, cow manure (12%) + biosolids (25%) + contaminated soil (63%); T13, cow manure (25%) + biosolids (6%) + contaminated soil (69%); T14, cow manure (25%) + biosolids (12%) + contaminated soil (63%); T15, cow manure (25%) + biosolids (25%) + contaminated soil (50%); T16, control.
Data are the mean of 3 replications. Same letters are not significantly different at $P < .05$ (n = 3) between different treatments according to Tukey test.

32.68 mg/kg, respectively, and the maximum concentration of Ni in their shoots was 11.50, 8.84, 6.67, 6.62, and 6.57 mg/kg, respectively. In Figure 4, the concentration of Ni is shown in the mid-term and at the end of the test period.

Simultaneous use of 2 modifiers of biosolids and cow manure in ratios of 25-6, 25-25, 12-25, and 25-12 in treatments (13, 15, 14, and 12) increased the absorption of Zn in both root and shoot of these treatments compared to other treatments. The maximum concentration of Zn in the roots of these treatments increased to 251.61, 217.62, 210.43, and 178.65 mg/kg, and the maximum concentration of Zn in the shoots of the treatments was 45.99, 157.36, 142.89, and 136.08 mg/kg, respectively. Comparison of Zn concentration in treatments 7, 8, 9, 11, and 12 during the mid-term and the end of the period is presented in Figure 5. According to the statistical analysis, the concentration of this element at the end of the period in both root and shoot parts decreased significantly compared to the mid-term ($P < .05$).

Effects of modifiers on heavy metal accumulation

Investigation of adsorption of Pb, Ni, and Zn in the root of the control group showed that the amount of absorption in this part is more than the shoot. The total amount of Pb, Ni, and Zn adsorption in various treatments is presented in Figure 6. In all treatments, aside from treatments 1 and 16, the Pb adsorption was in the shoots of the plants more than the roots. Similar results were obtained for Zn. In treatments 3, 6, 9, 13, 14, and 15, the absorption of Ni in the shoots was more than the roots, and in other treatments, the root absorbed more.

The effects of study of modifiers' addition on the absorption of Pb, Ni, and Zn in shoots of different treatments have shown that the Ni and Pb metals have the highest adsorption in the 2 treatments 14 and 15. Also, the amount of Zn absorption in shoots of plants 13 and 15 was higher than other treatments. The absorbed values in the shoots of plants compared to the shoots of the control group increased significantly, and the highest amount of absorption of all 3 metals was observed in treatment 15 in comparison with the control group. The average increase in Ni and Pb adsorption in the root and the shoot of treatment 15 was, respectively, 4.51, 14.54, and 3.98, 16.49 times the absorbance value in the root and the shoot of the control group. Also, the average Zn absorption in the shoot of plants in treatment 15 was 9.4 times the amount of shoot absorption of the control group.

Phytoremediation efficiency

To evaluate the ability of the studied plants to clear the soil heavy metals, the 3 indicators BCF, TF, and RF between the mid-term and the end of the period were used. They are given in Table 7. The highest amount of RF of Ni was observed at the end of the period in treatments 14 and 15, which was 0.86 and 1.32, respectively, while the RF value of the control group was 0.04. Also, the highest RF values of Pb were seen in treatments 12 and 15, with values of 4.10 and 5.23, respectively, and the RF value of the control group for Pb removal was obtained 0.14. Treatments 12, 11, 15, 9, and 14 had the highest average amount of Zn extraction in shoots with values of 2.95, 2.77, 2.77, 2.74, and 2.72, respectively. The average amount of Zn extraction in the control group was 0.23. Table 8 shows a comparison of phytoremediation indices in the treatment of 4, 5, 7, 8, 9, 11, and 12 in the mid-term and the end of the period. The phytoremediation indices of Ni (BCF and TF) decreased at the end of the period compared with the mid-term, and their reduction was statistically significant ($P < .05$).

Moreover, the BCF index and the amount of Pb metal stabilization in the roots of the treatments 4, 5, 7, 8, 9, 11, and 12 during the mid-term and the end of the test period were

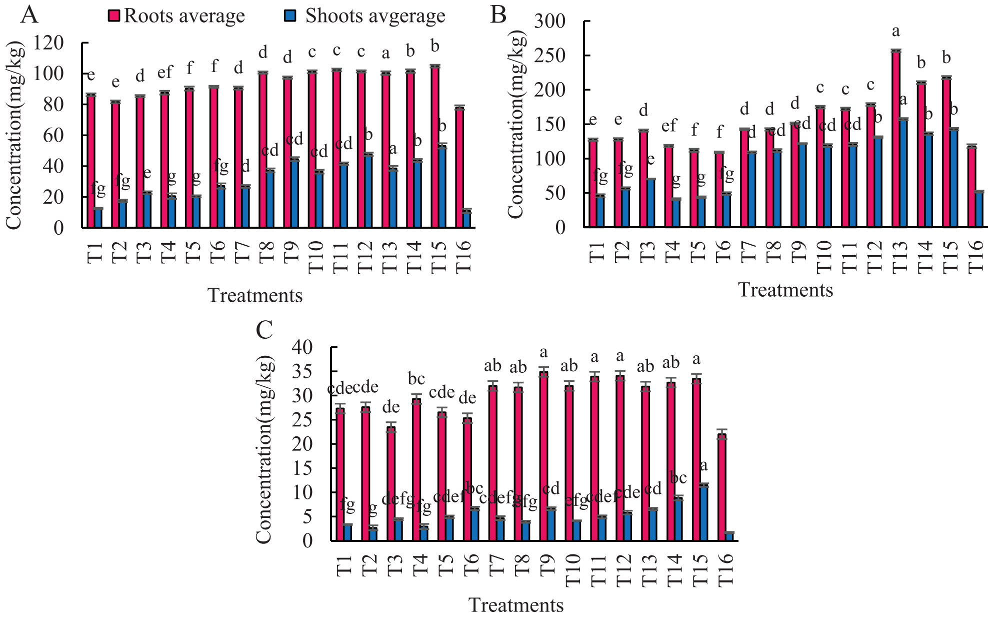

Figure 4. Soil heavy metals concentration in different treatments of (A) Pb, (B) Zn, and (C) Ni. Same letters are not significantly different at $P < .05$ (n=3) between different treatments according to Tukey test. The statistical analysis was a one-way ANOVA. T1 indicates cow manure (6%) + contaminated soil (96%); T2, cow manure (12%) + contaminated soil (88%); T3, cow manure (25%) + contaminated soil (75%); T4, biosolids (6%) + contaminated soil (96%); T5, biosolids (12%) + contaminated soil (88%); T6, biosolids (25%) + contaminated soil (75%); T7, cow manure (6%) + biosolids (6%) + contaminated soil (88%); T8, cow manure (6%) + biosolids (12%) + contaminated soil (82%); T9, cow manure (6%) + biosolids (25%) + contaminated soil (69%); T10, cow manure (12%) + biosolids (6%) + contaminated soil (82%); T11, cow manure (12%) + biosolids (12%) + contaminated soil (76%); T12, cow manure (12%) + biosolids (25%) + contaminated soil (63%); T13, cow manure (25%) + biosolids (6%) + contaminated soil (69%); T14, cow manure (25%) + biosolids (12%) + contaminated soil (63%); T15, cow manure (25%) + biosolids (25%) + contaminated soil (50%); T16, control; ANOVA, analysis of variance.

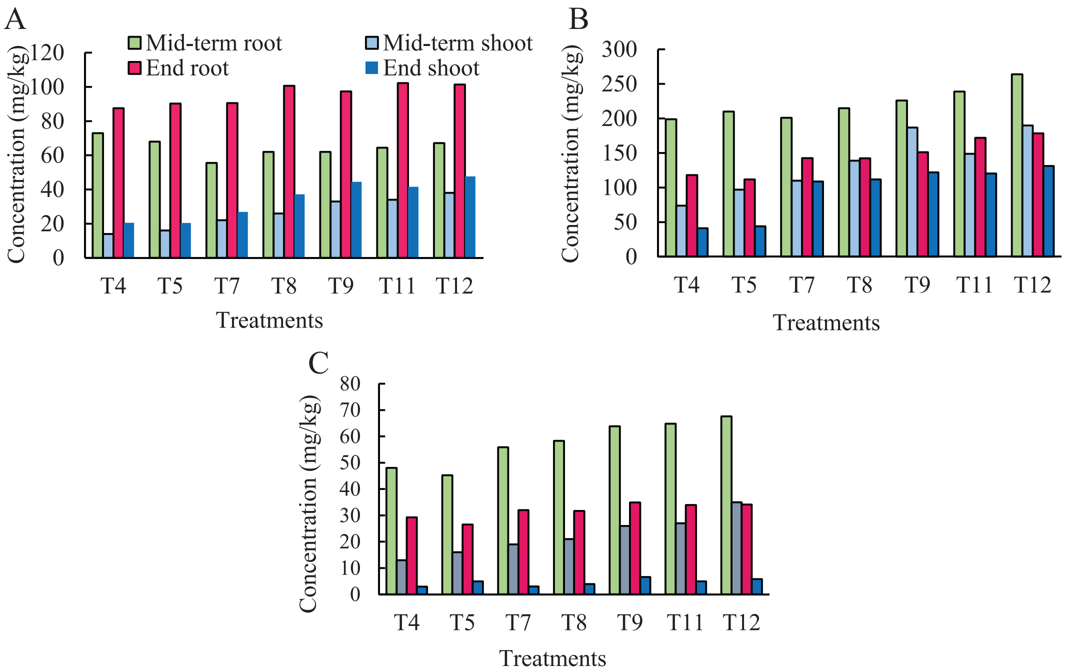

Figure 5. Soil heavy metal concentration of (A) Pb, (B) Zn, and (C) Ni in different treatments in the mid-term and the end of period. The statistical analysis was Wilcoxon. T4 indicates biosolids (6%) + contaminated soil (96%); T5, biosolids (12%) + contaminated soil (88%); T7, cow manure (6%) + biosolids (6%) + contaminated soil (88%); T8, cow manure (6%) + biosolids (12%) + contaminated soil (82%); T9, cow manure (6%) + biosolids (25%) + contaminated soil (69%); T11, cow manure (12%) + biosolids (12%) + contaminated soil (76%); T12, cow manure (12%) + biosolids (25%) + contaminated soil (63%).

investigated. During the growth period, the stabilization amount of Pb increased. Translocation factor study of this metal showed that, apart from treatment 4, the amount of transfer from the roots to shoots decreased over time, due to the loss of leaves at the end. The calculation and statistical analysis of BCF of Zn in the treatments during the mid-term

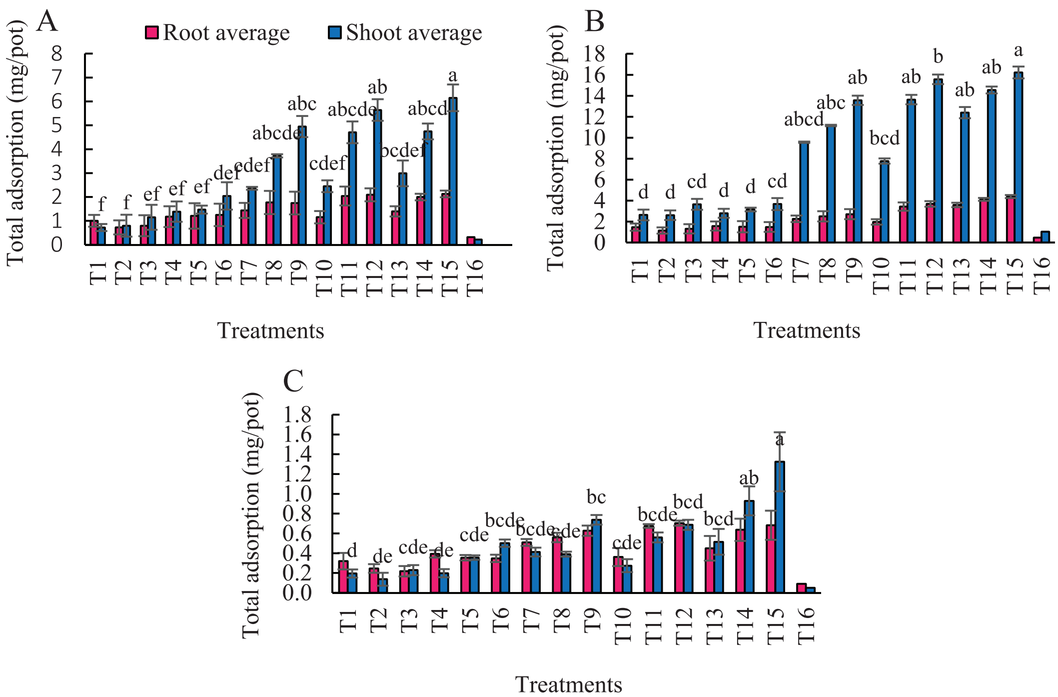

Figure 6. Total heavy metals adsorption in every pot in different treatments of (A) Pb, (B) Zn, and (C) Ni. Same letters are not significantly different at $P < .05$ (n = 3) between different treatments according to Tukey test. The statistical analysis was a one-way ANOVA. T1, cow manure (6%) + contaminated soil (96%); T2, cow manure (12%) + contaminated soil (88%); T3, cow manure (25%) + contaminated soil (75%); T4, biosolids (6%) + contaminated soil (96%); T5, biosolids (12%) + contaminated soil (88%); T6, biosolids (25%) + contaminated soil (75%); T7, cow manure (6%) + biosolids (6%) + contaminated soil (88%); T8, cow manure (6%) + biosolids (12%) + contaminated soil (82%); T9, cow manure (6%) + biosolids (25%) + contaminated soil (69%); T10, cow manure (12%) + biosolids (6%) + contaminated soil (82%); T11, cow manure (12%) + biosolids (12%) + contaminated soil (76%); T12, cow manure (12%) + biosolids (25%) + contaminated soil (63%); T13, cow manure (25%) + biosolids (6%) + contaminated soil (69%); T14, cow manure (25%) + biosolids (12%) + contaminated soil (63%); T15, cow manure (25%) + biosolids (25%) + contaminated soil (50%); T16, control.

and the end of the period indicated that the amount of stabilization of this metal decreased in all treatments at the end of the period ($P < .05$). Although, TF analysis showed that except for the treatments of 4, 5, and 9, over time, the transfer to shoots in other treatments was increased during this period. However, this increase was not statistically significant.

Discussion

Soil salinity affects the germination of the ornamental sunflower and reduces it.[38] As the studied soil showed a high electrical conductivity and was salty, the percentage of germination of the control plants decreased. Soil amendments are effective even at the high salinity on the growth and N assimilation in the sunflower;[16] therefore, adding biosolids and cow manure could decrease salt stress and increase the rate of the germination 50% to 176%. Biosolids can increasingly improve sunflower seedlings growth after 24 days of culturing.[39] Although the concurrent utilization of cow manure in high ratios with biosolids and cow manure with low biosolids decreased the sunflower survival, nonetheless, the simultaneous addition of organic amendments could increase the survival rate in other treatments. The application of these 2 modifiers increases the wet and dry weight of the root and shoot and the ratio of the shoot/root in the dry weight of the plants.[17] For this reason, with the increase in the application ratio of the modifiers, the root and shoot growth rate of the plant increased significantly.

Simultaneous use of biosolids and cow manure modifiers compared to the separate use of cow manure and biosolids and the control plant increases the concentration of Pb, Ni, and Zn in the total treated plants. The concentration of Pb was significantly different in the mid-term for both the roots and shoots of the treated plants (4, 5, 7, 8, 9, 11, and 12) with concentration at the end of the period and its value had increased at the end of the period.[40] Also, statistical analysis of Ni and Zn concentration between the mid-term and the end of the period in 2 parts of the plants indicated that at the end of the period, their concentrations decreased[40] and these decreases were statistically significant ($P < .05$). The decrease in the amount of Ni and Zn at the end of the period may have been due to the miss of leaves.[40]

BCF and TF are 2 critical indicators to assess a plant's ability to accumulate metals.[41] In the present study, the BCF of Pb in the mid-term and the end of the experiment period were 1 to 1.22 and 1.9 to 2.23, respectively, and the TF values in the mid-term and the end of the period were 0.19 to 0.57 and 0.15 to 0.51, respectively. Based on the results of all treatments, the amount of Pb uptake in the roots and shoots of the plants increased compared to the control group. Also, the study of Pb uptake in different treatments indicated that the addition of 2

Table 7. BCF, TF, and RF of heavy metals in ornamental sunflower at the end of test period in all different treatments.

METALS	PB			ZN			NI		
TREATMENTS	BCF	TF	RF	BCF	TF	RF	BCF	TF	RF
T1	1.4(0.03)hi	0.15 ± 0.01 h	0.5 ± 0.03 h	0.7 ± 0.02ghi	0.4 ± 0.02 g	0.6 ± 0.03gh	0.5 ± 0.03hij	0.1 ± 0.01de	0.15 ± 0.01f
T2	1.5 ± 0.03fgh	0.2 ± 0.01gh	0.6 ± 0.05gh	0.7 ± 0.02hij	0.4 ± 0.03ef	0.6 ± 0.02fg	0.6 ± 0.01fgh	0.1 ± 0.03e	0.1 ± 0.03f
T3	1.6 ± 0.02ef	0.3 ± 0.02fg	0.9 ± 0.07fg	0.7 ± 0.01ijk	0.5 ± 0.02e	0.7 ± 0.02f	0.6 ± 0.01ghi	0.2 ± 0.02 cd	0.2 ± 0.02ef
T4	1.3 ± 0.03i	0.2 ± 0.03fg	0.9 ± 0.07gh	0.65 ± 0.02jk	0.35 ± 0.01 g	0.6 ± 0.02 h	0.6 ± 0.02hij	0.1 ± 0.03e	0.15 ± 0.03f
T5	1.5 ± 0.04gh	0.2 ± 0.01fg	1 ± 0.03gh	0.6 ± 0.02k	0.4 ± 0.02fg	0.7 ± 0.02gh	0.5 ± 0.02ij	0.2 ± 0.02 cd	0.3 ± 0.01ef
T6	1.5 ± 0.02fgh	0.3 ± 0.03ef	1.4 ± 0.1fg	0.6 ± 0.01k	0.45 ± 0.02ef	0.9 ± 0.02fg	0.5 ± 0.02j	0.3 ± 0.02b	0.4 ± 0.03e
T7	1.6 ± 0.03de	0.3 ± 0.02ef	1.7 ± 0.06f	0.8 ± 0.01ef	0.8 ± 0.03ab	2.1 ± 0.03e	0.65 ± 0.02defg	0.15 ± 0.02cde	0.3 ± 0.03ef
T8	1.6 ± 0.02ef	0.4 ± 0.02de	2.4 ± 0.09fg	0.8 ± 0.02fg	0.8 ± 0.01a	2.4 ± 0.06d	0.65 ± 0.02efg	0.1 ± 0.01de	0.3 ± 0.02ef
T9	1.6 ± 0.02efg	0.5 ± 0.02abc	3.2 ± 0.07f	0.8 ± 0.02fgh	0.8 ± 0.02a	2.7 ± 0.09c	0.7 ± 0.03bcd	0.2 ± 0.02 cd	0.6 ± 0.03 cd
T10	1.7 ± 0.03 cd	0.4 ± 0.02de	1.7 ± 0.07e	0.9 ± 0.02c	0.7 ± 0.01c	1.6 ± 0.04d	0.7 ± 0.02cdef	0.1 ± 0.01de	0.2 ± 0.00ef
T11	1.65 ± 0.02de	0.4 ± 0.02bcd	3.0 ± 0.1d	0.9 ± 0.02 cd	0.7 ± 0.01bc	2.8 ± 0.09c	0.7 ± 0.02bcde	0.15 ± 0.02cde	0.5 ± 0.03de
T12	1.8 ± 0.02c	0.5 ± 0.02ab	4.1 ± 0.09b	0.85 ± 0.02de	0.75 ± 0.01ab	2.95 ± 0.04c	0.7 ± 0.02bc	0.2 ± 0.02 cd	0.6 ± 0.04 cd
T13	2.0 ± 0.04b	0.4 ± 0.02 cd	2.4 ± 0.12c	1.3 ± 0.02a	0.6 ± 0.01d	2.5 ± 0.04a	0.8 ± 0.02ab	0.2 ± 0.02bc	0.5 ± 0.03c
T14	1.95 ± 0.04b	0.4 ± 0.02bcd	3.65 ± 0.08b	1 ± 0.01b	0.65 ± 0.01 cd	2.7 ± 0.06b	0.8 ± 0.03ab	0.3 ± 0.02b	0.9 ± 0.07b
T15	2.2 ± 0.03a	0.5 ± 0.02a	5.2 ± 0.23a	0.9 ± 0.02bc	0.7 ± 0.01 cd	2.8 ± 0.04b	0.8 ± 0.02a	0.3 ± 0.01a	1.3 ± 0.07a
T16	1.3 ± 0.01	0.1 ± 0.02	0.1 ± 0.02	0.7 ± 0.02	0.4 ± 0.01	0.2 ± 0.07	0.4 ± 0.01	0.1 ± 0.01	0.04 ± 0.02

Abbreviations: BCF, bioconcentration factor; RF, remediation factor; SD, standard deviation; TF, translocation factor.
Data are the means ± SD of 3 replications. Same letters are not significantly different at $P < .05$ ($n = 3$) between different treatments according to Tukey test. T1, cow manure (6%) + contaminated soil (96%); T2, cow manure (12%) + contaminated soil (88%); T3, cow manure (25%) + contaminated soil (75%); T4, biosolids (6%) + contaminated soil (96%); T5, biosolids (12%) + contaminated soil (88%); T6, biosolids (25%) + contaminated soil (75%); T7, cow manure (6%) + biosolids (6%) + contaminated soil (88%); T8, cow manure (6%) + biosolids (12%) + contaminated soil (82%); T9, cow manure (6%) + biosolids (25%) + contaminated soil (69%); T10, cow manure (12%) + biosolids (6%) + contaminated soil (82%); T11, cow manure (12%) + biosolids (12%) + contaminated soil (76%); T12, cow manure (12%) + biosolids (25%) + contaminated soil (63%); T13, cow manure (25%) + biosolids (6%) + contaminated soil (69%); T14, cow manure (25%) + biosolids (12%) + contaminated soil (63%); T15, cow manure (25%) + biosolids (25%) + contaminated soil (50%); T16, control.

Table 8. BCF, TF, and RF of heavy metals in ornamental sunflower in the mid-term and at the end of the test period in some treatments.

METALS	TREATMENTS	T4	T5	T7	T8	T9	T11	T12
Ni	BCFm	0.9 ± 0.03	0.9 ± 0.06	1.1 ± 0.04	1.2 ± 0.03	1.3 ± 0.00	1.35 ± 0.03	1.5 ± 0.02
	BCFe	0.6 ± 0.02	0.5 ± 0.02	0.65 ± 0.02	0.65 ± 0.02	0.7 ± 0.03	0.7 ± 0.02	0.7 ± 0.02
	TFm	0.3 ± 0.02	0.35 ± 0.05	0.3 ± 0.14	0.4 ± 0.17	0.4 ± 0.22	0.4 ± 0.08	0.5 ± 0.05
	TFe	0.1 ± 0.03	0.2 ± 0.02	0.15 ± 0.02	0.1 ± 0.01	0.2 ± 0.02	0.15 ± 0.02	0.2 ± 0.02
	RFm	0.05 ± 0.02	0.07 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.03	0.2 ± 0.03
	RFe	0.15 ± 0.03	0.3 ± 0.01	0.3 ± 0.03	0.3 ± 0.02	0.6 ± 0.03	0.5 ± 0.03	0.6 ± 0.04
Zn	BCFm	1.1 ± 0.02	1.2 ± 0.01	1.1 ± 0.3	1.2 ± 0.47	1.1 ± 0.25	1.2 ± 0.03	1.25 ± 0.2
	BCFe	0.65 ± 0.02	0.6 ± 0.02	0.8 ± 0.01	0.8 ± 0.02	0.8 ± 0.02	0.9 ± 0.02	0.85 ± 0.02
	TFm	0.4 ± 0.03	0.5 ± 0.06	0.55 ± 0.04	0.65 ± 0.03	0.8 ± 0.00	0.6 ± 0.02	0.7 ± 0.02
	TFe	0.35 ± 0.01	0.4 ± 0.02	0.8 ± 0.03	0.8 ± 0.01	0.8 ± 0.02	0.7 ± 0.01	0.75 ± 0.01
	RFm	0.4 ± 0.02	0.6 ± 0.03	0.6 ± 0.03	0.8 ± 0.02	1.0 ± 0.02	0.8 ± 0.01	1.0 ± 0.01
	RFe	0.6 ± 0.02	0.7 ± 0.02	2.1 ± 0.03	2.4 ± 0.06	2.7 ± 0.09	2.8 ± 0.09	2.95 ± 0.04
Pb	BCFm	1.2 ± 0.06	1.1 ± 0.03	1.0 ± 0.03	1.0 ± 0.05	1.0 ± 0.03	1.0 ± 0.04	1.2 ± 0.02
	BCFe	1.4 ± 0.03	1.5 ± 0.04	1.65 ± 0.03	1.6 ± 0.02	1.6 ± 0.02	1.65 ± 0.02	1.8 ± 0.02
	TFm	0.2 ± 0.03	0.2 ± 0.01	0.4 ± 0.02	0.4 ± 0.02	0.5 ± 0.01	0.5 ± 0.02	0.6 ± 0.02
	TFe	0.2 ± 0.03	0.2 ± 0.01	0.3 ± 0.02	0.4 ± 0.02	0.5 ± 0.02	0.4 ± 0.02	0.5 ± 0.02
	RFm	0.2 ± 0.01	0.3 ± 0.01	0.4 ± 0.02	0.4 ± 0.02	0.6 ± 0.02	0.6 ± 0.01	0.75 ± 0.02
	RFe	0.9 ± 0.07	1.0 ± 0.03	1.7 ± 0.06	2.4 ± 0.09	3.2 ± 0.07	3.0 ± 0.1	4.1 ± 0.09

Abbreviations: BCF, bioconcentration factor; RF, remediation factor; SD, standard deviation; TF, translocation factor.
Data are the means ± SD of 3 replications. The statistical analysis was Wilcoxon. Data in the mid-term and at the end of the test period were shown by m and e suffixes, respectively.

modifiers, especially at high ratios, increases the amount of BCF and metal uptake in the roots. The values of BCF > 1 and TF < 1 indicate the ability of the ornamental sunflower to absorb Pb in the roots. As metal uptake in plants depends on species of the plant, type, and concentration of the metal, and soil properties, the researchers have obtained different values of BCF and TF in their studies. In the study by Kötschau et al,[40] the BCF of Pb in the sunflower was estimated to be 0.013. In the same study by Hamvumba et al,[42] the BCF of 0.108 to 0.5 obtained. Çelebi et al studied the uptake of Pb in the ornamental sunflower. They estimated the BCF of the shoot and root and TF of this plant in the range of 0.3 to 0.37, 19.6 to 38.6, and 0.0078 to 0.019, respectively.[43] Jadia et al,[46] used vermicompost to increase metal accumulation in sunflowers. The BCF of Pb in their study was 0.1 to 0.14. Rahmanian et al,[44] used 3 modifiers of ethylenediaminetetraacetic acid (EDTA), acetic acid, and poultry manure to increase Pb availability. The BCF and TF values in their study ranged from 5.55 to 10.42 and 0.11 to 0.35, respectively. Also, comparing the results of adsorption in the mid-term and the end of the period, it can be concluded that no clear and uniform time trend for all 3 metals can be presented.

In the case of Ni and Zn, BCF > 1 and TF < 1 in the mid-term of the growth period showed the maximum Ni and Zn uptake by the roots at this time. In agreement with the present study, Zalaghi et al[45] obtained the same results on BCF and TF of Zn in the sunflower. Also, other researchers by utilizing vermicompost[46] and biosolids[47] achieve a similar result to the present study on BCF of Ni in the sunflower.

The amount of Zn uptake in different treatments did not differ significantly in the mid-term of the growth period. However, at the end of the period, with the simultaneous addition of 2 modifiers, especially in treatments 13, 14, and 15, the absorption of Zn increased. Moreover, the addition of modifiers, especially their simultaneous application in the mid-term and the end of the period, provides more Ni absorption. Although the transfer factor of all 3 metals was less than 1, however, based on the results, the amount of this transfer enhanced with the increasing proportion of modifiers. Our results are consistent with those obtained from Mukhtar et al,[48] which found that utilization of organic amendments can increase the translocation of Pb and Zn from the roots to the above-ground parts of the sunflower.

Conclusions

In this study, the application of the 2 amendments, biosolids and cow manure, in improving the heavy metal accumulation within the sunflower was investigated. Moreover, the plants' dry weight was added with increasing modifiers. The results show that in treatments with 2 modifiers, phytoremediation has increased in comparison with other treatments, and thus, these treatments have absorbed more heavy metals than other treatments. Therefore, adding biosolids and cow manure simultaneously improves the ability of the ornamental sunflower plant to accumulate heavy metals significantly.

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Author Contributions

AY- supervised this study.
SNA and SAV - project advisors.
FK- experiment and the analysis.
ZK- collect the samples.
All authors helped to develop the content of the manuscript including reviewing/ editing of the final manuscript.

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