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Effects of wastewater Irrigation on Heavy Metal Uptake by Onions (*Allium cepa*).

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ABSTRACT

Onions are highly nutritious vegetables widely cultivated by small scale farmers worldwide and form the basis of everyday meals. The current study consisted of two sets, each with 10 replicates. One set was irrigated with wastewater and the other with tap water for 8 weeks. Onion samples were randomly selected from each pot and analysed for heavy metal (Cu, Mn, Pb, Cr, Cd and Ni) concentrations using Flame Atomic Absorption Spectrometry. Results obtained showed that heavy metal concentrations in bulbs and leaves of onions irrigated with wastewater exceeded the safe limits ($p < 0.05$) whilst heavy metal concentrations in bulbs and leaves of onions irrigated with tap water were lower and within the safe limits ($p > 0.05$). The study also showed that type of irrigation water (wastewater or tap water) had an effect on heavy metal concentration in bulbs and leaves ($p = 0.001$) whilst plant tissue type (bulb or leaf) had no significant effect on the concentration of heavy metals in onions. This study highlights the potential risks involved in the cultivation and consumption of vegetables using wastewater for irrigation, a practice which may place at risk the health of the urban population who consume these vegetables.

Introduction

Crop production through horticulture has greatly contributed to the rapid economic growth in Zimbabwe, and the demand for horticultural products has continued to increase with increasing population (Jackson, 1997). The increased demand for horticultural products has seen many small scale farmers in urban areas venturing into this business. In Zimbabwe, municipal water scarcity has pressed urban small scale farmers to resort to wastewater irrigation as an alternative. The same trend is also true for many developing countries where the productive use of wastewater has also increased, as millions of small scale farmers in urban and peri-urban areas depend on wastewater to irrigate high value food crops for urban markets, often as they do not have alternative sources of irrigation water. The availability of the wastewater all year round also enables small scale farmers to use wastewater irrigation during the dry season. Irrigation with treated municipal wastewater is considered an environmentally sound wastewater disposal practice compared to its direct disposal to the surface or water bodies (Mohammad and Mazahreh, 2003). Additionally, wastewater is a valuable source of plant nutrients and organic matter needed for maintaining fertility and productivity levels of the soil (Weber *et al.*, 1996). On the other hand, wastewater may contain undesirable chemical constituents, and pathogens that pose negative environmental and health impacts (Papadopoulos, 1995). Undesirable constituents of wastewater, especially heavy metals can harm human health and the environment. Hence, wastewater irrigation is an issue of concern to public agencies responsible for maintaining public health and environmental quality (McCornick, 2010).

The contamination of agricultural products with heavy metals has become an important concern throughout the world due to the potential adverse effects of heavy metals on human health. Growth media (soil, air and water) are the main routes of heavy metal transport to plants. The concentrations of heavy metals in agricultural soils have been found to be elevated due to continuous use of pesticides, fertilizers and municipal wastewater (Singh *et al.*, 2004). The accumulation of heavy metals in edible portions of vegetables has adverse effects on human health and plants (Wenzel and Jockwer, 1999). Several heavy metals such as cadmium (Cd), chromium (Cr), manganese (Mn), nickel (Ni) and lead (Pb) are reported to be carcinogenic in nature (Trichopoulos, 1997).

When wastewater is used continuously as the sole source of irrigation, excessive amounts of nutrients and toxic substances could simultaneously be applied to the soil-plant system. This can cause unfavourable effects on productivity and quality parameters of the crops and the soil (Vazquezmontiel *et al.*, 1996). Therefore, management of wastewater irrigation should consider the wastewater nutrient content and other soil fertility parameters (Mohammad and Mazahreh, 2003).

In Zimbabwe, particularly Harare and Chitungwiza, small scale vegetable farmers have made it a common practice to cultivate vegetables along banks of rivers near sewage works (Mapanda *et al.*, 2005). Water in these rivers is reported to be polluted by heavy metals (Nhapi, 2009). The major sources of these heavy metals are industrial effluents and the indiscriminate disposal of domestic or sewage drainage directed to the rivers untreated or partially treated (Bahemuka and Mubofu, 1998).

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The consumers' demand for quality vegetables has increased. However, many consumers judge the quality of these products basing on external morphology, which cannot guarantee safety from contamination. Vegetables also have a greater potential of accumulating heavy metals in their edible parts than grain or fruit crops (Kirkham, 1977).

The current study focused on lead, chromium, nickel, copper, manganese and cadmium as they have a number of undesirable properties that affect humans and the environment. Absorbed heavy metals are rapidly taken into the blood and soft tissue, followed by a slower redistribution to the bone. Effects of heavy metal consumption by humans may range from enzyme inhibition through lead uptake to the production of marked morphological changes and death. Heavy metal uptake by plants, specifically cadmium has a negative effect on processes such as transpiration, photosynthesis and stomatal opening (Goyer, 1986).

The study also aimed at providing valuable insight on heavy metal uptake by onions and how these heavy metals are transferred through the food chain to the consumers. It also aimed at informing the public on the hazards associated with wastewater irrigation and help farmers come up with alternative sources of water for crop production.

Materials and Methods

Study area

Chitungwiza sewage treatment plant discharges its partially treated wastewater into Nyatsime River. This research was conducted at a small scale plot located in Chitungwiza, along the banks of Nyatsime River. The site is covered by coarse grained loam soils of relatively low fertility. The majority of the crops grown around this area are vegetables that are grown all year round and irrigated using wastewater. The research was carried out within a period of 8 weeks from June to August 2015.

Experimental design

The project design consisted of two sets of potted onion plants. Each set consisted of 10 replicates and one set was irrigated with wastewater whilst the other set was irrigated with tap water. Tap water was collected in 20 litre plastic containers and the same volume of wastewater was also collected from the Chitungwiza Sewage treatment plant site after secondary treatment. The soil which was used as the growth medium in this experiment was garden loam soil with no wastewater or sludge amendment. The loam soil used was collected from a site remote from the study site where wastewater irrigation is not practiced. The soil was collected within a 2m by 2m grid to ensure uniformity. An equal amount (1kg) of soil was measured using a digital scale and was put in all the 20 pots. Five onion seeds were planted in each pot and these were irrigated with 300 ml of tap water or wastewater once every 24 hours till mature (8 weeks).

Chemical checks on tap water, wastewater as well as the soil were done at the Environmental Management Agency Laboratory. The chemical parameters of major concern were the heavy metals (Copper, Lead, Chromium, Manganese, Nickel and Cadmium). pH was also tested as it has an effect on assimilation of heavy metals by plants. The pH was measured using a portable pH meter (EcoSense, pH 100) and heavy metal concentration was determined by using a Flame Atomic Absorption Spectrometer (FAAS) (Varian Spectra AA, 200FS). Plant tissue analysis to determine the heavy metal concentration in the bulbs and leaves of the onions was also conducted at the Environmental Management Agency Lab using FAAS.

Testing for pH in soil and water samples

Soil pH was determined using a portable meter (EcoSense pH 100) in a ratio 1: 2.5 soil to water suspension. A mass of 4g of loam soil was measured and 10 ml of distilled water was added to obtain a saturated suspension. The mixture was left to set for 30 min after which a calibrated portable pH meter was used to measure the pH of the soil sample. The pH for wastewater and tap water was also determined using a pH meter (EcoSense pH 100). A volume of 100ml of each sample (wastewater and tap water) was pipetted into a volumetric flask and placed on a magnetic stirrer. The pH was then measured using a calibrated portable pH meter (EcoSense pH 100).

Flame atomic absorption spectrometry

Concentrations of heavy metals (Copper, Lead, Chromium, Manganese, Nickel and Cadmium) were analysed using Flame Atomic Absorption Spectrometry. A flame atomic absorption spectrometer (Varian Spectra AA, 200FS) was used to measure these relative concentrations using operating parameters summarized in Table 1.

Table 1. Operating parameters for flame atomic absorption spectrometer (FAAS)

Metal	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Fuel
Cu	324.7	0.5	4	Acetylene
Cr	357.9	0.2	7	Acetylene
Cd	228.8	0.5	4	Acetylene
Pb	217.0	0.1	5	Acetylene
Mn	213.9	1.0	5	Acetylene
Ni	232.0	0.2	4	Acetylene

Determination of heavy metal concentrations in soil and water samples by FAAS

A mass of 2 grams of loam soil was weighed in a Pyrex petri dish using a digital scale. A volume of 8 ml of aqua regia (HCl: HNO_3, 3:1 v/v) was added to the soil sample. Thereafter, the petri dish was closed and left for 20 minutes. The samples were then irradiated in a microwave digester for 30 minutes. The digested samples were poured through a filter paper into 50 ml volumetric flasks then filled up to the mark with distilled water. Solutions were analysed for heavy metals using a flame atomic absorption spectrometer (Varian Spectra AA, 200FS), with each metal using its respective light beam as summarized in Table 1. For water samples, a volume of 99 ml of wastewater and of tap water were separately poured into 100ml volumetric flasks. The samples were filled up to the mark with nitric acid (HNO_3) to acidify and these samples were analysed for heavy metal concentrations using a flame atomic absorption spectrometer (Varian Spectra AA, 200FS).

Determination of heavy metal concentration in bulb and leaf tissues by FAAS

A mass of 2g of each sample was weighed (leaves and bulbs) in a petri dish using a digital scale. These were transferred into separate conical flasks and mixed with 15 ml Perchloric acid ($HClO_4$) and Nitric acid (HNO_3) solution in the ratio 1:4 and left for cold digestion for 20 minutes. After cold digestion, the samples were placed in a microwave digester and irradiated for 30 minutes. The digested samples were then filtered through filter papers into 50 ml volumetric flasks and filled up to the mark with de-ionised water. The filtrates were analysed for heavy metals (Cu, Pb, Cr, Ni, Cd and Mn) using a flame atomic absorption spectrometer (Varian Spectra AA, 200FS) at different wavelengths as shown in Table 1.

Data analysis

A two way ANOVA was used to test the effects of two factors using the SPSS package version 21. Factor 1 was the type of water (wastewater or tap water) and factor 2 was the plant tissue type (bulb or leaf). The concentrations of copper, nickel, chromium, manganese, cadmium and lead were the response variables. A one sample t-test was also used to compare the heavy metal concentrations in wastewater irrigated onions against the permissible limits set by WHO/FAO. All statistical tests were done using a 5% significance level

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Results

Heavy metal concentrations in the garden loam soil

The concentrations of all the tested heavy metals were within the permissible standards according to the California metals concentration limits for soil containing non-hazardous concentrations. Lead had the highest concentration of 3.53 mg/kg and cadmium had the lowest concentration of 0.09 mg/kg. Results obtained from the soil heavy metal concentration tests are summarised in Table 2.

Table 2. Heavy metal concentrations in soil samples (mg/kg)

Parameters	Cd	Cr	Cu	Pb	Mn	Ni
Garden loam soil	0.09	0.85	2.13	3.53	0.76	2.87
Safe Limits	1.0	5.0	25.0	5.0	-	20

Heavy metal concentrations in tap water and wastewater

All the measured heavy metals in wastewater were extremely high and they exceeded the safe limits with lead having the highest concentration of 0.42 mg/l. Heavy metal concentrations in tap water were minimal and the values were all below the safe limits. Lead concentration in tap water had the lowest value of 0.003 mg/l. Table 3 shows the concentrations of cadmium, chromium, copper, lead, manganese and nickel in tap water and wastewater used in the experiment.

Table 3. Heavy metal concentrations in water samples (mg/L)

Parameters	Cd	Cr	Cu	Pb	Mn	Ni
Wastewater	0.228	0.24	0.196	0.42	0.226	0.23
Tap water	0.009	0.07	0.010	0.003	0.055	0.043
Safe Limits	0.01	0.1	0.05	0.05	0.1	0.1

Heavy metal concentrations in leaves of onions irrigated with wastewater and tap water

The study showed that the concentrations of heavy metals in leaves of onions irrigated with wastewater exceeded the safe limits ($p< 0.05$) and the concentrations of heavy metals in leaves of onions irrigated with tap water were within the safe limits ($p> 0.05$). The mean concentrations of heavy metals, Cu (0.456), Pb (1.192), Cr (0.509), Ni (0.592), Cd (0.493) and Mn (0.579) were higher in the leaves of onions irrigated with wastewater compared to mean concentrations, Cu (0.072), Pb (0.062), Cr (0.098), Ni (0.078), Cd (0.011) and Mn (0.095) in leaves of onions irrigated with tapwater (Figure 1).

Heavy metal concentrations in bulbs of onions irrigated with wastewater and tap water

There was enough evidence to suggest that concentrations of heavy metals in bulbs of onions irrigated with wastewater exceeded the safe limits ($p<0.05$) and the concentrations of heavy metals in bulbs of onions irrigated with tap water were within the safe limits ($p>0.05$). The mean concentrations of heavy metals, Cu (0.481), Pb (1.247), Cr (0.532), Ni (0.624), Cd (0.523) and Mn (0.594) were higher in bulbs of onions irrigated with wastewater compared to the mean concentrations, Cu (0.080), Pb (0.069), Cr (0.114), Ni (0.093), Cd (0.025) and Mn (0.1) in bulbs of onions irrigated with tap water (Figure 1).

Effect of irrigation water type (wastewater and tap water) on heavy metal concentrations in onions

The mean concentrations of heavy metals in bulbs and leaves of onions irrigated with wastewater and onions irrigated with tap water showed that heavy metal concentrations were higher in bulbs and leaves of onions irrigated with wastewater compared to onions irrigated with tap water (Figure 1). There was enough evidence at ($p=0.0001$) for all the tested heavy metals that type of water used for irrigation had an effect on the concentration of heavy metals.

Concentrations of heavy metals in different plant tissue types (leaves and bulbs)

The mean concentrations of heavy metals in bulbs, (Cu-0.481, Pb-1.247, Cr-0.532, Ni-0.624, Cd-0.523) and Mn-0.594) of onions irrigated with wastewater were insignificantly higher than the mean concentrations (Cu-0.456, Pb-1.192, Cr-0.509, Ni-0.592, Cd-0.493) and Mn-0.579) in the leaves (Figure 1). A similar trend was also observed when comparing the mean concentrations of heavy metals in the bulbs (Cu-0.080, Pb-0.069, Cr-0.114, Ni-0.093, Cd-0.025) and Mn-0.1) of onions irrigated with tap water and the mean concentrations of heavy metals (Cu-0.072, Pb-0.062, Cr-0.098, Ni-0.078, Cd-0.011) and Mn-0.095) in the leaves (Figure 1). There was enough evidence at $p > 0.5$ to suggest that plant tissue type had no effect on concentration of heavy metals in onions.

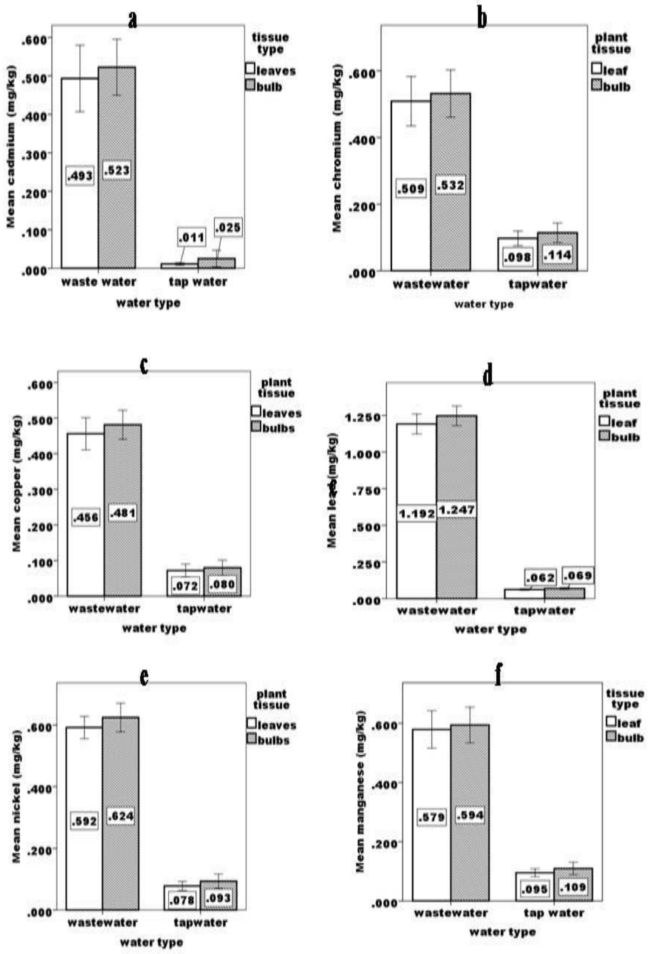

Figure 1. Mean heavy metal concentrations in the bulbs and leaves of onions (a) cadmium, (b) chromium, (c) copper, (d) lead, (e) nickel and, (f) manganese

Discussion

The concentrations of heavy metals in wastewater exceeded the safe limits set by WHO/FAO while heavy metal concentrations in tap water were below the WHO/FAO safe limits. Rattan *et al*, (2005) also found higher concentrations of heavy metals in sewage effluents compared to tap water. Many small scale industries such as dyeing, electroplating, metal surface treatment, and fabric printing discharge their effluents in sewage water through sewage drainages which may be the cause of elevated levels of heavy metals in the wastewater from Chitungwiza Sewage Treatment Plant. The application of wastewater leads to changes in heavy metal uptake by plants, particularly vegetables. The solid-solution partitioning of Pb, Cr, Mn, Ni, Cu and Cd is dependent on soil solution pH and total metal content (Brallier *et al*., 1996). Heavy metals are generally less mobile at pH>7 and their absorption by plants generally increases with decreasing pH, due to dissolution of metal-carbonate complexes, releasing free metal ions into solution (Connell and Miller, 1984).

Effects of water type (wastewater or tap water) on concentration of heavy metals

The results from this study showed that wastewater irrigation led to elevated levels of heavy metals in the bulbs and leaves of onions. Heavy metal accumulation by vegetables is a cause of serious concern due to the potential public health impacts (Bi *et al*., 2006). Liu *et al*, (2005) also reported an increase in heavy metal uptake in leaves of plants irrigated with wastewater compared to those irrigated with tube-well water in a study carried out in Beijing, China using spinach (*Spinacia oleracea*) as the test crop. This study also showed that water type used in irrigation has an effect on heavy metal uptake by plants. This result is supported by experiments carried out by Bahemuka and Mubofu, (1998) on heavy metal concentration in edible green vegetables grown along rivers in Dares-Salam using cabbage (*Brassica olerecea var. capitate*) as the test crop. Findings from this study suggested that heavy metal content was higher in cabbages irrigated with wastewater than those irrigated with groundwater. Adetogun (2010) also carried out an experiment on tomatoes (*Lycopersion esculentum*) in the Glen Valley, Botswana to determine chromium and nickel uptake and results obtained also suggested that type of water used for irrigation had an effect on heavy metal concentration in plants.

Results obtained from this study, showed that onions irrigated with wastewater accumulate more heavy metals than onions irrigated with tap water. This was also observed by Muchuweti *et al*, (2006) in a study using leafy vegetables. His results indicated higher levels of heavy metals in spinach irrigated with wastewater compared to spinach irrigated with ground water. Lui *et al*, (2005) also supported this hypothesis in his work done to investigate effects of long term irrigation using wastewater on quality of crops. He observed higher levels of nickel and lead in vegetables irrigated with wastewater compared to vegetables irrigated with tap water. All the onion samples irrigated with wastewater were highly contaminated with Pb, Cr, Mn, Ni, Cu and Cd and exceeded the permissible limits set by WHO/FAO.

Heavy metal concentration in leaves of onions irrigated with wastewater and those irrigated with tap water

Concentrations of heavy metals in leaves of onions irrigated with wastewater were higher than in leaves of onions irrigated with tap water and exceeded the safe limits whilst heavy metal concentrations in leaves irrigated with tap water were below the safe limits. The concentrations in both tap water and wastewater irrigated leaves followed the trend Pb> Ni>Mn> Cr> Cu> Cd. Pb concentration was higher than other heavy metals. According to Connel and Miller, (1984), Pb accumulates more in plants compared to other metals owing to its high transfer factor. According to (Luo *et al*., 2011), Pb has greater exchangeable capacity, easily becomes available and soluble in soils and becomes bio-available and accumulates in edible parts of the plant. Gupta *et al*, (2008) also found higher levels of Pb in wastewater irrigated crops compared to all other tested metals. Cd, according to Connel and Miller, (1984), is reported to have a low transfer factor; hence it was lower than other heavy metals. Findings from the current study were also similar to findings by Muchuweti *et al*, (2006) on an investigation on effects of wastewater irrigation on heavy metal uptake by Tsunga vegetable (*Catholea makoyana*) leaves and pepper (*Capsicum annuum*) at Firle Farm. Their results showed that Tsunga leaves and pepper irrigated with wastewater were heavily contaminated with lead, zinc, copper, chromium and nickel.

Heavy metal concentration in bulbs of onions irrigated with wastewater and those irrigated with tap water

The concentrations of all the six analysed heavy metals were high in bulbs of onions irrigated with wastewater compared to the bulbs of onions irrigated with tap water. The concentrations of heavy metals showed variations which may be attributed to the variation in heavy metal sources and the quantity of heavy metals in irrigated water. The concentrations of heavy metals in bulbs irrigated with wastewater exceeded the safe limits recommended by WHO/FAO. The effect of these high levels of heavy metals in the bulbs is justified by McBride (1995) who stated that the heavy metals become toxic in higher concentrations. The concentrations in bulbs irrigated with wastewater followed the trend Pb> Ni>Mn> Cr> Cu> Cd. This trend was also observed by Hillman (1988) in a similar study he did using wastewater and tube-well water to irrigate carrots. Generally, the concentrations of heavy metals were observed to be higher in bulbs of onions irrigated with wastewater compared to the bulbs of onions irrigated with tap water. Similar results were observed by Gupta *et al*, (2008) in a study carried out to determine heavy metal uptake by lettuce (*Lactuca sativa*) irrigated with wastewater. However, in their research, all heavy metals in lettuce irrigated with wastewater, though higher than in lettuce irrigated with ground water, were within safe limits recommended by WHO/FAO. This observed difference could be because of substandard conventional wastewater treatment being done at treatment plants in Zimbabwe before the water is discharged into streams

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Effects of plant tissue type (bulb or leaf) on concentration of heavy metals

Results obtained showed that plant tissue type (bulb or leaf) had no significant effect on accumulation of heavy metals. These findings contradict those of Sharma *et al*, (2006) that heavy metal concentration depends on the plant tissue being analysed in spinach (*Spinacia oleracea*). Adetogun, (2010), in an experiment done on tomatoes (*Lycopersion esculentum*) at Glen Valley Farm suggested that tissue type had an effect on heavy metal concentration as higher concentrations of nickel and chromium were observed in leaves of tomatoes irrigated with wastewater compared to the fruits. These differences could be accounted for by the differences in test crops used for the studies. Bahemuka and Mubofu (1998) observed that lead and cadmium concentrations were higher in lettuce (*Lactuca sativa*), followed by spinach (*Spinacia oleracea*) then lastly pepper (*Capsicum annuum*) in a study to determine heavy metal concentration in different crops being irrigated with wastewater. The differences could also be attributed to the different environmental conditions as well as the physio-chemical composition of the soil (Gupta *et al*., 2008).

Conclusion

A comparative assessment of heavy metal uptake between wastewater irrigated onions and tap water irrigated onions was done in this research. Results obtained showed higher levels of heavy metal contamination in the leaves and bulbs of onions irrigated with wastewater than those irrigated with tap water. The contamination of these onions by heavy metals was mainly due to the high heavy metal concentrations in the wastewater which was used for irrigation. All metals analysed in the bulbs and leaves of onions irrigated with wastewater were above the FAO/WHO recommended levels. This study also gave a brief insight into the current scenario of food crop contamination and possible future health risk estimates. There is a dire need to strictly monitor wastewater irrigation practices of the study area and develop different strategies to prevent heavy metal accumulation in food crops, this may ultimately minimize health risks to the exposed population.

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