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RESEARCH ARTICLE

The effect of sewage sludge on heavy metal concentrations in wheat plant (*Triticum aestivum* L.)

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Abstract The use of sewage sludge in agricultural soils can improve soil physical and chemical properties and soil fertility by increasing organic materials; however, the presence of heavy metals in sewage sludge is a significant problem for the quality of agricultural products and the environment. Most heavy metals, due to their inactive nature, are stable and can affect human health. This study investigates the effect of sewage sludge on the concentration of copper, zinc, cadmium, lead, iron, and manganese in two varieties of wheat, i.e., Sivand (*Triticum aestivum* cv. Sivand) and Roshan (*Triticum aestivum* cv. Roshan). Results were obtained from a factorial experiment in a completely randomized design with three replications and at three levels (0, 60, and 120 kg soil) and were carried out in a research greenhouse. This research concluded that in the treated seed and stem of Sivand variety, concentrations of Zn, Cd, Fe, and Mn were below the standard toxicity limit, and concentrations of Cu and Pb were above the standard limit. In the Roshan variety, the concentrations of Zn, Cd, Pb, Fe, and Mn were below the standard limit, and Cu concentration was above the standard. The results of this study lead to the recommendation that farmers avoid using sewage sludge in farming, as much as possible. Instead, it is far more appropriately employed as a fertilizer for green space, ornamental trees, and parks, where edible products for human consumption are not grown.

Keywords Arid and semi-arid regions · Agricultural soil · Sludge application · Sivand wheat · Roshan wheat

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Introduction

In industrial societies, improvement in living conditions leads to an increased amount of waste, with potential for environmental contamination. As the volume of urban sewage sludge grew, recycling became very significant (Bowszys et al. 2015). Purification of sludge and its disposal are the main problems of wastewater treatment plants, and its cost will be more manageable if it is used as fertilizer or in another economically viable application (Mahapatra et al. 2013). Using sewage sludge in agricultural soils increases soil physical and chemical properties, such as porosity, total stability, outward specific mass, soil water holding capacity (Silveira et al. 2003), and soil fertility by increasing organic materials and nutrition (Franco et al. 2010). However, the presence of heavy metals in sewage sludge is a significant problem for agricultural product quality and the environment, because most heavy metals, due to their inactive nature, are stable (Jamali et al. 2009).

pH, organic materials, and revival conditions are factors that generally affect the chemical properties of metals and their absorption by organisms. Among them, soil pH affects both availability and uptake of low consumption elements in plants (Förstner et al. 1991). Accumulation of heavy metals in soil can cause loss of soil functions and can increase concerns about maintaining the quality of the environment, preserving human health, and productivity. Soil contamination in high concentrations causes toxicity in plants; through animals' grazing of plants, heavy metals are transmitted into the animal diet and then conveyed into the human diet (Kabata-Pendias and Mukherjee 2007; Khan et al. 2017).

Bioaccumulation factor and transmission agent are important parameters in the absorption of heavy metals (Marchiol et al. 2004; Khan et al. 2016a). Long-term studies reveal conclusive evidence of an increase in the concentration of heavy metals in soil through use of sewage sludge (Nogueira et al. 2008). Concentrations of heavy metals in soils in tropical areas, even a short time after adding sewage sludge, may also significantly increase risks (Marques et al. 2007). Heavy metal uptake by plants depends on soil structure and plant variety. Moreover, most of the vegetative parts of plants, especially leaves, carry much more metal than seeds, nuts, or fruits (Melo et al. 2007; Khan et al. 2016b).

Concentration of heavy metals in the solid phase of wastewater is significantly greater than in the liquid phase. These elements can be found in sludge as minerals in different forms, including exchangeable, adsorbed and chelated by organic materials species, carbonate forms, and sulfide, iron oxide, and manganese. The dominant form of each element varies according to the type of sludge; several forms of each metal appear in different types of sewage (Luo and Christie 1998). In this case, the species found in sewage sludge depend on type of sludge processing and digestion, as well as the amount and type of added wastewater. Although some heavy metals are necessary for biological growth, concentrations higher than the threshold could be harmful to both plant and animal life (Krebs et al. 1998). The present study investigates the effect of sewage sludge on heavy metal concentration in the roots, stems, and seeds of Sivand and Roshan varieties of wheat.

Materials and methods

Test location

This study was conducted at the Department of Natural Resources and Environment, University of Yazd, in a research greenhouse during the years 2013–2014 in the city of Yazd, Iran, located at the geographical coordinates of 31° 53′ 50″ North, 54° 22′ 3″ East. Yazd has an average annual temperature of 19 °C and an average annual rainfall of 62 mm.

Wheat varieties used in this study

Since wheat is one of the main crops used for cultivation in Yazd, two varieties of wheat, namely Sivand and Roshan, were selected for the examination. Sivand is a new variety of bread wheat resistant to curvature and grain loss and is found in the black and yellow color range. It is suitable for cultivation in temperate regions such as Yazd Province (Esfandiari et al. 2015). Roshan wheat is cultivated in colder temperate regions and in somewhat hotter areas and is resistant to curvature, grain loss, drought, and salinity (Najafian et al. 2008).

Design and procedure

A factorial experiment in a completely randomized design with three replications and three levels, i.e., 0, 60, and 120 g/kg of soil (0, 20, and 40 t per hectare, respectively) was conducted on Sivand and Roshan wheat in January, 2013. The required soil was prepared from the Qasemabad region, located in Yazd. Soil samples were collected from a depth of zero to 30 cm; after air-drying, they were passed through a 4-mm sieve. The required sewage sludge was obtained from a wastewater treatment plant in Yazd; after air-drying, it was passed through a 2-mm sieve. Before planting, the amount of required sludge for each treatment was mixed with 6 kg of soil. Seeds of Sivand and Roshan wheat ($n = 10$) were planted in each vase after washing with hydrogen peroxide and distilled water. Plants were watered every other day. After they grew, the number of seeds in each pot was thinned to six.

Chemical analysis

In July 2014, after completion of the growth period, the wheat plants' roots, seeds, and stems were separately harvested; after washing with distilled water, they were placed in an oven to dry for 48 h at 65 °C. Then, the samples were ground using a powder mill and passed through a 1-mm sieve. For samples of sludge and soil, acidity was measured by pH meter and electrical conductivity by EC meter. Because the sludge is absorbent, ratio of 1:5 sludge:distilled water was used to measure the pH and electrical conductivity of the sludge (Kalra and Maynard 1991). The heavy metals of the soil were extracted using the DTPA method of distillation (Lindsay and Norvell 1978). Thus, 96.1 g of DTPA solution was mixed into 3.13 ml triethanolamine solution and 47.1 g of calcium chloride, with 100 ml of distilled water was added to the mixture. The solution's pH was brought to pH 3.7 by 1 N HCl, as determined by a pH meter. Afterwards, it was brought to a volume of 1 l by using distilled water. Twenty-five milliliters of DTPA solution was added to 25 g soil, which then was placed on a shaker for 2 h at a speed of 1200. The samples were filtered using Whatman Filter 42 Paper. Heavy metals in sewage sludge were also extracted through the distillate of the mixture of hydrochloric acid and nitric acid (Ryan et al. 2001). To this end, 10 ml aqua regia was added to 1 g of sludge and rested for 24 h. Then, it was heated at 80 °C for 30 min until its steam exited. After cooling, it was filtered through Whatman Filter 42 Paper and with distilled water it was brought to a volume of

25 ml. The concentration of heavy metals in soil and sludge was read using an atomic absorption spectrometer (novAA® 350, Analytik-Jena AG, Jena, Germany). In order to extract heavy metals from the roots, stems, and seeds of the plants, the dry ashing method (Westerman 1990) was used. Of each sample, 0.5 g was put in an electric furnace (Azar 1250 model, Azar Furnace Corp, the Netherlands) at 550 °C for 4 h. Then, 10 ml of 2NHCl were added and the composition was heated until two thirds of the acid evaporated. After cooling, the samples were filtered through Whatman Filter 42 Paper and by using distilled water, to a volume of 50 ml. Then, the concentrations of Cu, Zn, Cd, Pb, Fe, and Mn in different parts of the plant were measured by atomic absorption spectrometer. Finally, the amount of elements was determined in milligram per kilogram using the following equation:

$$\begin{aligned} &\text{Sample weight/Extract volume} \\ &\times \text{Element concentration in sample} \\ &= \text{Element concentration(mg/kg)} \end{aligned}$$

All samples were analyzed in three replicates. Analytical process precision was evaluated by relative standard deviation (RSD). RSD values were obtained for plant samples as follows: Cu (2.99%), Zn (3.41%), Cd (3.53%), Pb (3.35%), Fe (1.69%), and Mn (5.16%), and for soil samples as follows: Cu (0.56%), Zn (2.54%), Cd (5%), Pb (0.44%), Fe (5.08%), and Mn (7.16%).

Statistical analysis

Data were analyzed using SPSS v. 20 (IBM Corp. Released 2011, IBM SPSS Statistics for Windows, Version 20.0, Armonk, NY: IBM Corp.), and normality of data was measured using the Kolmogorov-Smirnov test. Two-way ANOVA was used to compare means. To investigate the significant of differences among treatments, Duncan's New Multiple Range Test was used at 5% level of significance.

Results and discussion

Some characteristics of the soil and sludge under study are presented in Table 1. The pH of used sewage sludge used is somewhat acidic (Table 1). Sewage sludge EC is relatively low and its salinity is not too problematic. Comparison of the amounts of heavy elements in the sewage sludge with the U.S. Standards of the Environmental Protection Agency (USEPA 503) (2002) shows that all of the heavy metals in this sewage sludge except lead are lower than the standard (Table 1).

Table 1 Characteristics of the study's soil and sewage sludge

Parameter	Soil	Sludge	Sludge Standard Limit (USEPA503 2002)
Soil tissue	Sandy loam	–	–
EC (ds m^{-1})	1.8	3	–
pH	7.7	6.3	–
Cu (mg kg^{-1})	1.8	437	1500
Zn (mg kg^{-1})	1.2	612	2800
Cd (mg kg^{-1})	0.03	12	39
Pb (mg kg^{-1})	10.6	375	300
Fe (mg kg^{-1})	61.8	3000	–
Mn (mg kg^{-1})	8.7	220	–

Heavy metal concentrations in root, stem, and seed of Sivand and Roshan wheat

The concentration of heavy metals in root

The mean ranges of heavy metals in the roots of Sivand and Roshan wheat are presented in Table 2.

The results reveal that by increasing the amount of sewage sludge, copper concentration in both Sivand and Roshan varieties decreased. The copper concentration of the control treatment was higher than the levels of 60 and 120 kg in sludge. The percentage of copper concentration decreased in both treatments compared to control treatment, with the concentration in Roshan variety higher than in the Sivand variety (Table 2). Results revealed that the effect of sewage sludge, variety, and their interaction effect on the copper concentration in the root was significant ($P \leq 0.01$; Table 3). Probably due to the strong binding of copper with organic materials and the formation of stable complexes, copper concentrations in control treatment are higher than its concentrations in the treatments containing sludge (Akbarnejad et al. 2010).

In comparison to control treatment, the use of sewage sludge reduces the concentration of zinc in both Sivand and Roshan wheat varieties. Therefore, the zinc concentration in the root of Sivand variety compared to control treatment at the levels of 60 and 120 g/kg sludge was reduced by 46 and 56%, respectively. In Roshan variety, the zinc concentration at the levels of 60 and 120 g/kg sludge compared to control treatment was reduced by 46 and 56%, respectively (Table 2). The effect of sewage sludge, variety, and their interaction effects were significant for zinc concentration in root ($P \leq 0.01$) (Table 3).

Compared to control treatment, the use of sludge caused a significant decrease in the concentration of cadmium in roots of both Sivand and Roshan wheat varieties. In Sivand variety, no significant difference between the levels of 60 and 120 g/kg sludge was observed. However, in Roshan variety, the cadmium concentration in the roots of the plants treated

Table 2 Effect of sewage sludge on heavy metal (mg kg^{-1}) concentrations in roots of Sivand and Roshan varieties of wheat [mean ± SD, (range)]

Metal	Sivand			Roshan		
	Control	60 (g kg^{-1})	120 (g kg^{-1})	Control	60 (g kg^{-1})	120 (g kg^{-1})
Cu	90.1 ± 2.7^{b} (85.3–94.8)	48.5 ± 1.6^{c} (43.7–53.3)	38.8 ± 2^{d} (34–43.6)	174.1 ± 4.1^{a} (169.3–178.9)	48.3 ± 7^{c} (43.5–53.1)	34 ± 2.4^{d} (29.2–38.8)
Zn	58.5 ± 2^{b} (56–61.1)	42.3 ± 1.6^{c} (39.8–44.8)	33.3 ± 3^{e} (30.8–35.8)	62.9 ± 2.2^{a} (60.3–65.4)	38 ± 1.4^{d} (35.5–40.6)	23.2 ± 0.92^{f} (20.7–25.7)
Cd	8.2 ± 0.29^{b} (7.7–8.8)	3.9 ± 0.34^{c} (3.4–4.4)	3.2 ± 0.16^{cd} (2.7–3.7)	11.9 ± 0.82^{a} (11.3–12.4)	3 ± 0.14^{d} (2.5–3.5)	1.4 ± 0.24^{e} (0.9–1.9)
Pb	77.6 ± 2.6^{b} (74.9–80.3)	38.1 ± 2.7^{b} (35.4–40.8)	30.2 ± 1.8^{d} (56–61.1)	85 ± 2.5^{a} (82.3–87.5)	35.6 ± 1^{c} (32.9–38.3)	29.7 ± 1.4^{b} (27–32.4)
Fe	1474 ± 25^{e} (1404.5–1543.5)	3877.5 ± 27.5^{a} (3808.1–3947)	2521.8 ± 30.9^{b} (2452.3–2591.2)	1826.1 ± 26.9^{d} (1756.6–1895.5)	2468.9 ± 62.7^{b} (2417.4–2556.3)	2325.7 ± 106.1^{c} (2256.2–2395.1)
Mn	27.1 ± 1.4^{d} (23.3–31)	103.7 ± 1.8^{b} (99.8–107.5)	65.4 ± 0.91^{b} (61.5–69.2)	168.3 ± 5.6 ^{a} (164.5–172.2	106.3 ± 3.9^{b} (102.5–110.2)	60.6 ± 1.4^{c} (56.8–64.5)

Means with at least one common letter in each column are not significantly different at the level of .05 of Duncan Test

with 120 g/kg sludge was significantly lower than plants treated with 60 g/kg sludge. The percentage of reduction of cadmium concentration in Roshan variety compared to control treatment was also higher than in Sivand variety at both sludge levels (Table 2). The effect of sewage sludge and the interaction effect of sewage sludge and variety were significant on the concentration of cadmium in roots ($P \leq 0.01$), but variety was not significant on cadmium concentration in roots (Table 3). Ahmadi et al. (2013) also reported the reduction of cadmium concentration in carrot plant because of the use of sewage sludge. The cause of lower cadmium concentration in sewage sludge treatments compared to the control treatment could be the transmission of this element into the stems. Several factors affect the uptake of cadmium in the plant. Among them are the cadmium concentration in soil and sewage sludge, pH of soil, plant type, weather conditions, and the physical and chemical properties of the soil (McBride 1995).

The results revealed that the use of sludge caused a significant decrease in lead concentrations in roots compared to control treatment in both Sivand and Roshan wheat varieties. In both varieties, 120 g/kg of sludge reduced lead concentration in roots significantly more than 60 g/kg of sludge (Table 2). The effect of sewage sludge and the interaction effect of sludge and variety was significant on the lead concentration in roots ($P \leq 0.01$), but the roots did not have significant effect on lead concentrations (Table 3). The obtained result demonstrated the previous results of Akbarnejad et al. (2010). These researchers suggested that the cause of lower lead concentrations in treatments containing sludge compared to control treatment was the strong bonds of lead with organic materials and the formation of stable complexes. In Roshan variety, the lead concentrations in roots were higher than in the stems and seeds. Dede and Ozdemir (2016) cited the increase of lead concentrations in the roots of *Brassica juncea* in their sludge treatments compared to control treatment. Keskin et al. (2010) showed that sewage sludge increased the lead concentration in *Bromus inermis* Leyss, a species of herbaceous plants. The higher accumulation amount of the most metals including lead in root compared to stem indicates the mechanism of plant resistance to high concentrations of heavy metals in the soil (Brunetti et al. 2011).

The use of sewage sludge increased iron concentration in roots of both Sivand and Roshan varieties compared to control treatment. In both wheat varieties, iron concentration in the

Table 3 ANOVA results of sewage sludge effect on the heavy metal concentrations in the roots of Sivand and Roshan wheat varieties

Mean squares							
Change sources	Degrees of freedom	Cu	Zn	Cd	Pb	Fe	Mn
Sewage sludge	2	16,324**	1615**	104.6**	4460**	3,521,579**	3022**
Variety	1	3117**	50.46**	0.426^{ns}	10.03^{ns}	762,151**	9672**
Sludge × variety	2	3750**	78.79**	12.63**	40.48**	1,191,121**	1038**
Error of measurement	12	14.54	4.08	0.167	4.63	3047	9.36

**Significant at the level of 0.01 of Duncan test. ns = not significant at the level of 0.01 of Duncan Test roots of plants treated with 60 g/kg of sludge was significantly higher than that of the level of 120 g/kg of sludge. Iron concentration in the roots of Sivand variety compared to the control treatment at the level of 60 g/kg of sludge increased 1.6 times and 71%, at the level of 120 g/kg. In Roshan variety, iron concentration at levels of 60 and 120 g/kg sludge, 36 and 27%, respectively, increased compared to control treatment (Table 2). The effect of sewage sludge, variety, and their interaction on the iron concentration at the level of 1% ($P \leq 0.01$) was statistically significant (Table 3). Iron accumulation in the wheat roots has been attributed to non-dynamicity of this element in the plant (Nazari et al. 2006). Increase in the amount of iron concentrations in the onion in treated soil with sludge was reported by Paresh et al. (2009).

Mean comparison showed that in Sivand variety, using 60 g/kg of sludge caused a significant increase of manganese concentration in roots. In Roshan variety, the use of sludge compared to control treatment reduced manganese concentration in roots. Consequently, no significant difference has been observed between levels of 60 and 120 g/kg sludge. Manganese concentration in root in Sivand variety compared to control treatment at levels of 60 and 120 g/kg sludge increased 2.8 and 1.4 times, respectively. However, in Roshan variety the manganese concentrations in root at the levels of 60 and 120 g/kg sludge compared to control treatment decreased 36 and 63%, respectively (Table 2). The effect of sewage sludge, variety, and their interaction effect on manganese concentration in roots was significant ($P \leq 0.01$) (Table 3). The results of a study by Nazmi et al. (2011) revealed an increase in manganese concentration in barley roots, resulting from using sludge. Studies have shown that roots have high vulnerability to increasing concentrations of heavy metals in soils treated with sewage sludge. Transferring metals from soil to root varies according to soil type. Low pH soils with a higher percentage of sand transferred some metals to the roots easily (Soriano-Disla et al. 2014).

Heavy metal concentrations in stem

Mean, standard deviation, and range of heavy metals in the stems of Sivand and Roshan wheat varieties in under-studied treatments are presented in Table 4.

The results revealed that in both Sivand and Roshan varieties, the use of sewage sludge compared to the control treatment caused a significant increase in copper concentrations in the stem (Table 4). The effect of sewage sludge, variety, and their interaction effect on concentrations of copper were significant ($P \leq 0.01$) (Table 5). Yonggi and Liu (2005) in their study concluded that copper concentrations in barley leaves increased by the use of sludge.

Zinc is one of the micronutrient elements required by plants. However, its high concentration in sludge may limit its use, because of the risk of contamination of soil and absorption by the roots and stems and then transmission to the food chain (Nogueira et al. 2008). In Sivand variety, the use of sludge in comparison to the control treatment caused a significant increase in zinc concentration in the stems. In Roshan variety, the use of 120 g/kg of sludge caused a significant decrease in zinc concentration in the stems, but the level of 60 g/kg of sludge did not have a significant effect on zinc concentration in the stems. The zinc concentration in Sivand variety was generally increased by increasing the amount of sludge, while in Roshan variety, decreasing zinc concentration was observed (Table 4). The effect of sewage sludge, variety, and their interaction effect on zinc concentrations in the stems were significant ($P \leq 0.01$) (Table 5). Nazari et al. (2006) reported an increase in the zinc concentrations in stems of wheat, barley, and corn because of the use of sewage sludge.

Table 4 Effect of sewage sludge on heavy metal concentrations (mg kg^{-1}) in the stems of Sivand and Roshan wheat varieties [mean ± SD, (range)]

Metals	Sivand			Roshan		
	Control	60 (g kg^{-1})	120 (g kg^{-1})	Control	60 (g kg^{-1})	120 (g kg^{-1})
Cu	42.5 ± 3.2^{bcd} (36–48.9)	50.1 ± 9.8^{b} (43.7–56.5)	73.3 ± 4.2^{a} (66.8–79.7)	33.9 ± 4.5^{d} (27.5–40.4)	39 ± 2.6^{cd} (32.6–45.5)	47.1 ± 2.2^{bc} (40.7–53.6)
Zn	16.1 ± 1^{e} (14.9–17.2)	28 ± 0.93^{b} (26.9–29.1)	33 ± 0.57^{a} (31.9–34.1)	25.5 ± 0.68^{c} (24.3–26.6)	25 ± 1^{c} (23.8–26.1)	22.1 ± 1^{d} (21–23.2)
Cd	2.6 ± 0.38^{c} (2.3–2.9)	2 ± 0.13^{d} (1.7–2.3)	4.6 ± 0.19^{a} (4.3–4.8)	4.4 ± 0.19^{a} (4.1–4.7)	4.2 ± 0.24^{a} (3.9–4.5)	3.7 ± 0.03^{b} (3.4–3.9)
Pb	50.3 ± 1.8^{a} (48.8–51.8)	48 ± 1.3^{b} (46.5–49.5)	26.7 ± 1.1^{c} (25.1–28.2)	21.5 ± 0.93^{d} (20–23)	21.6 ± 0.48^{d} (19.1–22.2)	21.7 ± 0.9^{d} (20.2–23.2)
Fe	136.5 ± 7.9^{d} (128–145)	139.6 ± 11.4^{d} (131.1–148.1)	162.6 ± 4.1^{c} (154–171.1)	143 ± 4.5^{d} (134.4–151.5)	222.1 ± 1.8^{b} (213.6–230.7)	238 ± 6.3^{a} (229.4–246.5)
Mn	4.5 ± 2.4^{c} (0.48–8.6)	13.7 ± 2.1^{b} (9.7–17.8)	8.4 ± 2.4^{bc} (4.3–12.4)	36.2 ± 5.1^{a} (32.1–40.2)	38.9 ± 3.3^{a} (34.9–43)	33.6 ± 2.8^{a} (29.5–37.6)

Means which have at least one common letter in each column are not significantly different at the level of .05 of Duncan Test

Table 5 ANOVA results of sewage sludge effect on the heavy metal concentrations in the stems of Sivand and Roshan wheat varieties

Mean squares							
Change sources	Degree of freedom	Cu	Zn	Cd	Pb	Fe	Mn
Sewage sludge	2	768.9**	80.16**	1.55**	236.8**	5727**	65.05*
Variety	1	10.26**	10.26**	4.57**	19.4**	13,516**	3366**
Sludge × variety	2	135.6**	156.3**	4.13**	275.6**	2647**	21.07^{ns}
Error of measurement	12	26.27	0.789	0.051	1.46	45.95	10.46

**Significant at the level of 0.01 and 0.05 of Duncan test, respectively. ns = not significant at the level of 0.01 of Duncan Test

Merrington et al. (1997) also reported that by increasing the amount of sewage sludge in soil, zinc concentration in the stems of wheat increased. It seems that zinc concentration in the stems is influenced by factors such as the absorbable zinc concentration of soil, absorption rate, transmission of zinc from root to stem, and growth rate of the plant (Najafi and Towfighi 2008). The reason for zinc concentration decrease in Roshan variety at both levels of sludge compared to control treatment is possibly due to growth dilution effect, which is caused by an increase in the yield of sludge treatments. In fact, by increasing the use of sludge, the dry weight of stem significantly increases; and despite an increase in metal uptake by soils, the proportion of each of the components of biomass diluted the total amount of metal uptake by the plant (Karami et al. 2009). Sewage sludge significantly increased absorption (multiplying plant yield by metal concentration) of zinc in wheat stem.

Cadmium concentration increased in stem of Sivand variety with 120 g/kg sewage sludge compared to the control treatment and decreased at the level 60 g/kg. In Roshan variety, using 60 g/kg sewage sludge compared to control treatment had no significant effect on cadmium concentration in the stem, but at the level of 120 g/kg sludge, a significant decrease was observed in cadmium concentration in stem (Table 4). The effect of sewage sludge, variety, and their interaction effect was significant on zinc concentration in stem ($P \leq 0.01$) (Table 5).

Cadmium is one of the most toxic metals in the environment. Cadmium is absorbed quickly and compared to other heavy metals moves easily in the soil. Many studies have shown that even small amounts of cadmium concentration can cause serious risk to human health through the soil-plant-human chain (Li et al. 2009; Ahmad et al. 2015). Low concentration of cadmium in sludge compared to control treatment can be attributed to the effect of dilution. Sewage sludge significantly increased cadmium absorption (multiplying plant yield by metal concentration) in wheat stems.

Dede and Ozdemir (2016) found that the concentration of cadmium in the stem of *Conyza canadensis* was significantly higher than the control treatment in sludge treatments. As shown in Table 4, in Sivand variety using sludge caused a significant decrease in lead concentration in stems. Thus, lead concentration at the levels of 60 and 120 g/kg sludge compared to control treatment decreased 4 and 46%, respectively. In Roshan variety, the use of sludge in comparison to the control treatment did not have a significant effect on lead concentrations in stems (Table 4). The effect of sewage sludge, variety, and their interaction effect was significant on lead concentrations of stems ($P \leq 0.01$; Table 5). Some researchers reported that lead absorbed by the plants remained in the roots, with only a small amount transferred to the stems of the plant (Malavolta 2006; Kabata-Pendias and Mukherjee 2007; Hooda 2010).

Results revealed that in Sivand variety, the use of the level of 120 g/kg sludge caused a significant increase in iron concentration in the stems. In Roshan variety, using sludge compared to control treatment caused a significant increase in iron concentration in the stems. Iron concentration in the stem of Roshan variety at the levels of 60 and 120 g/kg was also higher than in Sivand variety (Table 4). The effect of sewage sludge, variety, and their interaction effect was significant on iron concentration in the stem ($P \leq 0.01$) (Table 5). The results of a study by Najafi et al. (2012) on the common sunflower (*Helianthus annuus* L.) revealed that the use of sewage sludge caused iron absorption by plant stems.

According to the obtained results in Table 4, in Sivand variety using sewage sludge compared to the control treatment caused a significant increase in manganese concentration in the stems, so much so that no significant difference was observed between levels of 60 and 120 g/kg sludge. In Roshan variety, the use of sludge compared to control treatment had no significant effect on manganese concentration in the stems (Table 4). The results of ANOVA showed that the effect of sewage sludge on manganese concentration in the stems at the 5% level and the effect of variety at the 1% level were statistically significant, but the interaction effect of the sludge and variety had no significant effect on the concentration of manganese in the stems (Table 5). Nazmi et al. (2011) reported an increase in manganese concentrations in barley stem due to using sludge.

The concentration of heavy metals in seed

Mean, standard deviation, and range of heavy metals in seeds of Sivand and Roshan wheat varieties in the treatments discussed below are presented in Table 6.

The results revealed that in Sivand variety, by using sewage sludge, copper concentration in seeds at levels of 60 and 120 g/kg compared to the control treatment decreased 28%, but no significance difference was observed between levels 60 and 120 g/kg sludge. In Roshan variety, the use of 60 g/kg sludge compared to control treatment had no significant effect on copper concentration in the seeds, but at 120 g/kg, sludge caused a 16% increase in copper concentration in seeds (Table 6). The effect of sewage sludge, variety, and their interaction effect was significant on copper concentrations in the seeds ($P \leq 0.01$) (Table 7).

High concentration of copper decreases growth, biomass, and the total amount of chlorophyll in the plant (Chantachon et al. 2002). Jamali et al. (2009) reported an increase in copper concentration in wheat compared to control treatment in soil treated with sewage sludge.

Results revealed that in Sivand and Roshan wheat varieties, the use of 120 g/kg sludge compared to control treatment did not have a significant effect on zinc concentration in seeds, but the level of 60 g/kg sludge caused an 8% reduction in zinc concentration in seeds. Zinc concentration in seed compared to control treatment in concentrations of 60 and 120 g/kg, respectively, increased 59 and 100% (Table 6). The effect of sewage sludge and the interaction effect of sewage sludge and variety on zinc concentration in the seed was significant ($P \leq 0.01$), but the effect of variety on zinc concentration in the seeds was not significant (Table 7).

Results revealed that in the case of Sivand and Roshan wheat varieties, the use of 120 g/kg sludge compared to control treatment caused a significant increase in cadmium concentration in seeds, but the level of 60 g/kg sludge compared to control treatment caused a significant decrease in cadmium concentration in seeds. Cadmium in seeds of Sivand variety at the level of 120 g/kg compared to the control treatment increased 30%, and in Roshan variety compared to the control treatment increased 1.2% (Table 6). At the 1% level of significance, the effect of sewage sludge and the interaction effect of sewage sludge and variety on cadmium concentration in seed was significant, but the effect of variety on cadmium concentration in seed was significant at the 5% level of significance (Table 7). An increase in the concentration of cadmium in wheat seeds due to use of sludge was reported by Jamali et al. (2009). The results of a study on rice by Latare et al. (2014) showed that in treatments of 20 t/h^{-1} and more, sludge cadmium concentration in rice seeds was more than the limit. Chaudri et al. (2007) revealed that the increase of soil cadmium increased cadmium concentration in wheat seeds, while an increase of soil pH and soil organic carbon reduced its concentration in the plant. Thus, a high amount of soil pH and soil organic carbon reduced cadmium available to plant for absorption and transmission into seeds. Other studies have shown that total cadmium in soil and soil pH is the most significant factor in absorbing cadmium into products (McBride 2002; Adams et al. 2004).

Means comparison revealed that in Roshan variety, the use of sludge compared to control treatment did not have a significant effect on lead concentrations in the seeds. In Sivand variety, the use of 60 g/kg sludge compared to control treatment also had no significant effect on lead concentrations in seeds, but using 120 g/kg sludge compared to control

Table 6 Effect of sewage sludge on heavy metal concentrations (mg kg^{-1}) in seeds of Sivand and Roshan wheat varieties [mean ± SD, (range)]

Metals	Sivand			Roshan		
	Control	60 (g kg^{-1})	120 (g kg^{-1})	Control	60 (g kg^{-1})	120 (g kg^{-1})
Cu	61.5 ± 2.2 ^{a}	43.8 ± 1.4^c	44.4 ± 1.6^c	44.1 ± 1.5^c	44.5 ± 1.4^c	51.5 ± 1.1^b
	(59.5–63.4)	(41.8–45.9)	(42.3–46.4)	(42–46.1)	(42.5–46.6)	(49.5–53.6)
Zn	44.2 ± 1.4^b	40.2 ± 1.8^c	44.6 ± 1.6^b	27.3 ± 1^d	43.5 ± 1.5^b	56.5 ± 1.5^a
	(42.2–46.1)	(38.3–42.1)	(42.7–46.5)	(25.4–29.2)	(41.5–45.4)	(54.6–58.4)
Cd	2.8 ± 0.18^c	1.4 ± 0.21^e	3.7 ± 0.11^b	2.2 ± 0.14^d	1.4 ± 0.16^e	4.9 ± 0.1 ^{a}
	(2.6–3)	(1.2–1.6)	(3.5–3.9)	(2–2.4)	(1.2–1.6)	(4.7–5.1)
Pb	51.7 ± 2.8^a	50.3 ± 2^a	45.7 ± 1.3^b	21.9 ± 1.1^c	22.9 ± 1.3^c	23.8 ± 1.6^c
	(49.4–54)	(49–53.6)	(43.4–48)	(19.6–24.2)	(20.6–25.2)	(21.5–26.1)
Fe	111.4 ± 0.97^a	71 ± 1.4^{cd}	67.2 ± 1.4^d	94.6 ± 3.8^b	72.6 ± 2.4^c	72.9 ± 2.5^c
	(108.5–114.3)	(68.1–73.9)	(64.3–70.1)	(91.7–97.5)	(69.7–75.5)	(70–75.8)
Mn	19.3 ± 0.52^c	10.7 ± 0.58^d	10.1 ± 0.71^d	34.1 ± 2.4^b	40.2 ± 1.9^a	41.3 ± 1.1^a
	(17.5–21.1)	(8.9–12.6)	(8.3–11.9)	(32.3–36)	(38.3–42)	(39.5–43.1)

Means which have at least one common letter in each column are not significantly different at the level of .05 of Duncan Test

Table 7 ANOVA results of sewage sludge effect on the heavy metal concentrations in the seeds of Sivand and Roshan wheat varieties

Mean squares							
Change sources	Degree of freedom	Cu	Zn	Cd	Pb	Fe	Mn
Sewage sludge	2	112.6**	332.6**	12.82**	17.36*	2065**	2.67^{ns}
Variety	1	45.92**	1.46^{ns}	0.168*	3376**	45.06*	2847**
Sludge × variety	2	244.3**	325.7**	1.31**	33.98**	215.4**	121.1**
Error of measurement	12	2.68	2.33	0.026	3.30	5.32	2.11

**Significant at the level of 0.01 and 0.05 of Duncan test, respectively. ns = not significant at the level of 0.01 of Duncan Test

treatment caused an 11% decrease in lead concentrations in the seeds (Table 6). Results revealed that the effect of using sewage sludge on lead concentration in seeds was significant at a 5% significance level, while the effect of variety, interaction effect of variety, and sludge and variety were significant at the 1% significance level (Table 7). A study by Al Zoubi et al. (2008) disclosed that sewage sludge had no effect on lead concentration in seeds of wheat and corn. Lead concentrations in the seeds of both cultivars compared to stems were higher. Jamali et al. (2009) showed that the use of sewage sludge increased the lead concentration in wheat seeds. Singh and Agraval (2010) reported that the use of sewage sludge increased the lead concentration in rice seeds.

In both Sivand and Roshan wheat varieties, the use of sludge compared to control treatment caused a reduction in iron concentration in seeds. Therefore, there was no significant difference between levels of 60 and 120 g/kg sludge (Table 6). The results revealed that the effect of sewage sludge, and the interaction effect of variety and sewage and sludge on iron concentration, were significant at the 1% significance level. Furthermore, the effect of variety at the 5% significance level was significant (Table 7). Iron concentration in the roots was higher compared to that of stems and seeds. This can be attributed to iron's low mobility in the plant, which can cause accumulation of iron in the stem of the plant, and a lack of transmission to stems (Kashem and Singh 2001). There are some compounds, such as phytokeratin, in plants that consolidate some metals in roots or leaves and prevent dynamicity and their transmission to seeds (Marschner 1995).

Means comparison showed that in Sivand variety, the use of sludge compared to control treatment caused a 44% decrease in manganese concentration in seeds. No significant difference in manganese concentration occurred between levels of 60 and 120 g/kg sludge. Manganese concentrations in Roshan variety seeds compared to control treatment in concentrations of 60 and 120 g/kg increased 17 and 21%, respectively (Table 6). The results of ANOVA revealed that the effect of sewage sludge on manganese concentration in seeds was not significant, but the effect of variety and the interaction effect of variety and sewage sludge on manganese concentration in seeds were significant at the 1% significance level (Table 7). Bai et al. (2014) reported the increase of manganese concentration in ryegrass.

In Sivand variety, concentrations of heavy metals were respectively as follows: for root, Cu > Pb > Zn > Cd > Fe > Mn>; for stem, Fe > Cu > Pb > Zn > Mn > Cd; for seed, Zn > Fe > Pb > Cu > Mn > Cd. In Roshan variety, concentrations of heavy metals were as follows: for root, Cu > Pb > Zn > Cd > Fe > Mn>; for stem, Fe > Cu > Mn > Zn > Pb > Cd; for seed, Fe > Cu > Zn > Mn > Pb > Cd.

According to Table 8 and on the basis of the normal and toxicity ranges of heavy metals in plants, the concentrations of zinc, iron, and manganese in seeds and stems of Sivand and Roshan wheat varieties were less than the toxicity limit in the normal range. Cadmium concentration in seeds of both varieties for control treatment and treatment 60 g/kg sludge was in the normal range, and for treatment 120 g/kg was above the normal range but below the toxicity limit. Cadmium concentrations in stems of both varieties were above the normal and below the toxicity limit. Lead concentration in seed and stem of Roshan variety was above the normal range and below the toxicity limit; however, in Sivand variety, lead concentrations in seed and stem were in the range of toxicity. Copper concentrations in seed and stem of both wheat varieties were in the toxicity range.

Smith (1994) reported that the elements deposition in the form of insoluble hydroxide, carbonates, and organic complexes increased by increasing soil pH. Thus, the availability of soil heavy metals for plants in low pH soil compared to high pH soil was higher.

Bose and Bhattacharyya (2008) stated that the difference between the ratio of the amount of heavy metals and heavy metal

Table 8 Normal and toxic values from heavy metals observed in plants (mg/kg) (Kabata-Pendias and Mukherjee 2007)

Metal	Normal range	Toxicity range
Cu	20–5	20–100
Zn	1–100	100–400
Fe	400–500	500–1500
Mn	5–100	100<
Cd	.1–2.4	5–30
Pb	.2–20	30–300

uptake in wheat depends on many factors, including (a) the use of sludge in the soil, (b) concentration of heavy metals in soil treated with sludge, (c) soil physical and chemical parameters, and (d) the various parts of the plant. Keller et al. (2001) found that metal uptake from soils treated with sludge was significantly different between various products and various parts of a plant.

The long-term use of sludge in agricultural lands causes accumulation of heavy metals in soil and increases the possibility of absorbing these elements in plant (McBride 1995). Chang et al. (1987) reported that the highest amount of metal uptake by plants occurred during the first 2 years after sludge use. Then, this absorption decreases and constantly continues as time passes. Jamali et al. (2009), in an investigation of heavy metal concentrations in different wheat varieties in soil treated with sewage sludge, found that there were differences in absorption between various species, harvest time, metal concentrations, soil type, and the wheat plant outfit.

Conclusion

Farmers at times turn to sewage sludge as an affordable, easily obtainable fertilizer for their crops. Unfortunately, sewage sludge carries undesirable elements, which can make their way into the food chain. This study evaluated two levels of treatment of sewage sludge, in an effort to understand whether the uptake of undesirable elements could be overcome or reduced. The Sivand and Roshan varieties of wheat, widely used in Iran, were tested with these treatments.

This research concluded that in the treated seed and stem of Sivand variety, concentrations of Zn, Cd, Fe, and Mn were below the standard toxicity limit, and concentrations of Cu and Pb were above the standard limit. In the Roshan variety, the concentrations of Zn, Cd, Pb, Fe, and Mn were below the standard limit, and Cu concentration was above the standard.

The results of this study lead to the recommendation that farmers avoid using sewage sludge in farming, as much as possible. Instead, it is far more appropriately employed as a fertilizer for green space, ornamental trees, and parks, where edible products for human consumption are not grown.

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Compliance with ethical standards

Disclosure of potential conflicts of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals For this type of study, formal consent is not required. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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