Original Article

Effects of Sewage Sludge and Chemical Fertilizers on Pb and Cd Accumulation in Fenugreek (*Trigonella gracum*)

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ABSTRACT

Background: Reprocessing of sewage sludge to agricultural land is preferred to disposal through landfill or incineration due to its potentially beneficial effects on soil fertility from organic matter and plant nutrients, such as N and P, present in the sludge. Therefore, this study was conducted to investigate the impact of sewage sludge and chemical fertilizers on Pb and Cd accumulation in fenugreek, Trigonella gracum.

Methods: The experiment was carried out in earthen pots, 30 cm in diameter and 30 cm in depth. The study was done on five groups- control, S1: sewage sludge (50 tone/ha), S2: sewage sludge (50 tone/ha)+heavy metals [85 ppm of Cd(Cl)2; 840 ppm of Pb(NO3)2], F1: chemical fertilizer (250 kg/ha), and F2: chemical fertilizer (250 kg/ha)+ heavy metals [85 ppm of Cd(Cl)2; 840 ppm of Pb(NO3)2]- in three replicates.

Results: The results showed that total Pb concentrations ranged from 0.05 to 2 mg/kg, with the maximum level in the sewage sludge treatment (S2) while Cd concentrations in the plant ranged from 0.07 to 1.1 mg/kg with the maximum content in the chemical fertilizer treatment (F2). The concentrations of Pb and Cd in vegetables were significantly higher than the permissible limits.

Conclusion: This study highlights the potential risks involved in the cultivation and consumption of vegetables on plots irrigated with sewage sludge, a practice which may endanger the health of urban populations that consume these vegetables.

Keywords: Fenugreek, Health Risk, Heavy Metals, Sewage Sludge.

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INTRODUCTION

The increasing production of sewage sludge from wastewater treatment plants creates significant pressure concerning the optimal management and disposal of this by-product [1]. Cultivation of vegetables on soils amended with sewage sludge and fertilizers and irrigated with wastewater is a growing phenomenon. Excessive accumulation of heavy metals in agricultural soils may not only result in environmental contamination, but lead to elevated heavy metals uptake by crops, which may affect food quality and safety. Reprocessing of sewage sludge to agricultural land is preferred to disposal through landfill or incineration due to its potentially beneficial effects on soil fertility from organic matter and plant nutrients, such as N and P, in the sludge. Nevertheless, agricultural use of sludge has been debated for decades as some studies have reported detrimental effects on plant growth, forage quality, groundwater quality, and long-term soil fertility resulting from sludge application [2, 3]. These problems are often associated with trace metals in the sludge, particularly Pb, Ni, Cr, Sn, As, Cd, Cu, Mo, and Zn. It is important to understand the behavior of the metals in the sludge–soil system in order to predict their bioavailability, mobility, and toxicity over time [3, 4].

Due to their mobilizing characteristics and varied bioavailability to plants, the environmental impacts of heavy metals on sewage sludge cannot be effectively evaluated by the total concentration of heavy metals. Chemical specification of heavy metals can be defined as the identi-

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fication and quantification of the different defined species, forms, or phases in the sludge [5]. The main source of heavy metals in soil is the use of urban and industrial wastewater, chemical phosphate fertilizers, sludge from wastewater treatment plants in cities, and metal extraction mines [6]. Most heavy metals infiltrate and accumulate in the top soil. Accumulation of heavy metals in soil is detrimental and results in increased levels of contamination in the long run so that contamination levels may reach limits that can constitute a real threat to food safety for humans [6, 7].

Under natural conditions, the concentration of trace elements in the soil is relatively low, but certain human actions, such as agriculture, the application of tailings with residues when used as an organic amendment, the application of solid urban waste and sewage sludge (SS) as fertilizers, and atmospheric particle transport, as well as mining can lead to contamination above acceptable levels [8-14]. Therefore, health authorities in many parts of the world are becoming increasingly concerned about the effects of heavy metals on environment and human health and its potential implications to international trade [15]. Sewage sludge is a by-product of wastewater treatment. However, wastewater treatment plants in cities around the world encounter serious problems in disposing sewage sludge due to lack of public acceptance because of their unpleasant odor, presence of excessive salt, and acidity, as well as the levels of some heavy metal exceeding the critical limits. In general, SS is disposed in landfills [16, 17]. Decrease in available landfill sites and increase in costs associated with landfill operations has resulted in exploration of alternate disposal methods such as use in agricultural fields [18, 19]. Nevertheless, this may easily impact soils with heavy metals and could be a major risk for the growth of plants.

Flood irrigation of agricultural farms has been practiced for over 10 years at some agricultural farms in Isfahan province, Iran, as a form of tertiary treatment of sewage effluent, utilizing soil microorganisms to complete the treatment process. The cultivation of crops on the farm by workers using sewage sludge for fertilizing raises concerns about the safety of such vegetables with respect to their heavy metal content. The farm workers divert wastewater and the mixture of sewage sludge and wastewater from the flood irrigation channels by creating trenches to direct the flow to their crops. The vegetables produced are consumed by the villagers, largely supplying their entire dietary requirement. This study was carried out to evaluate threats associated with such practices. The study focused on the concentrations of heavy metals in soils and fenugreek (Trigonella gracum) grown on a large scale on these fields.

MATERIALS AND METHODS

Instrumentation

A pH meter (780, Metrohm, Herisau, Switzerland) equipped with combined Ag/AgCl glass electrode, 4510 bench conductivity meters, and automatic Kjeldahl analyzer apparatus CCATN-300 was used for pH, EC, and N measurements, respectively. Lead and Cd concentrations were measured using an inductively coupled plasma spectrometry (ICP) (Varian 710-Es Australia). All the operating parameters were those recommended by the manufacturers. The optimum operating conditions and measurement parameters for ICP include RF power: 1.5 kW, carrier gas flow rate: 0.9 L/min, mmakeup gas flow rate: 0.15 L/min, and iintegration time: 0.1s.

Study Location

Sewage sludge was obtained from Isfahan wastewater plant. The experiment was conducted under greenhouse conditions at the Faculty of Basic Sciences, Islamic Azad University of Hamadan, Hamadan,Iran. The main properties of the sludge are shown in Table 1.

Table 1. Sewage sludge properties.

Dry matter (%)	22.17
pH	6.10
EC (dS m^{-1})	8.70
TOC (%)	30.88
$N_{Kjeldahl}$	1.97
C/N	17.0

Experimental Design and Growing of the Plants

The experiment was carried out in earthen pots with 30 cm diameter and 30 cm depth. There were five treatment groups: control, S1: sewage sludge (50 tone/ha), S2: sewage sludge

(50 tone/ha)+heavy metals [85 ppm of Cd (Cl)2; 840 ppm of Pb (NO3)2], F1: chemical fertilizer (250 kg/ha), and F2: chemical fertilizer (250 kg/ha)+ heavy metals [85 ppm of Cd (Cl)2; 840 ppm of Pb (NO3)2], in three replicates. Details of treatments are given in Table 2.

Soil from agricultural farms was dug out up to the depth of 30 cm, left in the field for air-drying and then mixed properly. Sewage sludge was also air-dried and then ground to mix uniformly. For each treatment, three pots were provided, each filled with 4.5 kg prepared soil and amendments (sewage sludge or chemical fertilizer). Fenugreek, one of the most common vegetables in Iran, was selected. Thereafter, 30 seeds of fenugreek were sown manually at an equivalent distance in each pot. This was followed by irrigation of the pots applying municipal pipe water. The pots were kept in open field conditions to provide identical light and temperature to the treatments. Identical water regime was maintained for the treatments throughout the growth period of plants [20].

Estimations of Growth, Weight, and Heavy Metal Concentrations in Plants

After eight weeks, plants in each triplicate treatment were carefully harvested with roots from respective pots. Plant length was measured. For biomass determination, plants were separately washed to remove soil particles adhering to them and then separated from roots and shoots and oven-dried at 80 °C until constant weight was achieved.

The plant parts were then weighed separately. Their weight was expressed as g/plant. For heavy metal analysis, oven-dried samples were homogenized by grinding in blender and then passed through a sieve of 2 mm mesh size. Cd and Pb in plant samples were extracted by acid digestion. The process involved oven-drying of 1 g homogenized plant sample and digesting in a 10 ml tri-acid mixture (HNO3: H2SO4: HCLO4 in the ratio 5:1:1) until the solution becomes transparent [21]. Heavy metal concentrations were determined using ICP-AES (Varian, 710-ES).

The collected data were subjected to statistical analysis using one-way ANOVA test and the results were drawn accordingly.

Table 2. Description of experimental treatments.

Treatments	Description
1 (C)	Control soil (No Cd and Pb was added)
2 (S1)	Soil with sewage sludge (50 tone/ha)
3 (S2)	sewage sludge (50 tone/ha) with 85 ppm Cd and 840 ppm Pb
4 (F1)	chemical fertilizer (250 kg/ha)
5 (F2)	chemical fertilizer (250 kg/ha) with 85 ppm Cd and 840 ppm Pb

RESULTS

The results of this study indicated the effects of different treatments of Cd and Pb on their accumulation in fenugreek. Lead and Cd concentrations in root and shoot of fenugreek and dryness and wet weight of root, shoot, and length of fenugreek are given in Tables 3 and 4, respectively.

Table 3. Lead and Cd concentrations in root and shoot of the studied fenugreek (mg/kg).

Number	Treatment	Pb		Cd	
		Root	shoot	Root	shoot
1	C	0.02^{a}*	0.03^{a}	0.02^{a}	0.05^{a}
2	S_1	0.50^{c}	0.50^{b}	0.20^{b}	0.60^{d}
3	S_2	0.90^{d}	1.10^{d}	0.20^{b}	0.30^{b}
4	F_1	0.30^{b}	0.50^{b}	0.20^{b}	0.50^{c}
5	F_2	0.50^{c}	0.60^{c}	0.50^{c}	0.60^{d}

** The letters (a, b, c, d) represent the statistical differences among different treatments (P<0.05)*

Table 4. Dry and wet weight of root and shoot and length of the studied fenugreek.

Number	Treatment	Dryness Weight (g)		Wetness Weight (g)	Plant Length (cm)
		Root	shoot		
1	C	0.041[b*]	0.53[b]	4.60[b]	29.10[a]
2	S_1	0.043[bc]	0.62[c]	5.30[c]	30.20[a]
3	S_2	0.046[c]	0.53[b]	4.60[b]	29.10[a]
4	F_1	0.039[ab]	0.61[c]	5.30[c]	29.70[a]
5	F_2	0.037[a]	0.51[a]	4.40[a]	29.70[a]

** The letters (a, b, c, d) represent the statistical differences among different treatments (P<0.05)*

DISCUSSION

Heavy metals are of great significance in environmental chemistry and ecotoxicology because of their toxicity at low levels and tendency to accumulate in human organs [22]. The dietary limit in food and food stuff for Cd is 0.1 mg/kg. High concentration of Cd exerts detrimental effects on human health and causes severe diseases, such as kidney damage, cancer, diarrhea and incurable vomiting [23]. As it can be seen Table 3, Cd concentrations were higher than the permissible limits in all treatments, except treatment C. Among them, the maximum concentration of Cd (1.1 mg/kg) was found in F2 (chemical fertilizer+ heavy metals).

If exceed the maximum permissible limit (0.2 mg/kg), Pb in leafy vegetables may affect the nervous system, bones, liver, pancreases, teeth, and gum and causes blood disorders, when are consumed by human [19]. As shown in Table 3, the maximum concentration of Pb (2 mg/kg) was found in S2 (soil+ sewage sludge). In all treatment groups, except control, Pb concentrations in fenugreek were reported above the permissible limits. Accumulation of Pb and Cd in fenugreek was greater in the shoot of the plant (edible portion) than in its root. A similar trend was observed in group C.

The results of statistical analyses (Tables 3 and 4) showed that in treatment group 2 (chemical fertilizer), shoots present a notable statistical difference compared with the control sample. Moreover, it was shown that increase of sewage sludge does not have a significant effect on the amount of Pb absorption. In the case of Pb absorption in fenugreek roots, there was not a significant difference in any of the treatments except the control sample. Regarding Cd absorption rate in shoots and roots of the plant, all treatments showed significant differences compared with the control group, but adding sewage sludge to different treatments (S1, S2 and F1) did not indicate significant differences in Cd absorption in shoots and roots. Moreover, according to Table 4, fenugreek in all treatments presented a significant difference in comparison to the control sample in terms of plant operation (length, wetness, and dryness).

CONCLUSION

The results of this study demonstrate that by increasing the amount of sludge in different treatments, the rate of plant operation increases. Moreover, sewage sludge leads to greater weight in plants compared with chemical fertilizers. Thus, there is an urgent need to educate farmers and adopt new technologies for treating sewage sludge and other industrial effluents before their use in vegetables growth.

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