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RESEARCH ARTICLE

Heavy metals uptake and translocation by lettuce and spinach grown on a metal-contaminated soil

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Abstract

Nowadays, using sewage wastewaters in the irrigated agriculture is commune creating hazardous environment impacts. Assessment of these negative effects is vital issue to prevent heavy metals to be introduced in the food chain. Field and laboratory studies were conducted at Arab-El-Madabegh village, Assiut Governorate, Egypt, in order to evaluate the heavy metals concentrations in the edible parts of lettuce and spinach plants which irrigated with sewage water (SW). The obtained results indicated that, the soils of the studied site were contaminated by heavy metals. Zinc, Cu, Pb, Cd and Ni concentrations in the edible portions of the studied vegetables plants ranged between 75- 110, 15 - 17, 2 - 5, 1.0 -3.5 and 1.0 -2.5 mg kg^{-1}, respectively. The obtained results showed that the concentrations of Zn, Pb, Cd and Ni in the edible parts of the studied plants were higher than the permissible limit levels but those of Cu were within the safe limit levels. It is worthy to mention that the irrigated edible vegetable crops with SW should be avoided. This study highlights the potential hazard for human health due to the uptake of high concentrations of heavy metals especially Zn, Ni, Cd and Pb by the studied vegetable crops
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Keywords: Contaminated soils, Safe limits, Human consumption, Heavy metals.

1. Introduction

Water is important for all human activities. Water accounted for about 50-97% of plant and animal bodies; moreover, it is a vital for all biological processes in plant and animal cells (Buchholz, 1998). Fresh water recourses in the world are very limited and only 0.6% of the total world water resources is fresh water (Fakayode, 2005). Fresh water resources have been decreased in an alarming rate and they may not able to meet the requirements of the different human in the future (Qadir *et al.*, 2008). The agriculture sectored uses about 80% of the water resources in irrigation purposes. Most of agriculture lands located near urban are irrigated by wastewater because of the low availability of fresh water for crop production (Ghimire, 1994).

The increasing of population and human activities enlarged the volume of sewage wastewaters (SW) (Qadir *et al.*, 2008). In many developing countries, these water resources may, in most cases, use in the form of diluted raw sewage, even if it is considered unlawful (Huibers *et al.*, 2004). Wastewaters quality differs both between and within countries. In many poor countries in Africa, Asia and Latin America, the untreated wastewaters are used widdly in agriculture production, while in middle-income countries treated wastewater is used (Faruqui *et al.*, 2004).

The use of sewage wastewater in the irrigation provides the soil with nutrients and organic matter; moreover, it is an inexpensive system for wastewater disposal (Ghosh *et al.*, 2012). In many situations, Egyptian farmers use wastewater in irrigation even when the fresh one is available, due to the high profits earned by using waste one. Sewage wastewaters (SW) are usually rich in nitrogen (N), phosphorus (P) and potassium (K) and farmers use these waters as low price fertilizers (Chhabra, 1989). Nutrient concentrations in sewage waste waters are varied widely and Chhabra (1989) found that the SW contained 48.3, 7.6, 72.4 and 34.6 mg L^{-1} of N, P, K and S, respectively besides micronutrients contents of 0.34, 10.8, 0.2 and 0.36 mg L^{-1} for Zn, Fe, Cu and Mn, respectively. Therefore, ten SW irrigations of 7.5 cm each could add about 362, 58, 540 and 260 kg ha^{-1} of N, P, K and S, respectively, to the soil which are more than the nutrient requirements of most crops (Eissa, 2016). These findings recapitulate that wastewater has a great potential as a manure when it is used to irrigate crops (Khurana and Singh, 2012).

Wastewaters contain high levels of Cd, Ni, Pb, Cr and As. These elements are not essential for plant and animal nutrition (Kanwar and Sandha, 2000). The use of sewage wastewater can in the irrigation processes may cause remarkable increases in soil heavy metal concentrations (Khan *et al.*, 2008; Ghosh *et al.*, 2012). The raising of soil heavy metal content will lead to introduce the metals to the vegetables and cereals crops causing a potential health risk to human and animal (Singh *et al.*, 2010; Gupta *et al.*, 2011). The concentrations of heavy metals in plants cultivated on wastewater-irrigated soils are significantly higher than in those grown on fresh water-irrigated ones (Khan *et al.*, 2008; Singh *et al.*, 2010; Gupta *et al.*, 2011). The use of sewage wastewater to irrigate plants is an old acction in many areas in Egypt due to the complexity of its treatment and disposal as well as the scarcity of fresh irrigation water. Its use is obligatory in order to provide foods to the ever-increasing population. It may cause soils and plants to be contaminated with heavy metals. The present study was undertaken to assess heavy metal contents of lettuce and spinach plants irrigated with sewage wastewater.

2. Materials and Methods

2.1. Site description and sampling

Composite plant samples of spinach (*Spinacia olivera* L) cv. Balady and lettuce (*Lactuca sativa* L.) cv. Balady irrigated with sewage wastewater were collected from Arab Elmadabegh village, Assiut governorate, Egypt which are located at 27° 12^{-} $16.67^{=}$ N latitude and 31° 09^{-} $36.86^{=}$ E longitude to evaluate levels of some heavy metals (Zn, Cu, Pb, Ni and Cd) in these vegetables plants. The soils in this village have been irrigated by raw sewage water for more than 50 years. Table 1 shows the main properties of the studied site. Each composite sample included the edible portion of ten plants of each crop. Inorganic wastes were removed from plant samples by washing in 0.1 HCl and Tween 80. The plant samples were washed twice by tap water, rinsed by distilled water, air-dried, oven-dried at 70 ^{o}C to a constant weight, ground and then were kept for chemical analysis. Soil (0-30 cm) and sewage wastewater samples were also taken from the experimental site.

Table 1. Some physical and chemical characteristics of the soil in the studied site

Soil properties	0-30 cm	*Permissible limits
Clay instate of P	110	
Silt (g/kg)	180	
Sand (g/kg)	710	
Texture	Sandy loam	
$CaCO_3$ (g/kg)	70	
pH (1:2)	7.85	
CEC (cmol/kg)	18	
Total Organic-C (g/kg)	25.2	
EC (1:2) (dS/m)	1.8	
Available-Zn	7.5	
Available-Cu	5.0	
Available-Pb	6.4	
Available-Cd	0.40	
Available-Ni	1.20	
Total-Zn	620	200-300
Total-Cu	310	50-140
Total-Pb	300	300
Total-Cd	6.5	3.0
Total-Ni	50	50

*Permissible limits according to European Union Standards (EU, 2002) and U.S. Environmental Protection Agency (USEPA, 1997).

2.2. Soil, water and plant analysis

The physical and chemical properties of the studied soil samples were determined according to Burt (2004) as they are shown in Table 1. The available heavy metals (Zn, Cu, Pb, Cd and Ni) were extracted from the soil samples using a 0.005 M DTPA (diethylen triamine penta acetic acid) solution buffered at pH 7.3 as described by Lindsay and Norvell (1978). To determine the total heavy metals, the soil sample was digested according to the procedure given by the US EPA (1996). A known volume of sewage wastewater sample was oven-dried and then was digested using concentrated HNO_3 at 80 ^oC (Table 2). The soil sample was air-dried and sieved with a 2-mm diameter sieve and kept for analysis. The metals in the soil, water and plant digest as well as DTPA soil extract were measured using the Inductivity Coupled Plasma Emission Optical Emission Spectrometry (ICP−OES thermo iCAP 6000 series). The ground plant samples were digested using concentrated acids of HNO_3 and $HClO_4$. The reference material (SRM 1547) was measured at the same time for quality assurance. The obtained results of the reference material indicated that the determined levels are within the range of certified value. Recovery of Zn, Cu, Cd, Ni and Pb was 104, 101, 97, 98 and 105%.

2.3. Data analysis

The one-way ANOVA was run by SPSS software (version 15) to test the statistically significant difference between the three treatments. Moreover, Duncan test (at 5% probability) was performed to compare between means of the tested treatments.

Table 2. Chemical analysis of the irrigation sewage wastewater in the studied site

pH	EC (dS m^{-1})	Zn	Cu	Pb	Cd	Ni
7.25	3.5	0.45	0.28	1.3	0.06	0.06
PL*		0.20	0.20	5.0	0.01	0.20

*Permissible limits according to FAO (1985). Each value represents the mean of three replicates.

3. Results

3.1. Soil and sewage wastewater heavy metals

The heavy metal content of the irrigation source and soil in the experimental site was analyzed and the data are presented in Tables 1 and 2. The concentrations of Zn, Cu, Pb, Cd and Ni in the investigated wastewater sample were 0.45, 0.28, 1.30, 0.06 and 0.06 mg L^{-1}, respectively. The total concentrations of Zn, Cu, Pb, Cd and Ni in the studied soil sample were 620 to 310, 300, 6.5 and 130 mg kg^{-1}, respectively. The total content of soil heavy metal determines the level of contamination.

3.2. Heavy metals concentrations in the roots and shoots of lettuce and spinach plants

The concentrations of Zn in the shoot and root of the studied plants ranged between 75 and 200 mg kg^{-1} (Figure 1). The concentrations of Zn in the root and shoot of lettuce were 120 and 75 while those of spinach were 200 and 110 mg kg^{-1}, respectively. The highest Zn concentration was recorded in the root of lettuce and spinach plants. The root and shoot of spinach plants significantly (P< 0.05) contained higher levels of Zn rather than those of lettuce.

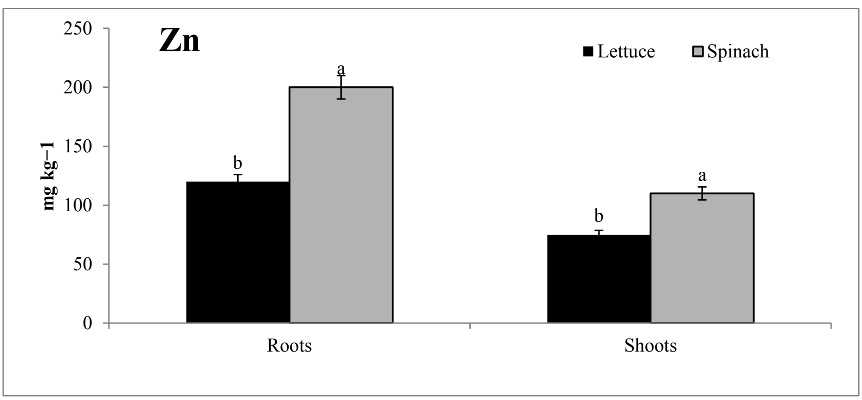

Figure 1. Zinc (Zn) concentrations (mg kg^{-1}) in the roots and shoots of lettuce and spinach plants

Means denoted by the same letter indicate no significant difference according to Duncan's test at P<0.05.

Copper (Cu) concentrations in the shoot and root of lettuce and spinach plants varied from 15 and 30 mg kg^{-1} (Figure 2). The highest value of Cu concentration was recorded for the root of spinach and lettuce (30 and 35 mg kg^{-1}) while the lowest concentrations were obtained in the shoots of the two mentioned plants (15 and 17 kg^{-1}). The root and shoot of spinach plants significantly (P< 0.05) contained higher levels of Cu rather than those of lettuce.

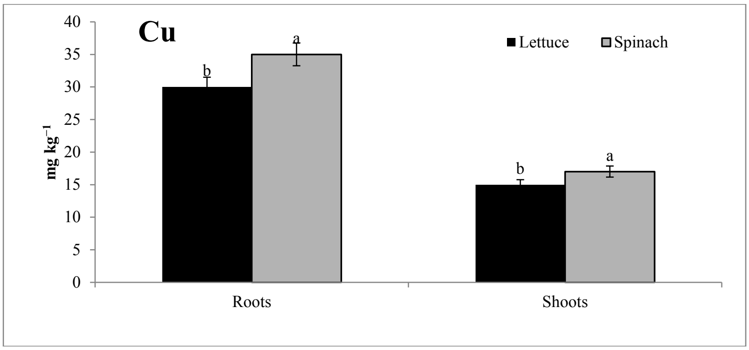

Figure 2. Copper (Cu) concentrations (mg kg^{-1}) the roots and shoots of lettuce and spinach plants

Means denoted by the same letter indicate no significant difference according to Duncan's test at P<0.05.

Lead (Pb) content of the root and shoot of lettuce and spinach plants differed between 2.0 and 20.0 mg kg^{-1} (Figure 3). The concentrations of Pb in the roots of lettuce and spinach were 14 and 20 mg kg^{-1} while in the shoots these concentrations were 2.0 and 5.0 mg kg^{-1}, respectively. Lead (Pb) concentrations in the root and shoots of spinach were significantly higher than those in lettuce; moreover, the root contained very higher concentrations compared to the shoot.

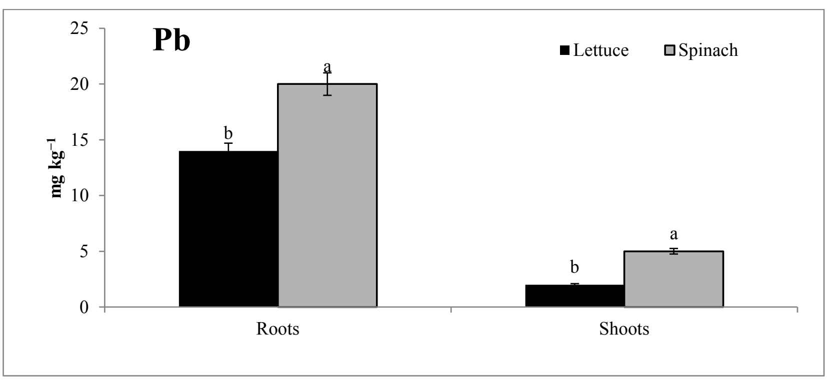

Figure 3. Lead (Pb) concentrations (mg kg^{-1}) in the roots and shoots of lettuce and spinach plants

Means denoted by the same letter indicate no significant difference according to Duncan's test at P<0.05.

The edible parts of lettuce and spinach plants contained Cd levels varied between 1.50 and 20.0 mg kg^{-1} (Figure 4). The concentrations of Cd in the shoot of lettuce and spinach were 1.50 and 3.0 mg kg^{-1}; while in roots were 15 and 20 mg kg^{-1}, respectively. The root and shoot of spinach plants significantly (P< 0.05) contained higher levels of Cd rather than those of lettuce

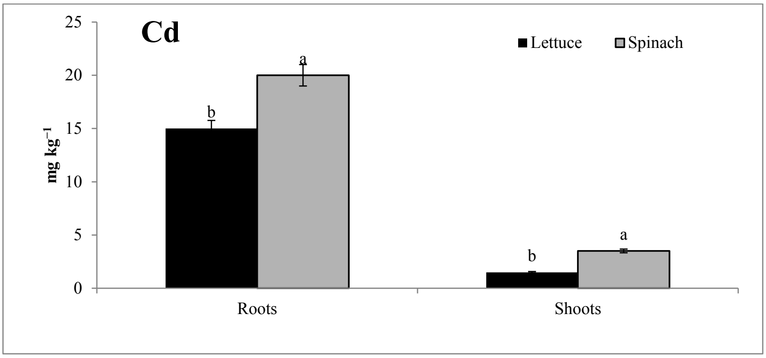

Figure 4. Cadmium (Cd) concentrations (mg kg^{-1}) in the roots and shoots of lettuce and spinach plants

Means denoted by the same letter indicate no significant difference according to Duncan's test at P<0.05.

Nickel (Ni) levels in the edible parts of lettuce and spinach plants varied from 1.5 to 15 mg kg^{-1} (Figure 5). The concentrations of Ni in the shoot of lettuce and spinach were 1.0 and 2.5 mg kg^{-1}; while in the roots were 12 and 15 kg^{-1} respectively. Nickel (Ni) concentrations in the root and shoots of spinach were significantly (P< 0.05) higher than those in lettuce; moreover, the root contained very higher concentrations compared to the shoot.

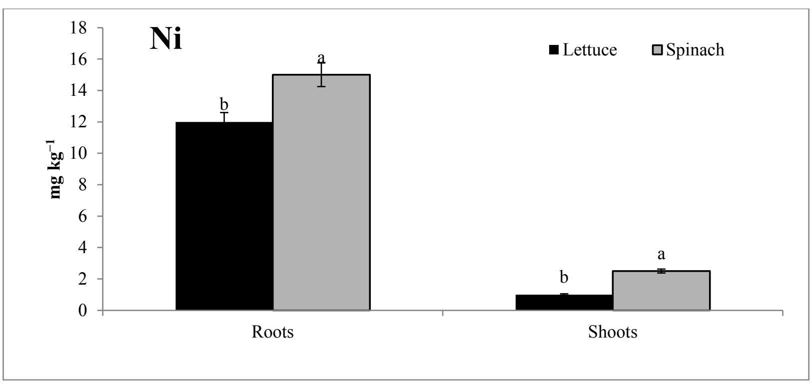

Figure 5. Nickel (Ni) concentrations (mg kg^{-1}) in the roots and shoots of lettuce and spinach plants

Means denoted by the same letter indicate no significant difference according to Duncan's test at P<0.05.

3.3. Translocation of heavy metals from soil to roots and from roots to shoots

The behavior of heavy metal accumulation by lettuce and spinach plants was investigated by calculation of root-shoot and soil-root transfer of the studied metals and the data are shown in Table 3. The bioaccumulation factor (BAF) was calculated from the metal in the root divided by the available metal in the soil. The BAC is used to assess the soil-root transfer of metal (Eissa and Ahmed, 2016). The values of BAC ranged between 1.9 and 50.0 and these values significantly differed between the investigated metals as well as between lettuce and spinach plants. The values of BAF of lettuce were 16.0, 6.0, 11.7, 37.5 and 1.9 for Zn, Cu, Pb, Cd and Ni, respectively. The values of BAF of spinach were 26.7, 7.0, 16.7, 50.0 and 2.30for Zn, Cu, Pb, Cd and Ni, respectively. The BAC of spinach was higher than that of lettuce for all the studied metals. The values of BAC of Cd were the highest either in the case of lettuce and spinach plants. The BAC values were found in the order: Cd >Zn >Pb>Cu>Ni.

The translocation factor (TF) was calculated from the metal in the shoot divided by that found in the root. The TF is used to assess the root-shoot transfer of metal (Eissa and Ahmed, 2016). In the current study the values of TF ranged between 0.08 and 0.63 and these values differed significantly from metal to metal as well as between the studied plants. The TF values were found in the order: Zn>Cu>Pb>Cd>Ni. The highest TF value was recorded in the case of Zn and Cu, while the lowest were found in the case of Pb, Cd and Ni. The TF values of Pb, Cd and Ni in the case of spinach were higher than those of lettuce.

Table 3 Translocation of heavy metals from soil to roots and from roots to shoots

Plant	BAF					TF				
	Zn	Cu	Pb	Cd	Ni	Zn	Cu	Pb	Cd	Ni
Lettuce	16.00 b	6.0 a	11.7 b	37.5 b	1.9 b	0.63 a	0.50 a	0.14 b	0.10 b	0.08 b
Spinach	26.67 a	7.0 a	16.7 a	50.0 a	2.3 a	0.55 b	0.49 a	0.25 a	0.18 a	0.17 a

BAF = metal concentration in the root/ the concentration of the available metal in the soil.

TF = metal concentration in the shoot/ metal concentration in the root.

Means denoted by the same letter indicate no significant difference according to Duncan's test at $P<0.05$.

4. Discussion

4.1. Heavy metals in the studied soil and sewage wastewater

The levels of Zn, Cu and Cd were higher than the permissible limits of the irrigation water according to the FAO (1985). On the other hand, Pb and Ni concentrations in this wastewater sample were lower than the FAO (1985) allowable limits. The higher Zn, Cu and Cd concentrations recorded in the studied water sample of Arab-Elmadabegh, where the sewage wastewater collection and treatment station of Assiut city is located.

The total concentrations of all the investigated metals (Zn, Cu, Pb, Ni and Cd) in soil were above the maximum permissible limits recorded by EU (2002) and USEPA (1997). These obtained values confirmed that the soil under study is contaminated with these heavy metals. Similar results were found by Eissa *et al.* (2016). The long-term use of treated and untreated wastewater in irrigation was reported to cause significant buildup of the heavy metals in the soils (Khan *et al.*, 2008; Ghosh *et al.*, 2012; Eissa, 2014).

4.2. Heavy metals in the roots and shoots of lettuce and spinach plants

The root and shoot of spinach plants contained higher levels of Zn rather than those of lettuce. These results were confirmed by Eissa (2016) who found that the concentrations of Zn in the leaves of spinach and lettuce grown on a metal contaminated soil were 225 and 78 mg kg^{-1}, respectively. According to WHO/FAO (2007) and EU (2006), the maximum permissible Zn limit for human consumption is between 60 and 80 mg kg^{-1} dry weights. Thus, the concentrations of Zn in the shoot of lettuce were less than the allowable level and these plants are safe for human consumption. The shoot of spinach contained Zn concentration more than the allowable levels and is not safe for human consumption.

The maximum permissible concentration of Cu in the edible parts for human consumption is 40 mg kg^{-1} dry weight (WHO/FAO, 2007; EU, 2006). Therefore, the edible parts of these plants are safe to be used by human being. The obtained results were confirmed by Eissa (2016) who reported that the concentrations of Cu in the leaves of lettuce and spinach grown on a metal contaminated soil were 16 and 15 mg kg^{-1}, respectively.

According to WHO/FAO (2007) and EU (2006) the maximum Pb permissible level for human consumption is 0.3 mg kg^{-1} dry weight. The concentrations of Pb in the shoot of lettuce and spinach were 2.0 and 5.0 mg kg^{-1}, respectively. Thus, the concentrations of Pb in the edible plant parts were higher than this permissible level indicating their unsafe to be used. The results indicated that sewage wastewater use in irrigating these vegetables caused a significant increase in Pb content in their edible portions. The obtained results are in an agreement with those of Rattan *et al.* (2005) and Lone *et al.* (2003). Eissa (2016) found that the concentrations of Pb in the leaves of spinach and lettuce grown on a metal contaminated soil were 1.9 and 0.3 mg kg^{-1}, respectively.

WHO/FAO (2007) and EU (2006) indicated that the maximum permissible level of Cd for human consumption is 0.2 mg kg^{-1} dry weight. The concentrations of Cd in the shoot of lettuce and spinach were 1.50 and 3.0 mg kg^{-1}, respectively. Thus, the concentrations of Cd in these edible parts were higher than that permissible limit which they are not safe to be consumed. The irrigation of these vegetables with sewage wastewater caused a significant increase in Cd concentrations in their edible portions. These coincide with those of Rattan *et al.* (2005) and Lone *et al.* (2003).

The maximum permissible Ni level for human consumption is 1.5 mg kg^{-1} dry weight (WHO/FAO, 2007; EU, 2006). The concentrations of Ni in the shoot of lettuce and spinach were 1.0 and 2.5 mg kg^{-1}, respectively. Thus, levels of Ni in the edible parts of these plants were higher than its permissible limit level. The use of sewage wastewater in irrigation these vegetables caused a significant increase in Ni concentrations in the edible portions. These results are in the same line with those of Rattan *et al.* (2005) and Lone *et al.* (2003). The prolonged application of treated and untreated wastewaters results in significant buildup of heavy metals in the soils (Khan *et al.*, 2008; Ghosh *et al.*, 2012; Durán *et al.*, 2016) and grown vegetables and cereals which they subsequently transfer to the food chain causing potential health risk to consumers (Singh *et al.*, 2010; Gupta *et al.*, 2011). Heavy metals concentrations in plants grown on wastewater-irrigated soils were reported to be significantly higher than those grown on fresh water-irrigated soils (Khan *et al.*, 2008; Singh *et al.*, 2010; Gupta *et al.*, 2011).

4.3. Translocation of heavy metals from soil to roots and from roots to shoots

The data of the current study indicated that the value of Cd soil-root transfer was the highest one. The high rate of the soil-root transfer in the case of Cd may be due to the high mobility of this metal compared to the other studied metals (Eissa *et al.*, 2016). The values of root-shoot transfer of the investigated metals were less than one indicating that the lettuce and spinach plants absorbed more metals in roots and transfered less concentrations to the shoots (Eissa and Ahmed, 2016). In general, the root-shoot transfer of Zn and Cu was higher than that of Pb, Cd and Ni. Similar results were found by Eissa (2016) and Eissa *et al.* (2014). The higher rate of the TF values in the case of Zn and Cu may be due to they are essential micronutrients (Marschner, 1995; Amin and Eissa, 2017) while Pb, Cd and Ni are toxic metals. The plants tend to accumulate the heavy metal in the roots and transfer a few concentrations to the shoots (Wozny, 1995); Voutsa *et al.*, 1996; Verdejo *et al.*, 2016). Kadukova *et al.* (2004) found that the Pb stored in plant roots was from 93 to 98 % from the total Pb absorbed by these plant roots. The root and shoot of spinach plants contained higher levels of Pb rather than those of lettuce. High concentrations of Cd and Pb in the roots of lettuce and spinach plants may be related to its low translocation within plants (Voutsa *et al.*, 1996, Kadukova *et al.*, 2004; Eissa, 2016).

5. Conclusion

It might be concluded that heavy metals could accumulated in the edible vegetables that irrigated with sewage wastewater. In this study, the obtained results showed that the concentrations of Pb, Cd and Ni in the edible parts of some vegetables were higher than their permissible limit levels. Therefore, the edible parts of lettuce and spinach plants are not safe for human consumption. It is worthy to mention that irrigated edible vegetable crops irrigated with sewage wastewater should be avoided and Egyptian guidelines should be developed for the reuse of these waters in agriculture. Therefore, it is recommended to never use sewage wastewater to irrigate vegetables unless it is obligated. Sewage wastewater might be used to irrigate other plants such as woody trees that can be used as a wind break as well as energy producer plants.

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