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Phytoremediation of Pb and Cd by Alfalfa (*Medicago sativa* L.): an Applied Study in the Presence of Lettuce Plants (*Lactuca sativa* L.)

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Abstract: A pot study was conducted to investigate the potential of alfalfa for phytoremediation of soil artificially contaminated with different concentrations of Pb and Cd and their combinations. Harvested plants were divided and used for two purposes: (1) separation into roots and shoots, which were digested to determine N, P, K, Pb and Cd concentrations, and (2) the use of fresh shoots to prepare alfalfa extract representing each treatment. Subsequently, lettuce seedlings were grown in the same pots with the same soil and without adding fertilizers; the alfalfa shoot extract prepared from each treatment was added to the treatment itself. Lettuce crops harvested after 67 days from transplanting were divided into roots and shoots which were digested to determine the previously studied elements. In addition, soil samples were collected after harvesting alfalfa and lettuce plants and prepared for chemical analyses. Results showed that alfalfa is an effective accumulator plant for the phytoremediation of Pb- and Cd-contaminated soils. In addition, using the alfalfa shoot extract to fertilize lettuce crops was beneficial to their growth without any risk of translocation of heavy metals. Thus, we recommend adding alfalfa to crop rotations, especially where soils are contaminated with heavy metals.

1 Introduction

Soils can be contaminated with heavy metals from various sources, including irrigation with wastewater (Abd-Elrahman 2017), excessive use of mineral fertilizers and pesticides that contain heavy metal impurities (Atafar et al 2010, Alengebawy et al 2021), and due to the proximity of agricultural soils to roads, which leads to the deposition of vehicle exhaust pollution containing Pb and Cd onto surrounding soils (Nawrot et al 2020). Soils contaminated with heavy metals are considered a major problem due to their potential negative effects on the environment and human health; however, such problems can be at least partially resolved using phytoremediation technology (Karimi 2013). Techniques for phytoremediation include the following types, *i.e.*, phytofiltration (known as rhizofiltration involves root adsorption/ absorption), phytovolatilization (the plants that can convert the contaminants inside their tissues into gasses and release them into the atmosphere), phytodegradation (known as phytotransformation which means the breakdown of pollutants inside the plants through metabolic processes, or for those found surrounding the plants in the ground by releasing enzymes from roots), phytostabilization, and phytoextraction by hyperaccumulator plants (Gwozdz 2003, Khan et al 2008) of these techniques, phytostabilization involves the use of plants to remove heavy metals from soils and polluted areas; it has become an important practical approach to dealing with soil pollution. Importantly, this form of phytoremediation is simple, efficient, cost-effective, and environmentally friendly (Yan et al 2020). However, an appropriate method for disposing of or benefiting from the plants used in phytostabilization is required; otherwise, they will become a burden on the environment and exacerbate the existing problem.

Alfalfa (*Medicago sativa* L.) is extremely tolerant to contaminants such as heavy metals and is considered a hyperaccumulator. Wang et al (2015) studied the effect of alfalfa that grew over different periods (10, 40, and 80 days) in different soils contaminated by Zn, Pb and Cd; they found that alfalfa could be applied for phytoremediation of moderately contaminated soil. Alfalfa was able to transfer heavy metals from roots to shoots during the seedling period, with the roots and shoots showing similar accumulation abilities, but relatively higher concentrations of the studied elements being found in the roots. The bioconcentration factor of alfalfa in relation to these elements was higher during the maturation period. In addition, the translocation factor (TF) of alfalfa was low and tended to decrease with increasing concentrations of Zn, Pb, and Cd in the studied soils. Karimi (2013) comparatively studied alfalfa and sorghum for their phytoremediation abilities in soils contaminated with different concentrations of Cr; they found that Cr concentration in the soil was reduced by 60%–74% and 51.2%–69.5% with phytoremediation by alfalfa and sorghum, respectively.

Lettuce (*Lactuca sativa* L.) is an economically important leafy crop with a short cultivation cycle. Lettuce plants accumulate heavy metals in their edible parts when such pollutants are present in the surrounding soil (Mostafa et al 2012, Abd El-Fattah et al 2022). Eissa and Negim (2018) reported that the concentrations of Zn, Ni, Pb, and Cd in the edible portions of studied lettuce plants were higher than the permissible limit levels due to irrigation of soil with wastewater.

This study aimed to determine (1) the effects of cultivating alfalfa in soil contaminated with Pb and Cd at various concentrations (either individually or in combination) and (2) the impact of using the subsequently produced alfalfa shoot extract to fertilize lettuce grown in the same soil after the alfalfa had been harvested. In addition, the effects on the availability of N, P, K, Pb, and Cd in the studied soil were assessed, as well as the nutrient concentrations in the roots and shoots of alfalfa and lettuce. Furthermore, the bioaccumulation factor (BAF), translocation factor (TF), daily metal intake (DMI), and risk index (RI) were calculated.

2 Materials and Methods

The current study involves two trials:

2.1 Cultivation of alfalfa

A pot experiment was conducted in the autumn season of the year 2020 under greenhouse conditions at the Faculty of Agriculture, Ain Shams University, Qalubia Governorate, Egypt, with the aim of determining the effects of using alfalfa as a plant remedy in soils contaminated with Pb and Cd on the pH, electrical conductivity (EC), and chemically available concentrations of N, P, K, Pb, and Cd in the soil. The total concentration of the studied elements in the roots and shoots of alfalfa after harvesting was also determined. At the end of the experiment, alfalfa fresh shoot extract was prepared and kept in the refrigerator until it was used in a later experiment.

Soil samples were taken at depths of 0–15 cm from agricultural soils located on both sides of the highway in Toukh Center (30° 22' 10.83" N, 31° 11' 39.01" E), Qalyubia Governorate, Egypt. After the samples were analyzed for diethylenetriamine-pentaacetic acid (DTPA)-extractable amounts of Pb and Cd, the most polluted sample was selected. After being air-dried and crushed to pass through a 2-mm-mesh sieve, the studied soil was packed uniformly into polythene-lined pots (5 kg pot^{-1}). Before plant cultivation, the basic physical and chemical properties of the studied soil were determined according to standard methods described by Page et al (1982) and Klute (1986); the results are presented in **Table 1**.

The following concentrations of Pb, *i.e.*, 1, 5, 10, and 15 mg L^{-1} in the form of Pb SO_4 (Mw= 303.265) and Cd, *i.e.*, 1, 2, 4, and 8 mg L^{-1} in the form of Cd $SO_4.8/3H_2O$ (Mw= 769.52) and their combinations were prepared and added to the soil using a completely randomized design with three replicates:

1. $Cd_0 \times Pb_0$ (control)
2. Cd_1
3. Cd_2
4. Cd_4
5. Cd_8
6. Pb_1
7. Pb_5
8. Pb_{10}
9. Pb_{15}
10. $Cd_1 \times Pb_1$
11. $Cd_1 \times Pb_5$
12. $Cd_1 \times Pb_{10}$
13. $Cd_1 \times Pb_{15}$
14. $Cd_2 \times Pb_1$

15. $Cd_2 \times Pb_5$
16. $Cd_2 \times Pb_{10}$
17. $Cd_2 \times Pb_{15}$
18. $Cd_4 \times Pb_1$
19. $Cd_4 \times Pb_5$
20. $Cd_4 \times Pb_{10}$
21. $Cd_4 \times Pb_{15}$
22. $Cd_8 \times Pb_1$
23. $Cd_8 \times Pb_5$
24. $Cd_8 \times Pb_{10}$
25. $Cd_8 \times Pb_{15}$

Table 1. Basic physical and chemical properties of the studied soil, before cultivation, (0−15 cm soil layer)

Property	Value
Particle size distribution, %	
Sand	27.3
Silt	39.4
Clay	33.3
Textural class	Silty clay
$CaCO_3$, g kg^{-1}	21.1
OM, g kg^{-1}	8.13
pH (1:2 soil: water suspension)	7.50
EC_e, dS m^{-1}	0.97
Available elements, µg g^{-1}	
N	61.4
P	6.60
K	213
Zn	1.37
Cu	0.71
Pb	0.57
Ni	0.52
Cd	0.64

The prepared solutions of Pb and Cd were added to the studied soil in pots at a rate of 500 mL pot^{-1} in two doses, with a 2-h gap between doses to confirm saturation of the soil with the full (combined) dose. At 4 h after adding the Pb and Cd solutions to the soil, alfalfa seeds obtained from the Legumes Crops Research Center, Ministry of Agriculture, Giza Governorate, Egypt, were planted. These seeds were cultivated on October 1, 2020, and the plants were harvested on December 5, 2020 (64 days after sowing). The harvested plants were divided into two groups: one group was separated into roots and shoots, which were digested to determine their content of N, P, K, Pb, and Cd; the fresh shoots of the second group were used to produce an extract from each treatment. To produce the extract, 10 g of fresh alfalfa shoots were used per liter of distilled water; these were mixed well with 2 g of EDTA in a blender, and the mixture was stored at 2°C in a refrigerator until it was used. Some of the chemical compositions of the alfalfa fresh shoot extract for each treatment are shown in **Table 2**.

Soil samples were collected after the alfalfa harvest; they were air-dried, crushed, and sieved through a 2-mm-mesh sieve, and the pH, EC of the soil extract (EC_e), and chemically available concentrations of N, P, K, Pb, and Cd were determined.

Table 2. Concentration of N, P, K, Pb, and Cd in the alfalfa fresh shoot extract of each treatment

Treatment	N	P	K	Pb	Cd
	mg L^{-1}				
$Cd_0 \times Pb_0$ (control)	0.70	4.40	21.2	0.024	0.026
Cd_1	0.84	4.42	27.7	0.065	0.252
Cd_2	0.56	4.39	31.7	0.067	0.256
Cd_4	0.28	4.33	26.4	0.068	0.257
Cd_8	0.98	4.45	31.6	0.069	0.259
Pb_1	0.84	4.45	28.6	0.230	0.081
Pb_5	0.42	4.56	29.3	0.235	0.084
Pb_{10}	0.56	4.53	27.3	0.237	0.087
Pb_{15}	0.28	4.52	24.6	0.238	0.089
$Cd_1 \times Pb_1$	0.42	4.47	22.8	0.234	0.252
$Cd_1 \times Pb_5$	0.70	4.57	26.9	0.234	0.255
$Cd_1 \times Pb_{10}$	0.84	4.47	26.2	0.236	0.257
$Cd_1 \times Pb_{15}$	0.84	4.50	26.9	0.239	0.259
$Cd_2 \times Pb_1$	0.70	4.59	23.9	0.234	0.253
$Cd_2 \times Pb_5$	0.42	4.64	23.0	0.237	0.257
$Cd_2 \times Pb_{10}$	0.98	4.64	25.4	0.239	0.258
$Cd_2 \times Pb_{15}$	0.70	4.55	23.9	0.242	0.261
$Cd_4 \times Pb_1$	0.84	4.64	24.3	0.239	0.254
$Cd_4 \times Pb_5$	0.56	4.62	27.3	0.242	0.263
$Cd_4 \times Pb_{10}$	0.42	4.71	22.6	0.251	0.272
$Cd_4 \times Pb_{15}$	0.56	4.67	20.6	0.246	0.268
$Cd_8 \times Pb_1$	0.56	4.61	24.7	0.242	0.260
$Cd_8 \times Pb_5$	0.42	4.61	27.1	0.242	0.265
$Cd_8 \times Pb_{10}$	0.42	4.63	23.7	0.257	0.274
$Cd_8 \times Pb_{15}$	0.28	4.75	21.8	0.254	0.271

2.2 Cultivation of lettuce

On December 15, 2020, iceberg lettuce seedlings were cultivated in the same pots used to cultivate alfalfa. At 2 weeks after cultivation, the first dose of alfalfa shoot extract prepared from each treatment was added to the same treatment at a rate of 250 mL pot^{-1}. A second dose at the same rate was added at 1 month after cultivation. Mineral or organic fertilizers were not applied. The lettuce plants were harvested on February 20, 2021 (67 days after transplanting); they were divided into roots and shoots, oven-dried at 65°C, and digested using a H_2O_2/H_2SO_4 mixture according to the procedure described by Chapman and Pratt (1961), after which the content of N, P, K, Pb, and Cd was determined.

Soil samples were collected after lettuce harvest, air-dried, crushed, and sieved through a 2-mm-mesh sieve, after which pH, EC_e, and the chemically available concentrations of N, P, K, Pb, and Cd were determined.

2.3 Plant and soil analyses

Total N in alfalfa and lettuce plants was determined using a micro-Kjeldahl method with 40% NaOH and 5% boric acid as outlined by Chapman and Pratt (1961). Total P content was determined using a Spectrophotometer and the ascorbic acid method described by Watanabe and Olsen (1965). Total K content was determined using a Flame photometer and a method outlined by Chapman and Pratt (1961). Total Pb and Cd were determined using inductively coupled plasma mass spectrometry (ICP-MS) as outlined by Jones (2001).

Soil was prepared in a 1:2 soil/water suspension to determine pH using a pH meter (Jackson 1973). The EC_e of salts in the soil extract was determined using an EC meter according to the method described by Page et al (1982). The chemically available concentration of N was extracted by 1-N KCl at pH 7 and determined using a micro-Kjeldahl method (Chapman and Pratt 1961). The chemically available concentration of P was extracted by 0.05-M $NaHCO_3$ at pH 8.5 and determined using a Spectrophotometer as described by Watanabe and Olsen (1965). The chemically available concentration of K was extracted by 1-N ammonium acetate at pH 7 and determined using a Flame photometer (Chapman and Pratt 1961). The chemically available concentrations of Pb and Cd were determined using ICP-MS with DTPA extract at pH 7.3 (Jones 2001).

The following equations were used, as outlined by Eissa and Negim (2018) to calculate the bioaccumulation factor (BAF) and translocation factor (TF) for alfalfa and lettuce plants:

$$\text{BAF} = \frac{\text{Metal concentartion in the root } (\mu g\ g^{-1})}{\text{Available metal concentration in the soil } (\mu g\ g^{-1})}$$

$$\text{TF} = \frac{\text{Metal concentartion in the shoot } (\mu g\ g^{-1})}{\text{Metal concentration in the root } (\mu g\ g^{-1})}$$

The following equations were used (as outlined by Khan et al 2008) to calculate daily metal intake (DMI) and risk index (RI) for lettuce plants as a leafy crop used widely as food:

$$\text{DMI} = \frac{\text{CM} \times \text{CF} \times \text{DFI}}{BAW}$$

Where: CM is the concentration of target metal in the vegetable ($\mu g\ g^{-1}$), CF is the conversion factor (0.085), DFI is the daily food intake (kg person^{-1} day^{-1}) of the vegetable (0.345 for adults; 0.232 for children; Rattan et al 2005), and BAW is body average weight (55.9 kg for adults; 32.7 kg for children; Wang et al 2005).

$$\text{RI} = \frac{DMI}{ROD}$$

Where: DMI is the daily metal intake calculated using the previous equation and ROD is the reference oral dose for the target metal ($\mu g\ g^{-1}$ day^{-1}), which is 0.0035 for Pb (Cui et al 2004) and 0.001 for Cd (US EPA 2020). The safety standard for humans is RI < 1.

2.4 Statistical analysis

SAS (version 9.1.3) was used to conduct statistical analysis of the data. Differences among means for all traits were tested for significance using a least significant difference (LSD) test, with significance set at $p \leq 0.05$ (SAS 2006).

3 Results and Discussion

3.1 Soil chemical characteristics after harvesting alfalfa and lettuce plants

Data in **Table 3** show the effects of the studied treatments on pH, EC_e, and chemically available NPK concentrations in the investigated soil after alfalfa and lettuce harvesting. Compared with the control treatment, soil pH decreased for both alfalfa and lettuce due to the applied treatments, with higher reductions caused by higher concentrations of Pb and Cd and by the interaction between the two elements rather than individual element applications. This may have been due to the effect of co-ions because Pb and Cd were added to the soil in the form of sulfate salts that form sulfuric acid in the presence of irrigation water (Andrade et al 2018). The EC_e increased as the applied concentrations of Pb and Cd salts in the studied soil increased **(Table 3)**, which is logical and consistent with previous findings (Abd-Elrahman et al 2012).

Table 3. pH, EC_e, and chemically available concentrations of N, P and K in the investigated soil after harvesting alfalfa and lettuce plants as affected by the studied treatments

Treatment	pH (1:2)	EC_e, dS m^{-1}	N	P	K	pH (1:2)	EC_e, dS m^{-1}	N	P	K
			µg g^{-1}					µg g^{-1}		
	Soil of alfalfa					Soil of lettuce				
$Cd_0 \times Pb_0$ (control)	8.35	0.46	59.5	5.77	543	8.09	0.56	24.5	5.47	326
Cd_1	8.27	0.50	56.0	5.73	362	7.89	0.58	24.5	6.13	509
Cd_2	8.32	0.41	42.0	5.80	360	7.93	0.34	38.5	5.27	394
Cd_4	8.15	0.67	38.5	5.60	437	7.84	0.49	21.0	5.30	190
Cd_8	8.17	0.61	45.5	5.63	547	7.94	0.55	38.7	6.17	440
Pb_1	8.20	0.60	73.5	5.57	126	7.99	0.47	56.0	5.33	79.0
Pb_5	8.27	0.32	45.0	5.77	90.0	8.01	0.68	17.5	6.13	394
Pb_{10}	8.11	0.78	49.0	5.70	130	8.06	0.48	24.5	5.43	441
Pb_{15}	7.59	0.76	45.5	5.00	543	8.11	0.47	38.5	5.17	472
$Cd_1 \times Pb_1$	7.99	0.43	35.0	5.73	325	8.06	0.67	17.5	5.43	331
$Cd_1 \times Pb_5$	7.92	0.35	31.5	5.70	393	7.79	0.54	31.5	5.60	373
$Cd_1 \times Pb_{10}$	7.93	0.39	38.5	5.63	492	7.93	0.45	38.5	5.60	181
$Cd_1 \times Pb_{15}$	7.78	0.83	66.5	5.67	290	8.01	0.57	24.5	5.47	387
$Cd_2 \times Pb_1$	7.91	0.68	112	5.83	415	8.20	0.49	59.5	5.73	367
$Cd_2 \times Pb_5$	8.15	0.35	73.5	5.87	480	8.12	0.53	24.5	5.60	368
$Cd_2 \times Pb_{10}$	8.23	0.36	52.5	5.80	537	8.14	0.66	45.5	5.47	123
$Cd_2 \times Pb_{15}$	7.15	0.58	31.0	5.73	468	8.04	0.73	31.5	5.57	327
$Cd_4 \times Pb_1$	7.69	0.35	45.5	5.73	125	7.97	0.83	24.5	5.70	175
$Cd_4 \times Pb_5$	7.94	0.74	38.0	5.73	284	7.94	0.53	52.5	5.43	232
$Cd_4 \times Pb_{10}$	8.06	0.71	59.5	5.87	101	8.09	0.63	66.5	6.30	455
$Cd_4 \times Pb_{15}$	7.96	0.73	31.5	5.77	437	8.07	0.73	52.5	5.50	491
$Cd_8 \times Pb_1$	8.01	0.49	34.5	5.93	501	8.06	0.55	66.5	5.77	492
$Cd_8 \times Pb_5$	7.92	0.59	59.0	5.83	293	8.22	0.66	42.0	5.60	368
$Cd_8 \times Pb_{10}$	7.87	0.79	73.5	5.73	261	8.14	0.59	24.5	5.67	510
$Cd_8 \times Pb_{15}$	7.87	0.86	66.5	5.63	78.0	8.20	0.58	31.5	5.83	82.0
$LSD_{0.05}$	**0.042**	**0.017**	**0.964**	**0.068**	**2.136**	**0.011**	**0.013**	**0.132**	**0.062**	**2.034**

Nitrogen concentration decreased as the applied concentrations of Pb and Cd increased in the soil **(Table 3)**. However, the opposite trend was observed when treatments were applied in combination, and this was more pronounced after harvesting alfalfa than after harvesting lettuce plants. Lin et al (2020) reported the opposite relationship between N and Cd with higher rates of N application in soil. In this study, P concentration decreased as the applied concentrations of Pb and Cd in the soil increased **(Table 3)**. The increased concentration of P in the soil after harvesting lettuce plants, despite no fertilizers being added, may have been due to the application of alfalfa fresh shoot extract, which contained appreciable amounts of NPK **(Table 2)**. Lamhamdi et al (2013) reported the opposite relationship between P and Pb. The current data for K concentration showed that it increased as the Pb and Cd concentrations in the soil increased; however, in most cases, K levels decreased when Pb and Cd were applied in combination. The $Cd_8 \times Pb_{15}$ treatment led to lower concentrations of available K (78.0 and 82.0 µg g^{-1}) in the soils of alfalfa and lettuce, respectively, when compared with K concentrations in other treatments. Chen et al (2007) studied the interaction between Cd, Pb, and K fertilizer in the form of K_2SO_4 in a soil system with wheat plants, and they found an opposite relationship among them. They noted that all application levels of K in soil reduced the phytoavailability of Cd and Pb.

3.2 Concentrations of N, P, and K in the roots and shoots of alfalfa and lettuce

Data in **Table 4** show the effects of the studied treatments on NPK concentration and translocation from the roots to the shoots of alfalfa and lettuce plants. The studied treatments affected the nutrient translocation from the roots to the shoots, with higher concentrations found in the shoots. N concentration increased in the plant parts with the application of Cd

Table 4. Concentration of N, P, and K in the roots and shoots of alfalfa and lettuce plants after harvesting, as affected by the studied treatments

Treatment	**Plant part**	**N**	**P**	**K**	**N**	**P**	**K**
		(%)			**(%)**		
		Alfalfa			**Lettuce**		
$Cd_0 \times Pb_0$ (control)	Root	1.44	0.46	1.12	0.92	0.49	0.74
	Shoot	1.50	0.46	1.45	3.44	0.48	0.91
Cd_1	Root	1.43	0.47	0.70	0.87	0.48	0.47
	Shoot	6.65	0.46	2.27	2.21	0.48	1.60
Cd_2	Root	1.13	0.47	0.73	0.31	0.47	0.94
	Shoot	6.47	0.46	3.38	0.92	0.47	1.22
Cd_4	Root	0.27	0.48	1.66	0.14	0.47	0.67
	Shoot	6.68	0.47	2.12	0.64	0.46	2.23
Cd_8	Root	0.06	0.48	0.98	0.18	0.47	0.63
	Shoot	6.73	0.46	1.16	1.12	0.47	1.47
Pb_1	Root	1.92	0.49	1.30	0.87	0.47	1.01
	Shoot	7.08	0.47	1.91	1.93	0.48	1.98
Pb_5	Root	2.29	0.47	1.34	0.90	0.47	0.64
	Shoot	6.05	0.48	1.87	2.04	0.48	1.67
Pb_{10}	Root	0.20	0.48	0.80	1.15	0.49	1.10
	Shoot	7.57	0.46	1.03	1.20	0.48	1.64
Pb_{15}	Root	1.86	0.47	1.09	0.98	0.47	0.81
	Shoot	7.07	0.47	1.49	1.71	0.47	1.33
$Cd_1 \times Pb_1$	Root	1.79	0.48	0.53	0.98	0.46	0.52
	Shoot	6.47	0.46	1.47	1.15	0.47	2.20
$Cd_1 \times Pb_5$	Root	1.30	0.48	1.42	0.73	0.49	0.67
	Shoot	4.66	0.47	2.14	2.58	0.48	1.16
$Cd_1 \times Pb_{10}$	Root	1.48	0.48	1.10	1.23	0.46	0.71
	Shoot	5.98	0.47	2.45	1.82	0.47	1.74
$Cd_1 \times Pb_{15}$	Root	2.17	0.47	0.70	0.98	0.46	0.51
	Shoot	4.24	0.47	1.85	1.43	0.48	1.19
$Cd_2 \times Pb_1$	Root	1.85	0.47	0.63	1.48	0.47	0.72
	Shoot	5.54	0.48	1.93	1.76	0.48	1.56
$Cd_2 \times Pb_5$	Root	1.01	0.49	0.86	0.70	0.48	0.40
	Shoot	3.39	0.47	1.99	2.77	0.48	0.93
$Cd_2 \times Pb_{10}$	Root	0.91	0.47	0.63	0.70	0.48	0.36
	Shoot	2.51	0.48	1.93	1.54	0.47	0.62
$Cd_2 \times Pb_{15}$	Root	0.71	0.47	0.88	0.84	0.47	0.30
	Shoot	3.12	0.46	1.32	1.32	0.47	1.17
$Cd_4 \times Pb_1$	Root	0.66	0.47	1.13	0.98	0.46	0.49
	Shoot	2.69	0.47	1.65	1.23	0.47	1.21
$Cd_4 \times Pb_5$	Root	0.63	0.48	0.62	0.84	0.47	0.35
	Shoot	4.82	0.46	1.69	1.43	0.47	1.41
$Cd_4 \times Pb_{10}$	Root	0.67	0.46	1.16	1.43	0.46	0.30
	Shoot	3.96	0.48	1.55	1.99	0.48	1.31
$Cd_4 \times Pb_{15}$	Root	1.57	0.47	0.63	0.70	0.48	0.44
	Shoot	1.65	0.48	2.77	2.02	0.47	0.72
$Cd_8 \times Pb_1$	Root	0.97	0.47	0.92	0.53	0.48	0.30
	Shoot	2.77	0.47	2.79	1.88	0.48	0.72
$Cd_8 \times Pb_5$	Root	1.19	0.48	0.99	1.48	0.48	0.60
	Shoot	2.13	0.47	1.87	1.82	0.48	0.78
$Cd_8 \times Pb_{10}$	Root	0.45	0.46	0.93	0.67	0.48	0.15
	Shoot	3.54	0.47	2.52	1.85	0.48	0.48
$Cd_8 \times Pb_{15}$	Root	0.90	0.47	0.65	1.20	0.48	0.53
	Shoot	3.00	0.47	1.01	1.60	0.47	0.87
$LSD_{0.05}$	**Root**	**0.063**	**0.001**	**0.036**	**0.053**	**0.001**	**0.016**
	Shoot	**0.087**	**0.042**	**0.115**	**0.022**	**0.001**	**0.082**

to the soil; specifically, higher N levels were detected following applications of lower concentrations of Cd. Lin et al (2020) studied the effects of applying N from both mineral and organic fertilizers on the uptake of Cd by a hyperaccumulator plant (*Sedum alfredii* Hance); they found that Cd accumulation in the shoots decreased with higher N fertilization rates and the effects were more pronounced with mineral fertilizer than with organic fertilizer. However, the appropriate concentration of N applied to the soil enhanced the uptake of Cd and its translocation from roots to shoots. In the present work, N concentration decreased within the studied plants as the applied Pb concentrations increased in the soil. Furthermore, N concentration and translocation inside alfalfa and lettuce plants decreased as the applied concentrations of Cd × Pb increased.

In terms of P concentration and translocation from the roots to the shoots of alfalfa and lettuce plants **(Table 4)**, there were no significant differences among the studied treatments. Lamhamdi et al (2013) studied the effects of applying three levels of Pb in the form of $Pb(NO_3)_2$, *i.e.*, 1.5, 3.0, and 15.0 mM on the nutrient uptake and metabolism of two plant species (wheat and spinach), which were grown under hydroponic conditions. They showed that Pb accumulation increased as the application rate increased, which in turn reduced plant growth and the uptake of all studied nutrients, including P, in both species. The equilibrium content among nutrients is one of the most important factors in nutrient uptake, translocation, and consequently crop productivity (Younis et al 2021).

In terms of K translocation inside alfalfa and lettuce plants **(Table 4)**, Cd application increased K concentration and translocation, with concentration increasing with lower concentrations of Cd. A similar trend was detected with the application of Pb concentrations in the studied soils. However, the effect of Cd was more pronounced than that of Pb on the translocation of K within the studied plants. In addition, K concentration decreased with an increase in the applied concentrations of Pb × Cd; for example, a lower concentration of K was detected following the application of the $Cd_8 \times Pb_{15}$ treatment. These results are consistent with those reported by Chen et al (2007).

3.3 BAF and TF of Pb and Cd for alfalfa and lettuce

Data in **Table 5** show the concentrations of Pb and Cd in the studied soil and alfalfa and lettuce plants with the calculated BAF and TF of the elements in each plant type according to the studied treatments. Pb and Cd concentrations increased in the soil as their ground applications increased; the highest concentrations of Pb (0.59 and 0.58 µg g^{-1}) and Cd (0.62 and 0.60 µg g^{-1}) were recorded following the application of $Cd_8 \times Pb_{15}$ in the soil of alfalfa and lettuce, respectively. In the edible portions of alfalfa and lettuce, the concentrations of Pb were 0.18 and 0.14 µg g^{-1} in the control treatment and 0.22 and 0.41 µg g^{-1} following application of the $Cd_8 \times Pb_{15}$ treatment. The concentrations of Cd were 0.17 and 0.13 µg g^{-1} in the control treatment and 0.22 and 0.39 µg g^{-1} with application of the $Cd_8 \times Pb_{15}$ treatment in alfalfa and lettuce, respectively. Alfalfa played a role in reducing Pb and Cd concentrations in the soil and lettuce plants, especially in the control treatment without continuous additions of the studied elements. Karimi (2013) and Wang et al (2015) also reported the effective role of alfalfa as a hyperaccumulator for heavy metals, such as Zn, Pb, Cr, and Cd.

In terms of the BAF of Pb and Cd in the alfalfa and lettuce plants according to treatment, the control treatment had the lowest BAF value compared with the other treatments, and BAF increased as the applied concentrations of Pb and Cd in the soil increased. The TF of the studied elements varied among treatments, e.g., it ranged from 0.38 for Cd in the shoots of alfalfa with the $Cd_8 \times Pb_5$ treatment to 0.92 for Pb and Cd in the shoots of lettuce with the $Cd_4 \times Pb_5$ treatment and for Pb with the $Cd_4 \times Pb_{10}$ treatment in the edible portions of lettuce **(Table 5)**. All treatments produced low TF values (<1) for all studied elements, which indicated ineffective element transfer. These results agreed with those obtained by Abu-Elela et al (2021), who calculated the TF of some heavy metals in vegetables irrigated with wastewater over long periods. Plants tend to accumulate heavy metals in the roots and transfer limited concentrations to the shoots (Abd-Elrahman 2017, Eissa and Negim 2018).

Table 5. Bioaccumulation factor (BAF) and translocation factor (TF) of Pb and Cd for alfalfa and lettuce plants as affected by the studied treatments

Treatment	Sample type	Pb, μg g^{-1}	BAF	TF	Cd, μg g^{-1}	BAF	TF	Pb, μg g^{-1}	BAF	TF	Cd, μg g^{-1}	BAF	TF
		Alfalfa						Lettuce					
$Cd_0 \times Pb_0$ (control)	Soil	0.48	0.41		0.53	0.36		0.40	0.43		0.49	0.45	
	Root	0.20		0.89	0.19		0.90	0.17		0.83	0.22		0.57
	Shoot	0.18			0.17			0.14			0.13		
Cd_1	Soil	0.51	0.43		0.58	0.60		0.43	0.56		0.54	0.59	
	Root	0.22		0.86	0.35		0.74	0.24		0.71	0.32		0.81
	Shoot	0.19			0.26			0.17			0.26		
Cd_2	Soil	0.52	0.48		0.58	0.66		0.43	0.60		0.55	0.67	
	Root	0.25		0.84	0.38		0.70	0.26		0.73	0.37		0.71
	Shoot	0.21			0.27			0.19			0.26		
Cd_4	Soil	0.52	0.50		0.60	0.70		0.44	0.59		0.55	0.70	
	Root	0.26		0.85	0.42		0.69	0.26		0.77	0.38		0.83
	Shoot	0.22			0.29			0.20			0.32		
Cd_8	Soil	0.49	0.59		0.60	0.75		0.43	0.63		0.57	0.70	
	Root	0.29		0.82	0.45		0.63	0.27		0.70	0.40		0.85
	Shoot	0.24			0.29			0.19			0.34		
Pb_1	Soil	0.50	0.76		0.51	0.43		0.49	0.71		0.51	0.45	
	Root	0.38		0.63	0.22		0.86	0.35		0.60	0.23		0.65
	Shoot	0.24			0.19			0.21			0.15		
Pb_5	Soil	0.52	0.86		0.54	0.53		0.51	0.75		0.50	0.48	
	Root	0.45		0.48	0.29		0.57	0.38		0.55	0.24		0.63
	Shoot	0.22			0.17			0.21			0.15		
Pb_{10}	Soil	0.56	0.87		0.58	0.54		0.55	0.74		0.52	0.52	
	Root	0.49		0.44	0.31		0.84	0.41		0.54	0.27		0.70
	Shoot	0.21			0.26			0.22			0.19		
Pb_{15}	Soil	0.56	0.81		0.54	0.63		0.56	0.79		0.53	0.51	
	Root	0.45		0.43	0.34		0.68	0.44		0.48	0.27		0.78
	Shoot	0.19			0.23			0.21			0.21		
$Cd_1 \times Pb_1$	Soil	0.51	0.96		0.53	0.96		0.50	0.95		0.51	0.77	
	Root	0.49		0.44	0.51		0.52	0.47		0.49	0.39		0.68
	Shoot	0.22			0.26			0.23			0.27		
$Cd_1 \times Pb_5$	Soil	0.57	0.85		0.59	0.86		0.55	0.89		0.57	0.68	
	Root	0.49		0.47	0.51		0.51	0.49		0.43	0.39		0.75
	Shoot	0.23			0.26			0.21			0.29		
$Cd_1 \times Pb_{10}$	Soil	0.58	0.89		0.60	0.89		0.55	0.78		0.56	0.68	
	Root	0.51		0.47	0.54		0.51	0.43		0.72	0.38		0.65
	Shoot	0.24			0.27			0.31			0.25		
$Cd_1 \times Pb_{15}$	Soil	0.55	0.91		0.57	0.91		0.54	0.83		0.56	0.74	
	Root	0.50		0.46	0.52		0.50	0.45		0.76	0.41		0.86
	Shoot	0.23			0.26			0.34			0.36		
$Cd_2 \times Pb_1$	Soil	0.59	0.71		0.61	0.70		0.55	0.73		0.56	0.75	
	Root	0.42		0.56	0.43		0.67	0.40		0.85	0.42		0.88
	Shoot	0.23			0.29			0.34			0.37		
$Cd_2 \times Pb_5$	Soil	0.56	0.70		0.58	0.83		0.56	0.64		0.56	0.69	
	Root	0.39		0.54	0.48		0.53	0.36		0.86	0.39		0.90
	Shoot	0.21			0.25			0.31			0.35		
$LSD_{0.05}$	**Soil**	**0.007**			**0.001**			**0.002**			**0.002**		
	Root	**0.009**			**0.007**			**0.004**			**0.004**		
	Shoot	**0.002**			**0.004**			**0.007**			**0.004**		

Table 5. Continued

Treatment	Sample type	Pb, µg g^{-1}	BAF	TF	Cd, µg g^{-1}	BAF	TF	Pb, µg g^{-1}	BAF	TF	Cd, µg g^{-1}	BAF	TF
		Alfalfa						Lettuce					
$Cd_2 \times Pb_{10}$	Soil	0.55	0.90		0.58	0.89		0.56	0.81		0.58	0.65	
	Root	0.50		0.46	0.52		0.54	0.45		0.88	0.38		0.90
	Shoot	0.23			0.28			0.40			0.34		
$Cd_2 \times Pb_{15}$	Soil	0.57	0.61		0.59	0.63		0.55	0.82		0.58	0.71	
	Root	0.35		0.58	0.37		0.54	0.45		0.91	0.41		0.81
	Shoot	0.21			0.20			0.41			0.33		
$Cd_4 \times Pb_1$	Soil	0.56	0.64		0.59	0.64		0.54	0.81		0.58	0.70	
	Root	0.36		0.60	0.38		0.53	0.43		0.90	0.41		0.86
	Shoot	0.21			0.20			0.39			0.35		
$Cd_4 \times Pb_5$	Soil	0.56	0.65		0.58	0.63		0.54	0.87		0.57	0.72	
	Root	0.36		0.57	0.36		0.56	0.47		0.92	0.41		0.92
	Shoot	0.21			0.20			0.44			0.38		
$Cd_4 \times Pb_{10}$	Soil	0.55	0.66		0.56	0.64		0.53	0.76		0.56	0.67	
	Root	0.36		0.69	0.36		0.72	0.40		0.92	0.37		0.81
	Shoot	0.25			0.26			0.37			0.30		
$Cd_4 \times Pb_{15}$	Soil	0.57	0.58		0.59	0.60		0.56	0.85		0.57	0.66	
	Root	0.33		0.63	0.35		0.57	0.47		0.84	0.38		0.85
	Shoot	0.21			0.20			0.40			0.32		
$Cd_8 \times Pb_1$	Soil	0.58	0.64		0.59	0.66		0.57	0.82		0.59	0.63	
	Root	0.37		0.63	0.39		0.54	0.47		0.75	0.37		0.90
	Shoot	0.23			0.21			0.35			0.33		
$Cd_8 \times Pb_5$	Soil	0.57	0.93		0.60	0.92		0.57	0.84		0.58	0.81	
	Root	0.53		0.44	0.55		0.38	0.48		0.83	0.47		0.80
	Shoot	0.24			0.21			0.40			0.38		
$Cd_8 \times Pb_{10}$	Soil	0.58	0.58		0.60	0.59		0.56	0.86		0.58	0.80	
	Root	0.34		0.63	0.36		0.57	0.48		0.85	0.46		0.84
	Shoot	0.21			0.20			0.41			0.39		
$Cd_8 \times Pb_{15}$	Soil	0.59	0.57		0.62	0.58		0.58	0.85		0.60	0.76	
	Root	0.34		0.65	0.36		0.61	0.49		0.84	0.45		0.85
	Shoot	0.22			0.22			0.41			0.39		
$LSD_{0.05}$	**Soil**	**0.007**			**0.001**			**0.002**			**0.002**		
	Root	**0.009**			**0.007**			**0.004**			**0.004**		
	Shoot	**0.002**			**0.004**			**0.007**			**0.004**		

3.4 Health risk assessment of Pb and Cd in lettuce plants

Data in **Table 6** show the health risk assessment of Pb and Cd in lettuce used as food for adults and children following the application of the studied treatments. The average Pb concentration in lettuce parts (roots and shoots) ranged from 0.16 µg g^{-1} in the control treatment to 0.45 µg g^{-1} with the $Cd_4 \times Pb_5$, $Cd_8 \times Pb_{10}$, and $Cd_8 \times Pb_{15}$ treatments. The average Cd concentration in lettuce parts ranged from 0.17 µg g^{-1} in the control treatment to 0.43 µg g^{-1} with the $Cd_8 \times Pb_{10}$ treatment. The adequate limits of Pb and Cd within plant tissues are 5.0–10.0 and 0.05–0.20 µg g^{-1}, respectively, whereas the toxic concentrations of these elements are approximately 30.0 and 5.0 µg g^{-1}, respectively (Kabata-Pendias 2000). Consequently, the concentrations of the estimated heavy metals in the different plant parts of lettuce, particularly the shoots, remained safe under the tested treatments. In contrast, the FAO/WHO (2023) and EU (2006) have reported that the maximum permissible levels of Pb and Cd for human consumption are 0.3 and 0.2 µg g^{-1} dry weight, respectively. Thus, the concentrations of Pb and Cd in the shoots of lettuce were higher than these concentrations, suggesting that they may be unsafe for consumption.

Table 6. Health risk assessment for Pb and Cd in lettuce as affected by the studied treatments

Treatment	Average Pb conc., μg g^{-1}	DMI		RI		Risk assessment	Average Cd conc., μg g^{-1}	DMI		RI		Risk assessment
		Adults	Children	Adults	Children			Adults	Children	Adults	Children	
$Cd_0 \times Pb_0$ (control)	0.16	0.00008	0.00009	0.023	0.027		0.17	0.00009	0.00010	0.091	0.104	
Cd_1	0.21	0.00011	0.00012	0.031	0.035		0.29	0.00015	0.00018	0.152	0.175	
Cd_2	0.23	0.00012	0.00014	0.034	0.039		0.31	0.00016	0.00019	0.165	0.189	
Cd_4	0.23	0.00012	0.00014	0.034	0.040		0.35	0.00018	0.00021	0.185	0.212	
Cd_8	0.23	0.00012	0.00014	0.034	0.040		0.37	0.00019	0.00022	0.195	0.224	
Pb_1	0.28	0.00015	0.00017	0.042	0.048		0.19	0.00009	0.00011	0.100	0.115	
Pb_5	0.30	0.00015	0.00018	0.044	0.051		0.20	0.00010	0.00012	0.102	0.118	
Pb_{10}	0.32	0.00017	0.00019	0.047	0.054		0.23	0.00012	0.00014	0.121	0.139	
Pb_{15}	0.33	0.00017	0.00019	0.049	0.056		0.24	0.00013	0.00015	0.126	0.145	
$Cd_1 \times Pb_1$	0.35	0.00018	0.00021	0.053	0.061		0.33	0.00017	0.00020	0.174	0.200	
$Cd_1 \times Pb_5$	0.35	0.00018	0.00021	0.052	0.060		0.34	0.00018	0.00020	0.178	0.204	
$Cd_1 \times Pb_{10}$	0.37	0.00019	0.00022	0.055	0.064		0.32	0.00017	0.00019	0.166	0.191	
$Cd_1 \times Pb_{15}$	0.39	0.00021	0.00024	0.059	0.068	Safe	0.39	0.00020	0.00023	0.202	0.233	Safe
$Cd_2 \times Pb_1$	0.37	0.00019	0.00022	0.055	0.064		0.40	0.00021	0.00024	0.207	0.238	
$Cd_2 \times Pb_5$	0.34	0.00018	0.00020	0.050	0.058		0.37	0.00019	0.00022	0.194	0.223	
$Cd_2 \times Pb_{10}$	0.43	0.00022	0.00026	0.064	0.074		0.36	0.00019	0.00022	0.188	0.216	
$Cd_2 \times Pb_{15}$	0.43	0.00023	0.00026	0.065	0.075		0.37	0.00020	0.00022	0.195	0.224	
$Cd_4 \times Pb_1$	0.41	0.00022	0.00025	0.062	0.071		0.38	0.00020	0.00023	0.198	0.227	
$Cd_4 \times Pb_5$	0.45	0.00024	0.00027	0.068	0.078		0.39	0.00021	0.00024	0.205	0.236	
$Cd_4 \times Pb_{10}$	0.39	0.00020	0.00023	0.058	0.067		0.34	0.00018	0.00021	0.178	0.205	
$Cd_4 \times Pb_{15}$	0.44	0.00023	0.00026	0.066	0.075		0.35	0.00018	0.00021	0.183	0.210	
$Cd_8 \times Pb_1$	0.41	0.00021	0.00025	0.061	0.070		0.35	0.00018	0.00021	0.184	0.212	
$Cd_8 \times Pb_5$	0.44	0.00023	0.00026	0.066	0.076		0.42	0.00022	0.00026	0.222	0.255	
$Cd_8 \times Pb_{10}$	0.45	0.00023	0.00027	0.067	0.077		0.43	0.00022	0.00026	0.223	0.257	
$Cd_8 \times Pb_{15}$	0.45	0.00024	0.00027	0.068	0.078		0.42	0.00022	0.00025	0.220	0.253	

DMI, daily metal intake; RI, risk index (RI < 1 is considered safe).

Accordingly, the highest DMI for Pb was 0.00024 for adults with the $Cd_4 \times Pb_5$ and $Cd_8 \times Pb_{15}$ treatments and 0.00027 for children with the $Cd_4 \times Pb_5$, $Cd_8 \times Pb_{10}$, and $Cd_8 \times Pb_{15}$ treatments. Exposure to high levels of Pb can cause adverse health effects, including brain and kidney damage, increased neurological abnormalities, and oxidative stress (Debnath et al 2019). The highest DMI for Cd was 0.00022 for adults with the $Cd_8 \times Pb_5$, $Cd_8 \times Pb_{10}$, and $Cd_8 \times Pb_{15}$ treatments and 0.00026 for children with the $Cd_8 \times Pb_5$ and $Cd_8 \times Pb_{10}$ treatments. High levels of Cd can have adverse effects on the salivary glands, lungs, heart, liver, spleen, and kidneys (Huang et al 2017).

The RI was <1 for the studied elements in lettuce considered food for adults and children with the application of all treatments. Accordingly, the associated risk from Pb and Cd contamination via treated lettuce consumed by children and adults was low, suggesting that the plants were relatively safe for consumption. These results are consistent with the findings reported by Satpathy et al (2014), Eissa and Negim 2018, Abu-Elela et al (2021).

4 Conclusion

The present study was carried out on soil samples of agricultural soil that were taken from both sides of the highway in Toukh Center, Qalubia Governorate, Egypt. In addition to their contents of Pb and Cd that precipitated due to their exposure to vehicle exhausts, they were artificially contaminated with different concentrations of Pb and Cd either alone or in combination with each other. Cultivation of alfalfa in the studied soil provided evidence to indicate that alfalfa is an effective accumulator plant for the phytoremediation of Pb and Cd-contaminated soils. Also, using alfalfa shoot extract in fertilizing lettuce that transplanted after alfalfa, revealed beneficial for growth without any risk to translocate heavy metals. The concentrations of Pb and Cd in lettuce shoots were relatively higher than the concentrations reported by FAO/WHO (2023) and EU (2006) which were considered unsafe to be consumed, as affected by the applied concentrations of Pb and Cd to the investigated soil. From this standpoint, monitoring heavy metals in study vegetable crops is demanded to minimize their excessive augmentation in the food chain. So, we recommend adding alfalfa to the crop rotations, especially in contaminated soils with heavy metals.

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