Journal of King Saud University – Science 35 (2023) 102382

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Heavy metals toxicity in spinach (*Spinacia oleracea*) irrigated with sanitary wastewater in rural areas

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ARTICLE INFO

Article history:
Received 8 September 2022
Revised 9 October 2022
Accepted 12 October 2022
Available online 17 October 2022

Keywords:
Heavy metals
Irrigation
Soil
Water
Spinach

ABSTRACT

Heavy metal contamination harms soil and plant growth, making it a serious global environmental issue. Due to the scarcity of canal water, people have become more reliant on groundwater, but aquifers are expensive and of poor quality. Therefore, wastewater has become one of the essential sources of irrigation in developing countries and rural areas. In rural areas, sanitary, sewage, and municipal wastewater are the most common sources of irrigation. The objective was to analyze and compare the concentration of heavy metals in Spinach (*Spinacia oleracea*) collected from two different sites, i.e., one field irrigated using sanitary wastewater and another by tube well water through drainage ditches in a rural area of Faisalabad. AAS (Atomic Absorption Spectrophotometry) analyzed heavy metals from plants through irrigated water, soil, and the collected edible parts of plant samples. The samples were digested by using acids and AAS-analyzed metals. The concentration of Cd (0.051 ± 0.029) mg/l found higher than the recommended values by WHO (0.01 mg/l) in sanitary wastewater, as well as Cd concentration (0.478 ± 0.276) mg/kg in edible parts of spinach, also detected beyond the permissible limits (0.2) mg/kg of WHO (2007). These exceeding values of Cd in edible parts of spinach may cause serious human health risks.

1. Introduction

Agriculture is critical not only for domestic food security but also for sustainable poverty prevention. Agriculture is acknowledged to be one of the most significant roles in the economy in all economies, particularly in developing countries (Soubbotina and Sheram, 2000). The agriculture sector is the backbone of Pakistan (Ahmad and Heng, 2012). Agricultural innovation "systems are an indispensable part of modern farming, yet they are helping" to optimize agricultural profitability and efficiency (Tingey-Holyoak et al., 2021). Agriculture, the quality of cultivated food, and population health are all factors that influence global food security (Salimova et al., 2020). Irrigation water is extensively used in agriculture. Pakistan possesses the world's most extensive irrigation system, the Indus Basin Irrigation System (IBIS). Due to water scarcity, wastewater has become a common application for irrigation. "In Pakistan, around 99 percent of sewage water is directly used for crop growth and released into various water bodies, with barely 1 % being cleaned in major towns" (Sardar et al., 2020). In rural Pakistan, wastewater is used in irrigation, which originates from sanitary and domestic waste. Heavy metals and dangerous organisms may be in sewage sludge (Zafar et al., 2020; Latosińska et al., 2021). Heavy metal refers to metals and metalloids with a density greater than 5 g/cm^3 that are toxic in small quantities, such as As, Cd, Cr, Cu, Fe, Pb, Hg, Ag, Zn, and other metals and metalloids. Heavy metals accumulate in nature due to their non-biodegradable habit, producing bio-accumulation in the food chain, which can lead to environmental and health problems (Leong and Chang, 2020). The heavy metals are necessary for "plant growth, development, and physiological life functions are Cu, Zn, Fe, Ni, Co, Mn, and Mo. Excessive concentrations of these elements and trace amounts of many other heavy metals reported as hazardous to plants (Liu et al., 2020; Çelik and Kunene, 2021).

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2022.102382
1018-3647/

Various plants have an odd capability to obtain a large number of heavy metals by adsorption from the soil. Plants have a higher ability to build metal than soil from wastewater. Plant metal expansion systems may also be due to defensive mechanisms like opposition to pathogens and herbivores (Khan et al., 2019; Rattan et al., 2005; Sherene, 2010). Spinach (*Spinacia oleracea*) is one of the essential leafy nutrient vegetables cultivated in Asian countries, especially Pakistan. It has the potential to absorb a higher quantity of heavy metals and toxic elements from the rhizosphere and translate them into edible parts compared to fruits and root vegetables (Bashir et al., 2020). Metals in soil are used in solid-phase processes. Soil texture, fissures, and cracks are the main elements that increase metal mobility. The sewage water is used in agricultural soil for economic and environmental gains. It includes the nutrients required for plant growth and is applied as a fertilizer (Alawsy et al., 2018). Vegetables are carriers of heavy metals when taken up by humans and ingested by the human body. The present study was planned (i) to estimate concentrations of heavy metals in the soil; (ii) to assess the concentration of heavy metals and their bioaccumulation in spinach irrigation type/site-wise, and (iii) to estimate the hazardous impacts of wastewater.

2. Methodology

2.1. Study area

This study was conducted in the selected rural area (the coordinates 31°33′59.7″N and 73°06′03.0″E) of Faisalabad. In rural areas, spinach is used as edible food and is highly dependent on spinach crops as food. Farmers used both tube well water and sanitary wastewater as irrigation sources. This study area was selected because of low traffic and other industrial pollution being away from the road.

2.2. Sample collection

This study was carried out in Faisalabad's rural areas, where sanitary effluent and tubewell water were used to cultivate vegetables and crops. For this purpose, spinach was exploited (*Spinacia oleracea*). The samples were collected from two locations: one field was irrigated with wastewater and with tube well water. "Soil samples were collected from roughly 0–20 cm depth, along with nine water samples from both crop zones from the selected sites". Twenty-one soil samples were obtained at the time of cultivation. Twenty-one specimens of spinach were collected from the field irrigated with sewage wastewater and tubewell water. After 60 days, the vegetables were harvested.

Plant leaves samples of spinach were randomly obtained from both locations after a 10-day interval, with site one watered with canal water and site two irrigated with sewage effluent. Water samples were collected in previously cleaned and dried bottles, while soil and vegetable samples were stored in clean polythene bags and labeled. All vegetables were cleaned in fresh running water to remove dust, grime, and foreign particles, and their edible parts were sliced into little pieces for air drying. After four days, all vegetable samples were collected and arranged in an oven at 65 °C for complete removal of moisture. Soil samples were collected from both plants' sites, dried in sunlight for four days, and then dried in a thermal oven for the complete removal of moisture. After drying, samples of vegetables and soil samples were grounded to powder form by using a grinder to homogenize size. The powder form of samples was stored in polythene zip lock bags.

2.3. Sample digestion procedure

Water sample digestion: 100 ml of the conical flask was used. 5 ml of nitric acid (HNO_3) was added and heat it on a hot plate until its volume was reduced to 20 ml. Remove the flask and let it cool down. After that 10 ml of perchloric acid ($HClO_4$) was added, and then 5 ml of nitric acid (HNO_3) was added and heated it again on a hot plate. Brown fumes appear immediately. No heat was applied after the volume was reduced to 2–3 ml, and white fumes appeared. A small portion (nearly 1 gm) was transferred into a conical flask, and then 5 ml of nitric acid (HNO_3), 2 ml of perchloric acid ($HClO_4$), and 1 ml of sulphuric acid (H_2SO_4) were added with 25 ml of distilled water. After this, samples were placed on a hot plate. The fumes appeared after some minutes. No more heat was applied as the volume was reduced to 2–3 ml, and white fumes appeared. Heavy metals from the samples were determined using the AB-DTPA method (Soltanpour and Workman, 1985).

In a 125 ml conical flask, 10 g of well-prepared soil was weighed. Ammonium bicarbonate and 0.005 DTPA were combined to make a 20 ml extraction solution. This solution was shaken for 15 min at 180 cycles min^{-1} on a reciprocal shaker. Filtration of the solution mentioned above via Whatman no.42 filter paper yielded the supernatant. The heavy metals were identified using an Atomic Absorption Spectrophotometer (AAS Trace A11200 Aurora, Canada). The principle on whom AAS work is that electromagnetic radiation passes through the substance that electromagnetic radiation may transmit or absorb by the substances depending on radiation wavelength. The samples were run in Atomic Absorption Spectrophotometer to determine the concentration of Pb, Cu, Cd, Zn, and As in spinach.

3. Results

Heavy metals toxicity was analyzed in Spinach (*Spinacia oleracea*) leaves irrigated with tube well water and sanitary wastewater using AAS. The results (Mean and S.D ± error) of seven replicas of randomly selected leave samples were compared. Four selected heavy metals were estimated in water, soil, and leaf sample. The following trend of heavy metals accumulation in Spinach (*Spinacia oleracea*) leaves was observed:

Irrigated with tube well water: Cu > Cd > Pb > Zn
Irrigated with sanitary wastewater: Cu > Pb > Zn > Cd

The following trend was observed in the selected study area's soil for plant growth:

Tube well water: Pb > Cu > Cd > Zn
Sanitary wastewater: Cu > Pb > Cd > Zn

The heavy metals in tubewell and wastewater were recorded in the following order: was observed in tubewell water; Cd > Cu > Pb > Zn

and wastewater; Pb > Zn > Cu > Cd, respectively.

Copper (Cu): Copper, an essential mineral found in many proteins and enzymes, plays a vital function in plant health and nutrition (Kasana et al., 2017). On the other hand, copper overload causes oxidative stress in plants by increasing the generation of reactive oxygen species (ROS). "Because of its dual nature (essential and potentially harmful), this metal needs a complicated network of absorption, sequestration, and transport (Mir et al., 2021). Cu deficiency or excess harms human health (Kumar et al., 2021). The highest (0.82 ± 0.473) and the lowest concentration (0.064 ± 0.037) mg/kg of Cu was recorded in edible parts of spinach irrigated with tube well water (Table 4). The highest

(1.396 ± 0.806) and the lowest concentration (0.12 ± 0.069) mg/kg of Cu was recorded in edible parts of spinach in irrigated wastewater water (Table 5). The highest (1.821 ± 1.051) and lowest concentration (1.144 ± 0.660) mg/kg of Cu was recorded in the selected study area of soil irrigated with tube well water (Table 2). The highest (0.442 ± 0.255) and the lowest concentration (0.036 ± 0.021) mg/l of Cu were detected in tube well water (Table 1). The highest (0.071 ± 0.041) and lowest concentration of Cu (0.033 ± 0.019) mg/l was recorded in the field irrigated with wastewater (Table 1).

Cadmium (Cd): The highest concentration of Cd (0.478 ± 0.276) mg/kg was observed in the edible part of spinach irrigated with tube well water, and the lowest concentration of Cd (0.087 ± 0.050) mg/kg was observed in spinach (Table 4) irrigated with tube well water. The highest concentration of Cd (0.123 ± 0.071) mg/kg was detected in edible parts of spinach irrigated with sanitary wastewater, and the lowest concentration of Cd (0.033 ± 0.019) mg/kg in spinach irrigated with sanitary wastewater (Table 5). The soil irrigated with sanitary sewage and tube well water shows a variation in Cd concentration. "The highest concentration of Cd (0.392 ± 0.227) and lowest concentration of Cd (0.110 ± 0.063) mg/kg was observed in the soil samples irrigated with tube well water (Table 2). Spinach that was grown in soil irrigated with sanitary wastewater" showed the highest concentration of Cd (0.566 ± 0.327) and the lowest concentration of Cd (0.068 ± 0.039) mg/kg (Table 3). The highest (0.548 ± 0.316) and the lowest concentration mg/l of Cd was detected in tube well water (Table 1). The highest concentration (0.051 ± 0.029) mg/l of Cd was estimated in irrigation water through sanitary wastewater, and the lowest concentration (0.029 ± 0.017) mg/l of Cd in the water of irrigation through sanitary wastewater (Table 1).

Lead (Pb): The highest concentration (0.396 ± 0.229) mg/kg of Pb was determined in edible parts of spinach irrigated with tube well water, and the lowest concentration (0.033 ± 0.019) mg/kg in spinach samples collected from the field irrigated with tube well water (Table 4). The lowest concentration (0.204 ± 0.118) mg/kg of Pb in spinach samples was determined in the field irrigated with wastewater water (Table 5). However, the highest (1.326 ± 0.766) and the lowest concentration (0.162 ± 0.094) mg/kg of Pb was observed in a selected field of soil irrigated with tube well w ater (Table 2). "The highest (0.876 ± 0.506) and the lowest concen tration (0.219 ± 0.126) mg/kg of Pb was recorded in the soil irri gated with wastewater water (Table 3)". The highest (0.329 ± 0.190) and the lowest concentration (0.209 ± 0.120) mg/l of Pb w as detected in tube well water (Table 1). The highest (0.739 ± 0.427) and lowest concentration (0.032 ± 0.018) mg/l of Pb was observed in the field irrigated with sanitary wastewater (Table 1).

Zinc: The highest Zn (0.157 ± 0.091) and the lowest concentration of Zn (0.032 ± 0.019) mg/kg was "detected in edible parts of spinach, mg/kg was estimated in the field of spinach irrigated with tube well water (Table 4)". The highest Zn (0.246 ± 0.142) and the lowest concentration of Zn (0.049 ± 0.028) mg/kg was recorded in edible parts of spinach irrigated with sanitary wastewater (Table 5). The highest Zn (0.129 ± 0.074) and the lowest concentration of zinc (0.056 ± 0.113) mg/kg, was observed in spinach samples from the field irrigated tube well water (Table 2). Spinach that was grown in soil irrigated with sanitary wastewater (Table 3) showed the highest Zn (0.175 ± 0.101) and the lowest concentration of Zn (0.037 ± 0.021) mg/kg. The highest concentration (0.278 ± 0.160) mg/l of Zn was detected in tube well water, and the lowest concentration (0.008 ± 0.004) mg/l of Zn was observed in irrigation water of tube well (Table 1).

4. Discussions

Results obtained from tube well water and sanitary wastewater irrigation clarify the concentration of Cu and Cd (Table 1) higher than the permissible limits of WHO/FAO (2007). In a previously reported study (Eid et al., 2017a,b), the concentration of heavy metal (Cd (1.17), Zn (667.6), Cu (162.6), and Pb (671.2)) mg/kg

Table 1
Heavy metals concentration (mg/L) in Tube well water and sanitary waste water

Heavy metals	Tube well water (mg/L)			Sanitary Wastewater (mg/L)			Safe limits
	1	2	3	1	2	3	WHO/FAO (2007)
Pb	0.209 ± 0.120	0.234 ± 0.135	0.329 ± 0.190	0.238 ± 0.137	0.032 ± 0.018	0.739 ± 0.427	5.0
Cu	0.036 ± 0.021	0.053 ± 0.030	0.442 ± 0.255	0.044 ± 0.025	0.033 ± 0.019	0.071 ± 0.041	0.20
Zn	0.278 ± 0.160	0.008 ± 0.004	0.027 ± 0.015	0.141 ± 0.081	0.102 ± 0.059	0.072 ± 0.042	2.0
Cd	0.512 ± 0.295	0.548 ± 0.316	0.202 ± 0.117	0.051 ± 0.029	0.047 ± 0.027	0.029 ± 0.017	0.01

Table 2
Heavy metals Concentration (mg/kg) in Soil samples irrigated with tube well water.

Heavy metals	1	2	3	4	5	6	7	Safe limits WHO/FAO (2007)
Zn	0.058 ± 0.033	0.067 ± 0.032	0.056 ± 0.113	0.072 ± 0.011	0.114 ± 0.066	0.102 ± 0.059	0.129 ± 0.074	300–600
Cd	0.365 ± 0.211	0.136 ± 0.078	0.137 ± 0.079	0.291 ± 0.168	0.110 ± 0.063	0.182 ± 0.105	0.392 ± 0.227	3.0–6.0
Cu	0.480 ± 0.277	0.624 ± 0.360	0.308 ± 0.178	0.820 ± 0.473	0.232 ± 0.134	0.091 ± 0.053	1.144 ± 0.660	135–270
Pb	0.162 ± 0.094	0.651 ± 0.376	1.008 ± 0.582	0.744 ± 0.430	0.246 ± 0.142	1.326 ± 0.766	0.447 ± 0.258	250–500

Table 3
Heavy metals concentration (mg/kg) in Soil samples irrigated with sanitary wastewater) mg/kg.

Heavy metals	1	2	3	4	5	6	7	Safe limits WHO/FAO (2007)
Zn	0.074 ± 0.043	0.043 ± 0.025	0.046 ± 0.027	0.090 ± 0.052	0.175 ± 0.101	0.097 ± 0.056	0.037 ± 0.021	300–600
Cd	0.068 ± 0.039	0.223 ± 0.129	0.269 ± 0.155	0.111 ± 0.064	0.566 ± 0.327	0.429 ± 0.248	0.272 ± 0.157	3.0–6.0
Cu	0.484 ± 0.279	0.724 ± 0.418	1.821 ± 1.051	0.592 ± 0.342	0.392 ± 0.226	0.262 ± 0.151	0.232 ± 0.134	135–270
Pb	0.687 ± 0.397	0.657 ± 0.379	0.876 ± 0.506	0.675 ± 0.390	0.675 ± 0.390	0.342 ± 0.197	0.219 ± 0.126	250–500

Table 4
Heavy metals concentration (mg/Kg) in edible part of spinach irrigated with tube well water.

Heavy metals	1	2	3	4	5	6	7	Safe limits WHO/FAO (2007)
Zn	0.058 ± 0.033	0.067 ± 0.039	0.056 ± 0.032	0.072 ± 0.041	0.074 ± 0.042	0.032 ± 0.019	0.157 ± 0.091	60.0
Cd	0.149 ± 0.086	0.090 ± 0.052	0.255 ± 0.147	0.087 ± 0.050	0.163 ± 0.094	0.239 ± 0.138	0.478 ± 0.276	0.2
Cu	0.288 ± 0.166	0.164 ± 0.095	0.82 ± 0.473	0.62 ± 0.358	0.288 ± 0.166	0.336 ± 0.194	0.064 ± 0.037	40.0
Pb	0.156 ± 0.090	0.222 ± 0.128	0.033 ± 0.019	0.165 ± 0.095	0.165 ± 0.095	0.252 ± 0.145	0.396 ± 0.229	5.0

Table 5
Heavy metals concentration (mg/Kg) in edible parts of spinach irrigated with sanitary wastewater.

Heavy metals	1	2	3	4	5	6	7	Safe limits WHO/FAO (2007)
Zn	0.049 ± 0.028	0.153 ± 0.088	0.094 ± 0.054	0.246 ± 0.142	0.208 ± 0.120	0.201 ± 0.116	0.061 ± 0.035	60.0
Cd	0.087 ± 0.050	0.107 ± 0.062	0.068 ± 0.039	0.033 ± 0.019	0.123 ± 0.071	0.081 ± 0.047	0.055 ± 0.032	0.2
Cu	0.612 ± 0.353	1.396 ± 0.806	0.12 ± 0.069	0.848 ± 0.490	0.256 ± 0.148	0.719 ± 0.415	0.208 ± 0.120	40.0
Pb	0.24 ± 0.139	0.339 ± 0.196	0.213 ± 0.123	0.542 ± 0.313	0.488 ± 0.282	0.204 ± 0.118	0.531 ± 0.307	5.0

was higher in sewage sludge which is not in line this present study. Cd, Zn, Cu, and Pb concentrations were higher than those in wastewater from Varanasi, India (Singh et al., 2010). Sobhan Ardakani et al. (2016) reported that Zn, Pb, Cd, and Cu concentrations in groundwater samples in the spring season were in the summer compared to winter, and these concentrations were higher than in the tube well water. Tables 2 and 3 show that heavy metal concentration (Cd, Cu, Pb, and Zn) was lower than the permissible limits of WHO/FAO (2007). The kind of soil in a site was influenced by local climate components (such as rainfall), terrain, regional water movement, mineral composition and rock fragments accumulating in the soil, animals living in the soil, plants growing in the area, and human activities nearby (Ding et al., 2019). The findings of this study were not in line with the results of Tasrina et al. (2015).

Tables 4 and 5 showed the concentration of heavy metals (Zn, Cd, Cu, and Pb) in a sample of the edible part of spinach irrigated tube well water and sanitary wastewater from the study area of Faisalabad. Table 4 showed that the concentration of Cd in edible parts of spinach samples collected from tubewell water was higher than the permissible limits suggested by WHO/FAO (2007). The concentration of heavy metals (Pb, Zn, and Cu) collected from edible parts of spinach irrigated with tubewell water and sanitary wastewater were below the permissible limits of WHO/FAO (2007). Zhang et al. (2020) reported the concentration of Pb (1.02 ± 0.20), Zn (7.03 ± 0.53), and Cu (1.84 ± 0.21) mg/kg accumulation in *Brassica campestris* L. that disagreed with the present study. Boamponsem et al. (2012) reported heavy metal accumulation in *Lactuca sativa*, *Daucus carrota*, and *Brassica oleracea* L." The results showed the order of accumulation of Cu in the three vegetables irrigated with wastewater was; *Lactuca sativa* (0.160 mg/Kg) > *Daucus carrota* (0.152 mg/Kg) > *Brassica oleracea* L. (0.066 mg/Kg) disagreed with the present study outcomes". Singh et al. (2012) reported that heavy metals (Pb, Cu, Zn Cd) exhibit different values of heavy metal accumulation in different edible crops, which disagreed with the present study.

5. Conclusion

This study revealed that wastewater was used for irrigation purposes. Heavy metals like Zn, Cd, Cu, and Pb in wastewater accumulate in soil and plant effects humans directly. By applying sanitary wastewater, cadmium exceeds the safe permissible limits and causes toxicity in edible parts of spinach and copper in irrigation sources. These metals were beyond the allowable limits established by FAO/WHO (2007). It has been further concluded that Cd concentration was higher in the edible part of spinach which was irrigated with sanitary. It is suggested that wastewater be used in moderate quantities to avoid possible health issues by consuming such spinach.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The current research was accomplished with the help of funds granted by the University of Agriculture, Faisalabad, Pakistan. The authors (FAM) express their sincere appreciation to the Researchers Supporting Project number (RSP-2021/24), King Saud University, Riyadh, Saudi Arabia.

References

Ahmad, K., Heng, A.C.T., 2012. Determinants of agriculture productivity growth in Pakistan. International Research Journal of Finance and Economics 95, 163–172.
Alawsy, W.S.A., Alabadi, L.A.S., Khaeim, H.M., 2018. Effect of sewage water irrigation on growth performance, biomass and nutrient accumulation in maize and barley. Int. J. Agric. Stat. Sci. 14 (2), 519–524.
Bashir, S., Ali, U., Shaaban, M., Gulshan, A.B., Iqbal, J., Khan, S., Husain, A., Ahmed, N., Mehmood, S., Kamran, M., Hu, H., 2020. Role of sepiolite for cadmium (Cd) polluted soil restoration and spinach growth in wastewater irrigated agricultural soil. J. Environ. Manage. 258, 110020. https://doi.org/10.1016/j.jenvman.2019.110020.
Boamponsem, G.A., Kumi, M., Debrah, I., 2012. Heavy metals accumulation in cabbage, lettuce and carrot irrigated with wastewater from Nagodi mining site in Ghana. Int. J. Sci. Technol. Res. 1 (11), 124–129.
Ding, H.J., Jiang, J., Zhang, J.c., 2019. Regional variation and factor analysis of soil permeability. J. Soil Water Conserv. 33 (1), 51–56.
Eid, E.M., El-bebany, A.F., Alrumman, S.A., El-latif, A., Taher, M.A., Fawy, K.F., Eid, E. M., El-bebany, A.F., Alrumman, S.A., El-, A., 2017a. Effects of different sewage sludge applications on heavy metal accumulation, growth and yield of Spinach (Spinacia oleracea L.). Int. J. Phytorem. 19 (4), 340–347. https://doi.org/10.1080/15226514.2016.122528.
Eid, E.M., El-Bebany, A.F., Alrumman, S.A., Hesham, A.E.L., Taher, M.A., Fawy, K.F., 2017b. Effects of different sewage sludge applications on heavy metal accumulation, growth and yield of Spinach (Spinacia oleracea L.). Int. J. Phytorem. 19 (4), 340–347.
Kasana, R.C., Panwar, N.R., Kaul, R.K., Kumar, P., 2017. Biosynthesis and effects of copper nanoparticles on plants. Environ. Chem. Lett. 15 (2), 233–240.

Khan, Z.I., Iqbal, S., Ahmad, K., Ashfaq, A., Bashir, H., Dogan, Y., 2019. ssessment of heavy metal content of wheat irrigated with wastewater in Sargodha, Pakistan: Implications for human health A. Trace Elem. Electrolytes 36 (04), 82–92. https://doi.org/10.5414/tex01530.

Latosińska, J., Kowalik, R., Gawdzik, J., 2021. Risk assessment of soil contamination with heavy metals from municipal sewage sludge. Appl. Sci. (Switzerland) 11 (2), 1–13. https://doi.org/10.3390/app11020548.

Leong, Y.K., Chang, J.-S., 2020. Bioremediation of heavy metals using microalgae: recent advances and mechanisms. Bioresour. Technol. 303, 122886.

Liu, Y., Sun, X., Li, S., Li, S., Zhou, W., Ma, Q., Zhang, J., 2020. Influence of green waste compost on Pb-polluted soil remediation, soil quality improvement, and uptake by Pakchoi cabbage (Brassica campestris L. ssp). Environ. Sci. Pollut. Res. 27 (7), 7693–7701. https://doi.org/10.1007/s11356-019-07505-9.

Mir, A.R., Pichtel, J., Hayat, S., 2021. Copper: uptake, toxicity and tolerance in plants and management of Cu-contaminated soil. Biometals 34 (4), 737–759.

Rattan, R.K., Datta, S.P., Chhonkar, P.K., Suribabu, K., Singh, A.K., 2005. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater - A case study. Agric. Ecosyst. Environ. 109 (3–4), 310–322. https://doi.org/10.1016/j.agee.2005.02.025.

Salimova, G., Ableeva, A., Lubova, T., Zalilova, Z., Sharafutdinov, A., 2020. The role of agriculture in gross added value. Montenegrin J. Econ. 16 (1), 183–191. https://doi.org/10.14254/1800-5845/2020.16-1.12.

Sardar, A., Shahid, M., Natasha, Khalid, S., Anwar, H., Tahir, M., Shah, G.M., Mubeen, M., 2020. Risk assessment of heavy metal(loid)s via Spinacia oleracea ingestion after sewage water irrigation practices in Vehari District. Environ. Sci. Pollut. Res. 27 (32), 39841–39851.

Sherene, T., 2010. Mobility and transport of heavy metals in polluted soil environment. Biol. Forum 2 (2), 112–121.

Singh, A., Sharma, R.K., Agrawal, M., Marshall, F.M., 2010. Risk assessment of heavy metal toxicity through contaminated vegetables from waste water irrigated area of Varanasi, India. Trop. Ecol. 51 (2), 375–387.

Singh, S., Zacharias, M., Kalpana, S., Mishra, S., 2012. Heavy metals accumulation and distribution pattern in different vegetable crops. J. Environ. Chem. Ecotoxicol. 4 (4), 75–81.

Sobhan Ardakani, S., Yari, A.R., Taghavi, L., Tayebi, L., 2016. Water quality pollution indices to assess the heavy metal contamination, case study: groundwater resources of Asadabad Plain in 2012. Arch. Hyg. Sci. 5 (4), 221–228.

Soltanpour, P.N., Workman, S., 1985. Modification of the NaHCO3 DTPA soil test to omit carbon black. Communications in Soil Science and Plant Analysis 10, 1411–1420.

Soubbotina, T.P., Sheram, K., 2000. Beyond Economic Growth: Meeting the Challenges of Global Development. World Bank Publications.

Tasrina, R.C., Rowshon, A.A.M.R., Mustafizur, A.M.R., Rafiqul, I., Ali, M.P., 2015. Heavy metals contamination in vegetables and its growing soil. J. Environ. Anal. Chem. 2 (142), 2.

Tingey-Holyoak, J.L., Pisaniello, J.D., Buss, P., 2021. Embedding smart technologies in accounting to meet global irrigation challenges. MEDAR 29 (5), 1146–1178.

WHO, 2007. The world health report 2007: a safer future. 72p.

Zafar, S., Farooq, S., Qazi, H.A., Jaweed, T.H., Kadam, A.K., Lone, F.A., 2020. Evaluation of nutrient status of kale and spinach as affected by sewage sludge and mineral fertilizers. J. Plant Nutr. 43 (17), 2633–2644. https://doi.org/10.1080/01904167.2020.1783306.

Zhang, Z., Wu, X., Tu, C., Huang, X., Zhang, J., Fang, H., Lin, C., 2020. Relationships between soil properties and the accumulation of heavy metals in different Brassica campestris L. growth stages in a Karst mountainous area. Ecotoxicol. Environ. Saf. 206, 111150.

Çelik, H., Kunene, S.S., 2021. Assessment of heavy metal amounts of Spinach plants (Spinach oleracea l.) grown on cd and chicken manure applied soil conditions. Pol. J. Environ. Stud. 30 (2), 1105–1115. https://doi.org/10.15244/pjoes/125481.