Heliyon 6 (2020) e05241

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

# Accumulation of heavy metals and bacteriological indicators in spinach irrigated with further treated secondary wastewater

Moatlhodi Wise Letshwenyo [a,b,*], Gobusaone Mokokwe [a,b]

[a] Botswana International University of Science and Technology, Faculty of Engineering and Technology, Private Bag 16, Palapye, Botswana
[b] Department of Civil and Environmental Engineering, Private Bag 16, Palapye, Botswana

ARTICLE INFO

*Keywords:*
Bacteriological indicators
Health index
Heavy metal
Spinach
Transfer factor
Engineering
Food science
Agricultural science
Environmental science

ABSTRACT

Shortage of water for agriculture has resulted in the need to explore the use of wastewater for irrigation, however this can pose a health problem to the people emanating from the produced food as a result of the accumulation of pollutants. The purpose of this research was to investigate the accumulation of some heavy metals and bacteriological indicators in the different parts of spinach vegetable irrigated with secondary wastewater effluent treated through a pilot filtration system. There was a variation of heavy metals accumulation in the roots, stem and leafy parts of the vegetable. Bioaccumulation factors of some metals were greater than 1 in the roots and stems but not in the leaves. Three heavy metals being copper, iron and zinc revealed high translocation factors in the stems than leaves; whereas arsenic, chromium, nickel, manganese and lead had high translocation factors in the leaves than stems. Health index coefficients in the stem during the first, second and third months were 2.33, 0.18 and 3.57 respectively, and corresponding values in the leaves were 0.68, 0.09 and 6.75 if consumed by adults. The health index values greater than 1 in children were 2.68 in the stem during the first month and then 4.1 and 7.76 in the stem and leaves during the third month for spinach consumed by children. There was no bacteriological indicators detected in the aboveground parts of the vegetable. To conclude, irrigation of vegetables should be practiced using secondary treated wastewater and monitoring over time intervals in order to safe guard human health.

## 1. Introduction

Scarcity of water around the world has resulted in some countries experiencing long droughts and in turn low production of food, hence global food shortage. Some countries have opted to the use of wastewater for irrigation in order to increase food production (Gupta et al., 2012). Though wastewater is used to alleviate water shortage especially for agricultural purpose, its physio-chemical and biological composition are associated with the contamination of crops and groundwater (Aiello et al., 2007). Soils receiving wastewater effluent for irrigation may accumulate toxic heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr) and Nickel (Ni) (Anwar et al., 2016). Heavy metals easily accumulate in edible parts of leafy vegetables compared to grain or fruit crops and this can cause health problems to animals and human beings (Arora et al., 2008) Studies have been conducted on the accumulation of heavy metals in plant tissues irrigated with wastewater. For instance, a study conducted by Arora et al. (2008) reported higher concentrations of heavy metals in wastewater irrigated vegetables than fresh water irrigated vegetables. Different orders of magnitude of the heavy metals in food crops have been reported. For instance, Emurotu and Onianwa (2017) reported the order of heavy metals concentrations in magnitude as Zn > Ni > Cu > Pb > Cd > Co for maize and sugar cane and the same order for passion fruit and cassava except that Ni had a higher value than Zn. The order in the pumpkin was Zn > Cu > Ni > Co > Cd > Pb indicating that metals accumulate differently in some plants. Mazhari et al. (2019) conducted a study on the geochemical and environmental investigation of soils and crops and reported concentrations of Cr, Cu, Ni and Pb exceeding maximum permissible levels in alfalfa and maize crops. Some of the studies have investigated the human health risks associated with vegetables grown on soil irrigated with treated wastewater (Gupta et al., 2008). For instance, Balkhair (2016) reported that the Health Risk Index (HRI) was high (>1) in the okra vegetable crop, thus posing a health risk to human beings and animals. A Study by Ametepey et al. (2018) reported variations in the order of metal accumulations in vegetable or plant parts where it was observed that the metals concentrations in such vegetables were below World Health Organization (WHO) guidelines. Similarly studies have investigated the bioaccumulation and translocation factors of different heavy metals in plant parts or organs.

* Corresponding author.
*E-mail address:* letshwenyom@biust.ac.bw (M.W. Letshwenyo).

https://doi.org/10.1016/j.heliyon.2020.e05241
Received 31 July 2020; Received in revised form 9 September 2020; Accepted 8 October 2020

Qureshi et al. (2016) reported bioaccumulation factor (BCF) in different plants irrigated with treated wastewater in the order Fe > Zn > Cu > Cr. Translocation or transfer factor (TF) of heavy metals in plants have shown different values indicating differing mobility of these metals in plants. Gupta et al. (2010) has reported TF values >1 for Cd compared to other metals suggesting that this metals was easily absorbed by vegetables.

Studies conducted on the microbial loading in food crops irrigated with tertiary treated wastewater have revealed different loading of coliforms. A study conducted by Qureshi et al. (2016) observed high levels of total coliforms (TC) in spinach followed by radish and egg plants. The mode of irrigation has an influence on the microbial contamination of plants. Plants irrigated with drip irrigation will have different pollution load compared to those irrigated with sprinkling or flooding using the same quality of treated wastewater for irrigation. Tripathi et al. (2019) conducted a study on the accumulation of microbial pollutants in different plant parts and soil and observed high counts of *E. coli* and TC in the soil and plant roots of cauliflower and eggplant irrigated with wastewater. It was observed that plants irrigated with wastewater treated through filtration had lower *E. coli* and TC loads compared to those irrigated with untreated wastewater.

Compared to previous studies, many studies conducted did not monitor the accumulation of the heavy metals and bacterial indicators into or on plant tissues at different time intervals over time. Such studies can help in estimating the time the vegetables will have high risks to human health and even recommend harvest time. The purpose of this study was to investigate the accumulation or build-up of heavy metals and bacteriological indicators in different spinach parts at different time intervals. The study was motivated by the increasing vegetable horticultures in the country that are using reclaimed wastewater for irrigation purposes and such project use secondary treated wastewater for irrigation. The study was conducted through a pilot scale wastewater treatment system comprising filtration system and pilot garden.

## 2. Material and methods

### 2.1. Study area

The study was conducted at a pilot scale treatment system located at the end of the wastewater treatment facility of the university campus. The wastewater treatment system is a waste stabilisation type treating domestic wastewater and comprises two anaerobic ponds in parallel, one facultative pond and four maturation ponds all in series with anaerobic ponds. The pilot system consisted a holding tank for effluent pumped from secondary treated wastewater maturation pond, roughing and slow sand filters and a holding tank for the treated tertiary effluent (Figure 1). The roughing filter consisted of gravel and coal clinker of varying size. Gravel was sourced from local suppliers and coal clinker from a mining company. Gravel, copper smelter slag and sand media were used in the slow sand filter.

At the end of the system, there were experimental plots on which the spinach (Ford hook Giant) was grown for a period of 4 months. On these experimental plots, spinach seeds were directly sown 10–20cm apart. The crop was drip irrigated with secondary treated wastewater. The drip irrigation system supplied crops with irrigation water of about 3.5mm–5mm per day. Fertilisers were not applied in the experimental plots.

### 2.2. Sample preparation

Spinach was collected from the plot and then separated into roots, stem and leaf organs and placed into different sampling polyethylene bags. Spinach samples were washed with deionised water to get rid of air pollutants and dirt and then allowed to dry at room temperature (20 °C). Sample preparation was as per Alghobar and Suresha (2017) protocol. Individual organs of spinach were thoroughly mixed together to represent one sample. The first samples were collected at the beginning of November 2019, second sampling in December 2019 while last sampling was in January 2020. Sampling was conducted every last week of the month.

Plant samples were dried at 105 °C for an hour and then at 60 °C for 24 h in a furnace and then pulverised into finer particles by grinding using mortar and pestle. The ground particles were then passed through a 1.0 mm sieve after which 0.5 g of each sample was subjected to acid digestion as described by Ramachandra et al. (2018) using perchloric acid ($HClO_4$) and Nitric acid ($HNO_3$) at a ratio of 1: 4 as per Alghobar and Suresha (2017). Digested samples were cooled at room temperatures and filtered with Whatman filter paper No 42. Filtrates were diluted with deionised water (DI) to make a total volume of 25 mL. In the case of soil sample digestion, HF-$H_2SO_4$ -$HClO_4$ were used for digestion following the procedure of Alghobar and Suresha (2017).

In addition, soil samples were also collected around the areas the plant samples were collected at a depth of 10 and 15 cm using stainless steel crab. Samples were air dried and passed through a 2.0 mm sieve and media passing ground using a hand mortar. The samples were prepared in the same way described by Alghobar and Suresha (2017).

The irrigation water samples were collected from the storage tank using either plastic or glass bottles. The samples were filtered through a 0.45 μm Whatman filter paper and analysed as per APPHA 1998 procedure.

### 2.3. Analytical procedure

For metals analysis, the solutions were subjected to Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (I-CAP 7000 SERIES). The instrument was calibrated before analysis for accuracy and quality control using the standards recommended by the supplier.

#### 2.3.1. Bioconcentration factor

The bioconcentration factor (BCF) which is the soil to plant metal transfer or the efficiency of a plant in accumulating metals in its tissues from the surrounding environment was calculated as per Boechat et al. (2016) description:

$$BCF = \frac{\text{Metal concentration in plant tissue}\left(\frac{mg}{kg}\right)}{\text{Metal concentration in soil}\left(\frac{mg}{kg}\right)} \tag{1}$$

#### 2.3.2. Translocation factor

The translocation factor (TF) indicating the ability of plants to translocate or transport heavy metals from roots to stem and leaves was calculated according to equation described by Boechat et al. (2016):

$$TF = \frac{Cp}{Cr} \tag{2}$$

Where Cp is the concentration of heavy metals in the plant parts (mg $kg^{-1}$), Cr is the concentration of heavy metals in roots (mg $kg^{-1}$). The TF values of all the heavy metals were calculated to assess their transport in the different parts of the vegetable.

#### 2.3.3. Daily intake of metals

The daily intake of metals (DIMs) in mg/kg/day was calculated according to Khan et al. (2008):

$$DIM = \frac{\text{Cmetal} \times \text{Cfactor} \times \text{Dfoodintake}}{\text{Baverage weight}} \tag{3}$$

Where $C_{metal}$, $C_{factor}$, $D_{food}$ intake and average weight are heavy metal concentration in spinach (mg/kg), conversion factor, daily intake of food in vegetable (mg) and average body weight (kg) respectively. The $C_{factor}$ was 0.085, the average daily intake of vegetables for adults and children adopted were 0.345 and 0.232 kg $person^{-1}$ $day^{-1}$ respectively. The corresponding adult and child body weights were 55.9 and 32.7 kg respectively and this was as per the protocol of Wang et al. (2005).

#### 2.3.4. Hazard Quotient (HQ)

The HQ, which has been defined is a proportion of probable exposure to heavy metals and concentration at which no negative effect is expected was calculated as per Ametepey et al. (2018):

$$HQ = \frac{DMI}{RfD} \tag{4}$$

Where HQ is Hazard Quotient, DIM is the average daily intake of metal (mg/kg/day) and $R_fD$ is the oral reference dose of the metal (mg/kg/day). The $R_fD$ values of 0.004, 0.3, 0.04, and 0,7, 1.5, 0.0003, 0.02 and 0.14 mg/kg/day for Pb, Zn, Cu, Fe, Cr, As, Ni and Mn were adopted from Ametepey et al. (2018).

#### 2.3.5. Hazard Index (HI)

The HI which is a summation of exposures to more than one pollutant was determined by the addition of HQ values as described by Ametepey et al. (2018). HI is used to estimate the potential heavy metals have on the human risks and is the summation of THQs:

$$HI = (THQi + THQii + THQiii........THQn) \sum THQ \tag{5}$$

Where HI is the hazard index, THQ is the target hazard quotient, and ΣTHQ is the summation of the target hazard quotients.

#### 2.3.6. Bacteriological analysis

Bacteriological indicators analysed in irrigation water, plant tissues and soil samples were Total Coliforms, Faecal Coliforms, *E. coli* and Faecal Streptococci according to Federation (1999). The results were expressed as Coliform Forming Units (CFU)/100 mL.

#### 2.3.7. Statistical analysis

The data was analysed through Microsoft Excel Analysis of variance (ANOVA) test to determine any significant differences between the means of heavy metals in spinach parts. The level of significance set was $p \leq 0.05$, means and standard deviations of metal concentrations in soil and vegetable parts were computed as well.

## 3. Results and discussion

### 3.1. Irrigation water and unplanted soil quality

The concentrations of the heavy metals in sewage water, treated water and soil before irrigation are as shown in Table 1. From the Table, the concentrations of the metals in the treated water were below the national thresholds values for irrigation water set by Botswana Bureau of Standards (BOBS). The mean concentrations of As, Cr, Cu, Fe, Mn, Ni, Pb and Zn were 77, 71, 1.8, 833, 3.3, 2.5, 250 and 100 times below the recommended maximum limit in the national standards, respectively. This was an indication that the water could be used for irrigation purposes. It is interesting to note that in the sewage water, there were no Cu, Fe, Mn, Pb and Zn detected. However, these metals were present in the treated water and this might have been due to leaching from the materials used for filtration in the pilot treatment system. Woldetsadik et al. (2017b) reported similar findings for Cr, Cu, Ni, Pb and Zn in irrigation water used for irrigation vegetables farming sites. In contrast, Achak et al. (2009) observed mean concentrations of Ni, Cd and Co above Food and Agricultural Organisation (FAO) irrigation guidelines for irrigation water. Similarly, the concentration of heavy metals in the sewage and treated water were far below those reported by Jin et al. (2020) which were above FAO limits. Though the concentrations of the heavy metals in the water was low, its continuous use might lead to accumulation in soil and then uptake into the different parts of spinach and accumulate there.

The mean concentrations of As, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the soil before irrigation were 0.32 ± 0.04, 0.92 ± 0.50, 0.56 ± 0.22, 912.6 ± 792.9, 3.7 ± 2.9, 0.56 ± 0.003, 0.63 ± 0.06 and 8.2 ± 0.81 mg/kg respectively. But with the use of effluent, the concentrations are expected to increase due to accumulation overtime in the soil. It was observed that all the heavy metals concentrations in the irrigated soil had increased and this was due to accumulation from irrigation water.

Other parameters such as TDS, turbidity, EC and salinity revealed a decrease after treatment in the pilot plant. The observed decreases were by 23.5, 93.6, 23.9 and 24.82% respectively, indicating that the pilot plant remarkably reduced these parameters. All the parameters were within the national limits of irrigation water.

### 3.2. Heavy metals uptake by spinach

The mean concentrations of the eight heavy metals in the different vegetable parts are shown in Table 2. The different crop parts of roots, stem and leaves are reported to play important roles in the storing and transportation of heavy metals in crop parts (Fujimaki et al., 2010). The results reveal varying concentrations of the heavy metals in the vegetable parts. It was observed that during the first month As, Fe, Ni and Pb had the highest concentrations in the roots of the vegetable than other parts. During the same period, stem had high concentrations of Cu and Zn compared to other heavy metals. The concentrations of Cr and Mn metals were higher in the leafy parts than stems and roots during the same period. The trend changed during the second month where it was observed that six of the eight heavy metals were dominant in the roots than the other parts of the vegetable. The dominant heavy metals were As, Cr, Cu, Fe, Mn and Zn. Of the remaining two heavy metals, Ni was not detected in any plant part but Pb had high concentration in the stems. There was a further change observed during the third month of monitoring as Cr, Fe and Pb had a high concentrations in the roots and the remaining five heavy metals were dominant in the leaves instead. A study by (Tasrina et al., 2019) found the level of Pb in plants exceeding the toxic levels with the level in spinach amarantha being the highest at 1.596 mg/kg exceeding WHO standard of 03 mg/kg. The findings in the study also revealed high Pb levels during the first and third month exceeding WHO threshold. The results suggest that the concentrations of the heavy metals in the vegetable parts varied with time, but that variation was either high in the roots of leafy parts. The reason might be due to the roots being the first parts to transport the ions from the soil, and because of transpiration the metals are quickly transferred to the leaves. The concentrations of As in the roots increased by 941% during the second month but reduced by 60% during the third month. The trend was different in the stem as there were increases of 1475 and 567% during the second and third month respectively. In the leaves, the concentration of the same heavy metals reduced by 85% during the second month and then increased by 5285% during the third month. This trend was similar to the other heavy metals during the period of the study.

The results of this study are comparable to the findings of Meng et al. (2016) who reported different trends of heavy metals concentrations in root, stem and leaves of some plants. The same authors reported high accumulation of Zn, Cr and Ni in leafy vegetables such as spinach and amaranthas and the concentrations of these heavy metals had increased in all the vegetable parts during the third month which was in agreement with the findings by Meng et al. (2016). However, in terms of concentrations, the results contrasts the finding by Meng et al. (2016) who reported Cu concentration of 39.02 mg/kg in tomatoes after being irrigated with sewage water for a long term. The difference might have been due to the high concentration of Cu observed in the used water, which ranged between 10.92 – 61.32 mg/kg compared to 0.11 ± 0.002 mg/kg used in this study, which was tertiary treated. Similarly, Khan et al. (2008) reported concentrations of Cd, Cr, Ni and Pb in plant parts exceeding State Environmental Protection Administration limit in China in contrast to the findings in this study further indicating the need to further treat secondary effluent before use for irrigation. Similar observations were

**Table 1.** Heavy metal and physiochemical parameters concentrations in sewage, treated irrigation water and soil.

| Heavy metals (mg $L^{-1}$) | Secondary effluent | Treated secondary effluent | Unirrigated soil | Botswana Bureau of standards (BOBS) | Food and Agriculture Organisation (FAO) |
|---|---|---|---|---|---|
| Arsenic (As) | 0.12 | 0.0013 ± 0.0020 | 0.32 ± 0.04 | 0.1 | - |
| Chromium (Cr) | 0.57 | 0.0014 ± 0.0005 | 0.92 ± 0.50 | 0.1 | 2.3 |
| Copper (Cu) | 0.00 | 0.11 ± 0.0020 | 0.56 ± 0.22 | 0.2 | 73 |
| Iron (Fe) | 0.00 | 0.006 ± 0.0001 | 912.6 ± 792.9 | 5.0 | 425 |
| Manganese (Mn) | 0.00 | 0.06 ± 0.0009 | 3.7 ± 2.9 | 0.2 | 500 |
| Nickel (Ni) | 0.01 | 0.08 ± 0.0020 | 0.56 ± 0.003 | 0.2 | 67 |
| Lead (Pb) | 0.00 | 0.008 ± 0.0010 | 0.63 ± 0.06 | 2.0 | 0.3 |
| Zinc (Zn) | 0.00 | 0.02 ± 0.0010 | 8.2 ± 0.81 | 2.0 | 100 |
| Temperature (°C) | 23.00 ± 3 | 20 ± 4.0000 | ND | 25 | |
| pH (pH units) | 8.20 ± 0.21 | 8.10 ± 0.2000 | | 6.5–8.4 | - |
| Chemical Oxygen Demand (COD) (mg/L) | 135 ± 10 | 9 ± 3.0000 | | Not defined | - |
| Biochemical Oxygen Demand (BOD) (mg/L) | 32 ± 2 | 5 ± 0.5000 | | Not defined | - |
| Total Dissolved Solids (TDS) (mg/L) | 622 ± 79 | 476 ± 60.0000 | | 200 | - |
| Total Suspended Solids (TSS) (mg/L) | 140 ± 120 | 0 | | 100 | - |
| Turbidity (NTU) | 78 ± 58 | 5 ± 3.0000 | | Not defined | - |
| Electrical conductivity (EC) (μS/cm) | 876 ± 109 | 667 ± 87.0000 | | 300 | - |
| Salinity (mg/L) | 419 ± 48 | 315 ± 47.0000 | | Not defined | - |

**Table 2.** Concentration of heavy metals in the vegetable parts (mg/L).

| Heavy metals (mg/kg) | | As | Cr | Cu | Fe | Mn | Ni | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|
| Months | Soil and Parts | | | | | | | | |
| 1st month | Irrigated soil | 0.26 ± 0.020 | 0.49 ± 0.110 | 0.61 ± 0.260 | 238 ± 66.3 | 3.60 ± 2.2 | 0.28 ± 0.001 | 0.44 ± 0.004 | 16.10 ± 0.6 |
| | Roots | 0.32 ± 0.009 | 0.44 ± 0.007 | 0.62 ± 0.050 | 199 ± 7.2 | 1.60 ± 0.13 | 0.30 ± 0.007 | 0.60 ± 0.03 | 7.20 ± 0.24 |
| | Stem | 0.01 ± 0.006 | 0.39 ± 0.310 | 0.90 ± 0.740 | 2904 ± 0 | 2.50 ± 2.0 | 0.09 ± 0.008 | 0.10 ± 0.01 | 59.15 |
| | Leaves | 0.22 ± 0.020 | 0.49 ± 0.110 | 0.61 ± 0.260 | 238 ± 66 | 3.60 ± 2.2 | 0.28 ± 0.001 | 0.44 ± 0.004 | 16.10 ± 0.6 |
| 2nd month | Irrigated soil | 0.032 ± 0.010 | 4.04 ± 1.200 | 2.11 ± 0.500 | 464 ± 15.3 | 17.80 ± 3.5 | 1.90 ± 0.43 | 0.62 ± 0.15 | 1.55 ± 0.05 |
| | Roots | 0.41 ± 0.002 | 0.71 ± 0400 | 3.10 ± 0.600 | 45.10 ± 5.4 | 9.30 ± 1.5 | 0.00 | 0.14 ± 0 | 3.03 ± 0.05 |
| | Stem | 0.02 ± 0.0 | 0.26 ± 0.01 | 0.89 ± 0.15 | 9.98 ± 2.3 | 3.60 ± 0.25 | 0.00 | 0.85 ± 0.02 | 0.96 ± 0.35 |
| | Leaves | 0.004 ± 0.0 | 0.33 ± 0.01 | 0.98 ± 0.05 | 16.43 ± 4.5 | 8.10 ± 2.5 | 0.00 | 0.23 ± 00 | 0.95 ± 0.05 |
| 3rd month | Irrigated soil | 0.35 ± 0.030 | 65.40 ± 10.5 | 33.40 ± 5.6 | 437.54 ± 55.6 | 331 ± 45.3 | 741 ± 96.5 | 56.40 ± 10.3 | 89.21 ± 10.65 |
| | Roots | 1.81 ± 0.500 | 12.50 ± 3.5 | 19.50 ± 4.5 | 615.54 ± 45.6 | 135 ± 13.4 | 9.70 ± 1.1 | 2.93 ± 0.001 | 18.50 ± 4.67 |
| | tem | 1.5 ± 0.41 | 3.87 ± 0.65 | 14.90 ± 3.3 | 595.40 ± 100.34 | 44.90 ± 2.5 | 2.26 ± 0.03 | 0.69 ± 0.003 | 22.20 ± 7.6 |
| | Leaves | 2.5 ± 0.25 | 9.32 ± 1.5 | 28.50 ± 4.5 | 481.21 ± 55.5 | 197 ± 33.5 | 12.50 ± 0.55 | 2.85 ± 0.92 | 166 ± 20.5 |

reported by Ackah et al. (2014), and the differences with this study is that there was no monitoring of heavy metals uptake by the vegetables with time which can help in minimising public health risks. The heavy metals that were in leaves were as a result of absorption by roots and then transfer to leaves from roots via stems. The variability of heavy metal concentrations in the different vegetable parts have been linked to the characteristics of the plant as well as the availability of these different heavy metals (Tom et al., 2014). The results also indicate that the same crop or plant type can have different levels of heavy metals.

There were significant differences between the concentrations of As in the roots, and stem ($p < 0.05$) and there were no significant differences between the leaves and roots and stem and leaves. However, in the case of other heavy metals there was no significant difference between the plant parts ($p > 0.05$). This was expected as the accumulation of these heavy metals varied in each vegetable part over a period either increasing or decreasing. The overall significant differences between heavy metal concentrations overtime in the vegetable parts was only observed during the second month of the study ($p = 0.009$) but no significant differences were observed during the first and third months with $p = 0.225$ and $p = 1.32$ respectively. Posthoc tests on the different parts of the vegetable to determine the significant difference observed during the second month showed no significant differences of metal uptake by the different parts of the vegetable. The concentrations of the heavy metals in the vegetable parts were similar to that reported by Zhou et al. (2016) in the edible parts of different vegetable species. It has been reported that Pb binds to root surfaces and cell walls and therefore reducing transfer to shoots or leaves (Obb et al., 2000). This observation was in agreement to the findings of this study except during the 2nd month during which Pb accumulation was high in the stems. In contrast, a study by Baldantoni et al. (2016) observed that leafy vegetables showed significantly higher accumulation of cadmium in leaves compared to other parts. A study by Pajevi and Arsenov (2018) reported high concentrations of heavy metals in spinach leaves compared to other organs. The observation was similar to the findings of this study in some instances where for example, As, Cr, Cu, Mn, Ni and Zn concentrations were observed to be high in spinach leaves than roots and stems, therefore a need to seriously monitor vegetables irrigated with wastewater. A study by Gan et al. (2017) reported that in general, leafy and root vegetables have high concentrations of heavy metals compared to fruit crops and attributed this to large distribution of stomata on the leaves and evaporation taking place on the leaves surfaces.

The irrigated soil samples were monitored over time, and an increase in the concentrations of the heavy metals was observed. For instance, the increases of Cr were 724 and 1519% during the second and third month and the corresponding increases for Cu were 246 and 1483% respectively. This trend was observed mostly for the other heavy metals. The increase might have been due to the accumulation from irrigating water over time. The same was reported by Meng et al. (2016) who reported

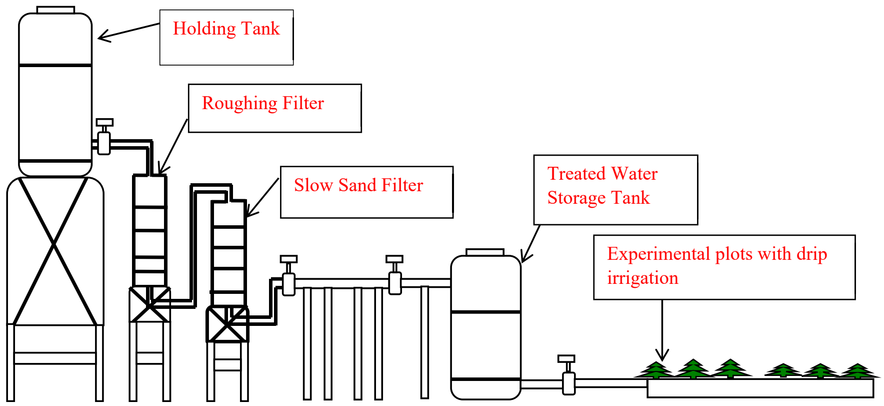


**Figure 1.** Flow diagram for the pilot wastewater treatment plant used for irrigation of spinach.

accumulation of heavy metals in the soils irrigated with wastewater over a period of time. The results suggest that over a period of time using wastewater effluent, the heavy metals concentrations will build up in the soil, hence posing a risk to public health when consuming vegetables irrigated with the water. If the wastewater used is not treated to the required standard before use there is a possibility of accumulation, resulting in increased risks to public health Chauhan and Chauhan (2014) also reported accumulation of heavy metals in all wastewater irrigated soils over time. The variability of metal concentrations in soils has been attributed to each plant's characteristics and the concentrations of these metals in the soil (Tom et al., 2014).

### 3.3. Bioaccumulation factor

Figure 2 shows the accumulation of the different heavy metals in the spinach roots, stem and leaves overtime. During the first month, the accumulation of As, Cu, Ni and Pb in the roots were >1.0 and in the stem Cu, Fe and Zn the BCF values of these metals were also >1 whereas Cr, Cu, Fe, Ni, Pb and Zn values were 1 in the leaves. The results during the second month indicate that only As, Cu and Zn having BCF values in roots >1 and only the BCF values in the stem exceeding 1. In the case of leaves, there was no BCF value exceeding 1. During the third month of the study, only As and Fe had BCF values >1 in the three vegetable parts (roots, stem and leaves). It has been reported that heavy metals accumulation in plants depend on the plant species with uptake from soil occurring with a mass flow of water (Boechat et al., 2016). With regard to the edible parts, it was observed that Cu, Fe, Pb and Zn had high accumulations in the stem but the other metals had BCF values <1 in both parts during the first two months of the study.

During the third month only As and Fe had BCF values were >1 in both the stem and the leaves, indicating that the accumulation of other metals in these parts was low. BCF values of all the metals except Pb were observed to be high in the roots than the aboveground parts during the second month of the study. The same results were reported by Ferniza-García et al. (2017) in their study of accumulation of heavy metals in *Typha latifolia* and this was attributed to passive absorption process and metal accumulation in the rhizosphere. It has been reported that the accumulation of heavy metals in plants can affect their growth rate and development as they can bring changes in plant hormones (Mi et al., 2019). The results in this study suggest that those metals ions with higher BCF might affect the growth of spinach either positively or negatively through metabolism processes. Khan et al. (2008) observed high BCF values of Cd, Cu, and Ni than other heavy metals, and these were varying between plant species indicating high uptake by the plants. BCF >1 indicate that the heavy metals are easily taken up by plants from the soil through the plants organs and these organs accumulate these heavy metals and BCF <1 indicate that spinach absorbed these heavy metals but did not store them (Chopra and Pathak, 2012). It has been reported that BCF ratio >0.20 indicates high contamination of vegetables by human activities hence high risk to health (Ahmed et al., 2019). The BCF ratios of the heavy metals during the first month were >0.2 but the spinach was not ready for harvest during the time hence low public health risk. During the second month, only As, Cr, Fe and Ni BCF values in leaves were <0.2 and during the third month, Cr, Ni, Pb and Zn recorded <0.2 BCF values in leaves. The results further show the variability of heavy metal accumulation in plants with time which might be due to different levels of nutrient requirements in plants or vegetables. There is a need to monitor BCF of vegetables in order to predict the time the accumulations might be a risk to public health. There were significant differences of heavy metal uptake between the different organs or parts, roots, stem and leaves ($\rho < 0.05$) observed. The significant differences observed were between roots and leaves and stem and leaves, for the accumulation of As. The other significant differences observed were between roots and stem, stem and leaves for Fe accumulation and roots and stem for Mn accumulation. There were no significant differences ($\rho > 0.05$) between these organs for the accumulation of other heavy metals. The results indicate that the

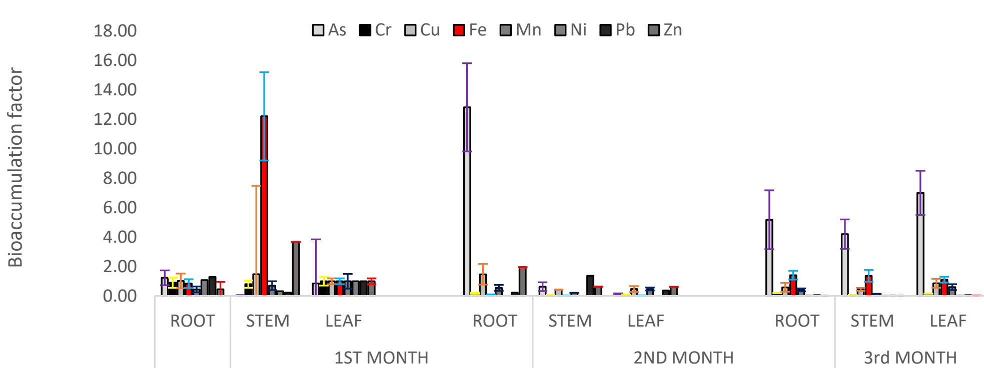


**Figure 2.** Bioaccumulation factor of heavy metals in different plant parts overtime.

accumulation of the heavy metals in the different parts of spinach did not vary significantly. A significant difference ($p = 0.02$) was observed on the overall accumulation of the heavy metals in the vegetable parts. In addition, further analysis indicated that the differences occurred during the third month of the study period. This suggests that the overall uptake and storage of the heavy metals by the different spinach plants differed and indicating that the metals have different mobility into plant tissues and the requirement of these nutrients differ.

### 3.4. Transfer factor

The values of transfer factors of the different metals from soil to the different vegetable parts varied (Figure 3). During the first month, Fe showed the highest TF in stems than leaves as TF in stem was 12.16 times more than in leaves. Similarly, Zn was 3.7 times in stems than the leaves of the plant and Cu was observed to be 1.48 times higher instead. In contrast, all the remaining 5 metals had higher TF values in leaves than in stems. As, Pb, Ni, Mn and Cr values were 22, 6.0, 3.1, 1.44, and 1.3 times higher in the leaves than the stems, respectively. Heavy metals having TF values >1 in the stem during the first month were Cu, Fe, Mn, and Zn in the case of leaves the heavy metals having TF values >1 were Cr, Fe, Mn and Zn. The results suggest that Fe, Mn and Zn were had high mobility during the early stages of the plant growth as they might be in demand nutrients at that stage. There were changes of TF values observed during the second month of the study (Figure 3). Only, As and Pb TF values were higher in stems than leaves during the second month with As and Pb being 5.0 and 3.7 times higher respectively. As for the remaining heavy metals, it was observed that their TF values were higher in the leaves than the stems in the decreasing order of magnitudes Mn > Fe > Cr > Cu > Zn > Ni with corresponding TF magnitudes of 2.25, 1.65, 1.27, 1.10, 1.01 and 0.0, respectively. Only Pb had TF value >1 in both the stem and the leaves of the vegetable. During the third month, only Fe had high TF value in the stem but the remaining heavy metals indicated higher TF values in the leaves than the stem. The TF value in the stem was 1.65 times than in the leaves. The values in the leaves decreased as Zn > Ni > Mn > Pb > Cr > Cu > As with the corresponding values of TF magnitudes in the leaves than stem being 7.5, 5.5, 4.4, 4.1, 2.4, 1.9 and 1.7 respectively. A study conducted by Rehman et al. (2018) observed high transfer factor of heavy metals in spinach with Pb, Cu, Zn, Mn and Fe having high TF values similar to this study suggesting high mobility of these heavy metals in spinach. High TF values indicate the capability of the plant to transport the heavy metals in their parts and also poor retention by soil (Tasrina et al., 2019). The results of this study show that during the first and second months roots were the barriers to As mobility since TF values were low during that period, this was also true for Ni during the second month. Leafy vegetables have been reported to have a high translocation rate and high transpiration rate compared to other vegetables (Sridhara Chary et al., 2008). Also the transfer of metals from roots to stem and then fruits takes longer compared to leaf vegetables. It has been reported that the enrichment of heavy metals ability is related to growth characteristics and genetic characteristics of different vegetables (Meng et al., 2016). In this study, both the stem and leaves of spinach are edible parts of the vegetable, hence care should be taken on the transfer of the metals to both plant parts. The results of this study indicate that the transfer of heavy metals was mainly to the leaves than to the stem. But it should be noted that there are people who consume both the stem and the leaves of spinach hence both vegetable parts will pose health risks to the consumers. In addition the results indicate that TF values of the different metals in the two parts of the vegetable can differ at any time. However, significant differences ($\rho < 0.05$) were observed between stem and leaves only for the transfer of Pb and Fe, no significant difference ($\rho > 0.05$) were observed for the other heavy metals. The results suggest that the transfer of the six heavy metals by the stem and leaves of the spinach did not differ except in the case of Pb and Fe. This suggests that the mobility of these heavy metals through the stem and leaves were the same but that of Pb and Fe differed through these vegetable parts.

The metals that had high TF values (>1) during the third month were As, Cu, Mn, Ni and Zn which were recorded in the leaves of the vegetable and only Zn had TF > 1 in the stems. This suggests that the heavy metals were transferred to the leafy parts overtime.

### 3.5. Daily intake of metals

Table 3 shows the daily intake of heavy metals estimated basing on the average vegetable consumed by either adults or children. The DIM values were evaluated in the stem and the leaves since both parts of the plants are sometimes all consumed by people though leaves are mostly consumed. But since sometimes only leaves are cooked, it was necessary to divide the two parts and find the values of each. For adults during the first month, the highest DIM values in the stem were those of Cu, Mn, Fe and Zn whereas in the leaves it was found that the highest DIM values in adults were those of As, Cr, Ni and Pb. As for children, the highest DIM values in the stem were those of Cu, Fe, and Zn with As, Cr, Mn, Ni and Pb dominant in leaves. The trend changed during the second month where it was observed that Cu and Zn had the same DIM values in an adult for both the stems and the leaves meaning. This suggests that, if it is eaten by an adult the intake levels of these heavy metals will be the same. It was observed that As and Pb were high in the stem whereas Cr and Fe were high in the leaves if consumed by adults. The situation in children was that As and Pb had the highest DIM values in the stem and Zn had the same values in both stem and leaves and the remaining heavy metals (Cr, Cu and Fe) had high values in the leaves of the vegetable if consumed by

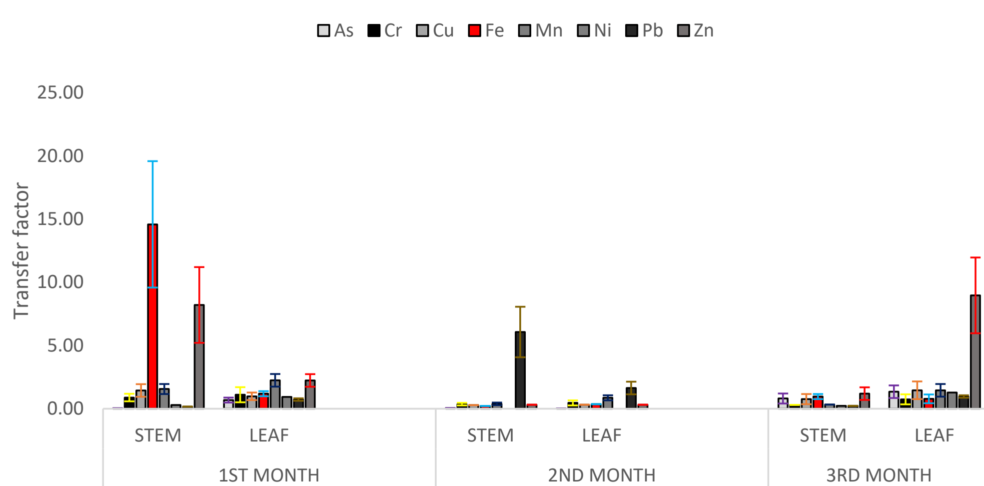


**Figure 3.** Translocation factor of heavy metals in different plant parts of spinach overtime.

**Table 3.** Daily intake of heavy metals by adults and children overtime (mg/kg/day).

| Time | Plant part | Individuals | As | Cr | Cu | Fe | Mn | Ni | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|---|
| 1st Month | Stem | Adult | 5.2E-06 | 2.0E-4 | 5.0E-4 | 1.5 | 1.3E-02 | 4.7E-05 | 5.2E-05 | 3.1E-02 |
| | Leaves | | 1.0E-04 | 3.0E-04 | 3.0E-04 | 1.2E-01 | 1.9E-03 | 1.0E-04 | 2.0E-04 | 8.4E-03 |
| | Stem | Children | 6.0E-06 | 2.4E-04 | 5.4E-04 | 1.8 | 1.5E-03 | 5.4E-05 | 6.0E-05 | 3.6E-02 |
| | Leaves | | 1.0E-04 | 3.0E-04 | 4.0E-04 | 1.4E-01 | 2.2E-03 | 2.0E-04 | 3.0E-04 | 9.7E-03 |
| 2nd Month | stem | Adult | 1.0E-05 | 1.0E-04 | 5.0E-04 | 5.0E-03 | 2.0E-03 | 0.0 | 4.0E-04 | 5.0E-04 |
| | leaves | | 2.1E-06 | 1.7E-04 | 5.1E-04 | 8.6E-03 | 4.2E-03 | 0 | 1.2E-04 | 5.0E-04 |
| | Stem | Children | 1.2E-05 | 1.6E-04 | 5.4E-04 | 6.0E-03 | 2.2E-03 | 0.0 | 5.1E-04 | 5.8E-04 |
| | Leaves | | 2.4E-06 | 2.0E-04 | 5.9E-04 | 9.9E-03 | 4.9E-03 | 0.0 | 1.4E-04 | 5.7E-04 |
| 3rd Month | Stem | Adult | 1.0E-03 | 2.0E-03 | 8.0E-03 | 3.1E-01 | 2.4E-02 | 1.0E-03 | 0.0 | 1.2E-02 |
| | Leaves | | 1.0E-03 | 5.0E-03 | 1.5E-02 | 2.5E-01 | 1.0E-01 | 7.0E-03 | 1.0E-03 | 8.7E-02 |
| | Stem | Children | 9.0E-04 | 2.3E-3 | 9.0E-03 | 3.6E-01 | 2.7E-02 | 1.4E-03 | 4.0E-04 | 1.3E-02 |
| | Leaves | | 1.0E-03 | 6.0E-03 | 1.7E-02 | 2.9E-01 | 1.2E-01 | 8.0E-03 | 2.0E-03 | 1.0E-01 |

children. During the third month, if adults consumed the stems and the leaves of the vegetable, there would be high intakes of Fe. The same is true for children and in addition high DIM values of Mn and Zn were observed in the leaves if consumed by children. For children, the highest DIM values of the heavy metals will be found in the leaves except for Fe which had high value in the stem. The trend is almost similar for adults suggesting that overtime DIM values will be high for both groups if leaves of the spinach are consumed. Rehman et al. (2018) observed DIMs in the order Fe > Mn . Pb > Ni > Zn > Cu which differed to the findings in this study, and this might be due to influence of many factors such as BCF and TF values which are influenced by many factors such as soil characteristics.

Human exposure to heavy metals is through food intake in the food chain which is the primary pathway for the exposure (Khan et al., 2008). All the DIM values were <1 in both adults and children except for Fe in the stem for both groups indicating risk to Fe if the stem was consumed and this was only during the first month. The results suggest that it will be safe to consume the spinach without any health risks except for Fe during the first month. The irrigation water used was tertiary treated to remove most of the pollutants and this might be the reason of low exposure to health risk. The results are comparable to the findings by Rehman et al. (2018) who reported DIM values <1 for both adults and children for exposure to vegetables grown on contaminated soils in Pakistan. In this study, it was important to calculate DIM values for both leaves and stem since locally some people cook and consume both hence need to know risks associated with their consumption, unlike in other studies where only the leaf part is investigated, for instance, a study by Chauhan and Chauhan (2014) on spinach leaves. There was no overall significant differences be between DIM values for stems and leaves during the study period ($p > 0.05$) if consumed by adults, the same was observed for the first, second and third months. Similar trends were observed in the case of children consuming stems or leaves of the vegetable.

#### *3.5.1. The HQ and HI*

The HQ of the heavy metals that exceeded 1 were Fe (2.18) in the stem and As in both stems (2.6) and leaves (4.3) during the third month if these vegetable parts are consumed by adults (Table 4). The health index in the stem during the first, second and third months were 2.33, 0.18 and 3.57 respectively and corresponding values in leaves were 0.68, 0.09 and 6.75 if consumed by adults. The results suggest that Fe has the potential of non-carcinogenic risk for adults when the stems are consumed during the first month of growth, whereas other heavy metals have no obvious individual risks in both the stems and the leaves in the second month. During the third month, only As showed potential carcinogenic risks in the stem and leaves but no risks for the other heavy metals. The combined HI values of the eight heavy metals was >1 (2.33) in the stems during the first month and then 3.57 and 6.75 in the stems and leaves respectively during the third month.

Similarly, it was observed that during the first month of plant growth Fe had HQ value >1 in the stem and then As in both the stem and leaves of the vegetable during the third month if consumed by children (Table 5). Consumption of vegetables with single heavy metals will only pose a health risk during the first month for Fe and the third month for As. The combined HI values of the heavy metals exceeded 1 in the stems during the first month if consumed by both adults and children and then during the third month HI > 1 in both stems and leaves for both cases. Just in the same way as for adults, it was found that HI values >1 were 2.68 in the stem during the first month and then 4.1 and 7.76 in the stem and leaves during the third month. The absorption of metals by plants

**Table 4.** Hazard quotients of the different heavy metals in stem and leaves of spinach if consumed by adults and corresponding hazard indices.

| Time (months) | 1st month | | 2nd month | | 3rd month | |
|---|---|---|---|---|---|---|
| Vegetable parts | Stem | Leaves | Stem | leaves | Stem | leaves |
| Heavy metals | | | | | | |
| As | 0.02 ± 0.001 | 0.39 ± 0.1 | 0.035 ± 0.001 | 0.0067 ± 0.0 | 2.6 ± 0.05 | 4.23 ± 0.25 |
| Cr | 0.00014 | 0.0002 ± 0.0 | 9.09302E-05 | 0.0001 ± 0.0 | 0.0014 ± 0.0 | 0.0033 ± 0.0 |
| Cu | 0.01 ± 0.002 | 0.008 ± 0.0 | 0.012 ± 0.001 | 0.013 ± 0.0 | 0.20 ± 0.003 | 0.37 ± 0.0 |
| Fe | 2.18 ± 0.005 | 0.18 ± 0.002 | 0.007 ± 0.00 | 0.012 ± 00 | 0.45 ± 0.015 | 0.36 ± 0.015 |
| Mn | 0.009 ± 0.0 | 0.013 ± 0.0 | 0.013 ± 0.00 | 0.03 ± 0.001 | 0.17 ± 0.003 | 0.74 ± 0.025 |
| Ni | 0.0024 ± 0.0 | 0.007 ± 0.0 | 0.00 | 0.00 | 0.06 ± 0.002 | 0.33 ± 0.016 |
| Pb | 0.013 ± 0.003 | 0.058 ± 0.002 | 0.11 ± 0.05 | 0.03 ± 0.001 | 0.09 ± 0.0 | 0.37 ± 0.018 |
| Zn | 0.103 ± 0.0 | 0.028 ± 0.0 | 0.002 | 0.002 ± 0.0 | 0.04 ± 0.005 | 0.29 ± 0.05 |
| HI | 2.3 ± 0.006 | 0.68 ± 0.008 | 0.18 | 0.09 | 3.57 | 6.75 |

**Table 5.** Health Index of the different heavy metals in stem and leaves of spinach if consumed by children and corresponding hazard indices.

| | 1st month | | 2nd month | | 3rd month | |
|---|---|---|---|---|---|---|
| | Stem | Leaves | Stem | Leaves | Stem | leaves |
| Heavy metals | | | | | | |
| As | 0.02 | 0.44 | 0.04 | 0.0080 | 2.96 | 4.92 |
| Cr | 0.0 | 0.0 | 0.0001 | 0.0001 | 0.002 | 0.0 |
| Cu | 0.01 | 0.01 | 0.013 | 0.015 | 0.23 | 0.43 |
| Fe | 2.5 | 2.50 | 0.0086 | 0.014 | 0.51 | 0.41 |
| Mn | 0.01 | 0.02 | 0.016 | 0.035 | 0.19 | 0.85 |
| Ni | 0.0 | 001 | 0.0 | 0.0 | 0.068 | 0.38 |
| Pb | 0.02 | 0.07 | 0.13 | 0.035 | 0.10 | 0.43 |
| Zn | 0.12 | 0.03 | 0.0019 | 0.0019 | 0.045 | 0.33 |
| HI | 2.68 | 0.78 | 0.21 | 0.11 | 4.1 | 7.76 |

differ and the physical and chemical soil characteristics differ and also affect the transformation and bioavailability of heavy metals (Huang et al., 2018).

The results from both adults and children suggest that the health risks associated with the consumption of vegetables are high during the early and later stages of plant growth. The reason might be due to the fact that during the early stages of growth the plant needs the heavy metals as nutrients at a high demand but then over time there is an accumulation of these heavy metals in the plant parts because they are no longer needed at high concentrations. The results indicate that as the vegetable matures, there is a need for high attention on the monitoring of heavy metal accumulation in vegetables and design has to be taken on when to stop harvesting the spinach. The results suggest that both groups (adults and children) will be safe when consuming leaves of spinach during the first month and stems and leaves during the second month. In general, it will be safe to harvest the vegetable during the second month when HI $< 1$ therefore the population will be safe from carcinogenic exposure. There was no significant differences ($P > 0.05$) of HI between stem and leaves during the first and second months but significant differences ($P < 0.05$) were observed during the third month of the study.

### 3.6. Bacteriological indicators

#### 3.6.1. Irrigation water

Table 6 below shows the bacteriological indicator results before and after secondary wastewater treatment in a pilot scale comprising a holding tank, roughing filter and slow sand filter system. The tertiary effluent was then used for irrigating the vegetable. The results reveal that the pilot plant was able to reduce the bacteriological indicator organisms to a level suitable for irrigation water requirements which is <1000 cfu/100 ml for *E. coli* and hence if *E. coli* is isolated it means faecal coliforms are present (BOS 463-2011). The percentage reductions were all 100% for FC, FS and *E. coli*. *E. coli* in the effluent for irrigation should be <1000 for crops cooked before consumption irrigated with drips. FC concentration in the raw wastewater, 4.48 log units was in the range reported by Woldetsadik et al. (2017a) which was in the range 4.29–5.61 log counts used for irrigation of lettuce in Ethiopia. The quality of irrigation water used during this study is also better than that used by Aiello et al. (2007) whose quality was 132, 132 and 137 CFU/100 ml of FC, *E. coli* and FS respectively for irrigating tomatoes in Sicily, Italy compared to that used in this study where no FC, *E. coli* and FS were observed except total coliforms. The results confirm the recommendation by Cirelli et al. (2012) who reported that the use of tertiary treated wastewater is suitable for vegetable irrigation.

The results suggest that secondary effluent should be treated before irrigation, hence the need to use secondary treated effluent instead. Though not shown, other parameters such as suspended solids, turbidity were very low in the effluent. A study by Letshwenyo and Lebogang (2019) has reported the successful reduction of these parameters using the same system.

#### 3.6.2. Soil and vegetable parts

Analysis of bacteriological indicators results is shown in Table 7. The bacteriological indicators (TC and *E. coli*) were present in the irrigated soil and plant roots (TC). The presence of TC was expected because the effluent used contained TC, and these might have increased in the soil and roots due to natural multiplication in the environment. TC count in roots was over 830 times more than in irrigated soil. The results suggest that TC were attached to the roots than soil. Furthermore, this suggests that the roots were a conducive environment for TC multiplication than soil. The same was also true for FS where it was observed that FS count in roots was 200 times more than in the soil. However, the situation was different for *E. coli* where it was observed that the count in soil was 73 times more than in roots suggesting a favourable soil environment compared to roots. It has been reported by Lonigro et al. (2016) that usually during spring–summer periods, weather conditions are favourable for bacterial persistence and regrowth which might have been true in this study since it was conducted in summer.

The results contrast the findings by Woldetsadik et al. (2017a) who reported FC concentration in lettuce ranging between 3.46-5.03 log counts. In their study the authors reported that furrow method was used for irrigating the vegetables and this might have contributed to high FC counts reported. In this study, drip irrigation method was used, hence reducing the chance of polluting vegetable leaves. Drip irrigation has been reported to minimize the spread of pathogens which might be due to surface flow and aerosolisation (Sadovski et al., 1978). Balkhair (2016) reported a considerable reduction in bacterial counts when using subsurface irrigation system compared to surface system, which is similar to this study. Tripathi et al. (2019) reported high population of TC and *E. coli* on the plant roots of cauliflower. Another finding by Woldetsadik

**Table 6.** Results of bacteriological indicator organisms analysis in wastewater used for irrigation.

| Treatment unit | Total coliforms | Faecal coliforms | Faecal streptococci | *E. Coli* |
|---|---|---|---|---|
| Raw wastewater | Too numerous to count | 30000 ± 10000 | 16000 ± 2000 | 31600 ± 19648 |
| Holding tank | Too numerous to count | 25 ± 10 | 35 ± 12 | 585 ± 233 |
| Roughing filter | 54 ± 5 | 0 | 15± | 500 ± 381 |
| Slow sand filter | 40 ± 42 | 0 | 0 | 0 |

**Table 7.** Results of bacteriological indicator organisms' analysis in the irrigated soil and vegetable.

| Parameters | TC | FC | FS | *E. coli* |
|---|---|---|---|---|
| Irrigated soil | 300 ± 5 | 0 | 1 | 73 ± 30 |
| Roots | 250000 ± 10 | 0 | 200 ± 14 | 0 |
| Stem | 0 | 0 | 0 | 0 |
| Leaves | 0 | 0 | 0 | 0 |

et al. (2017a) report about washing of vegetables using the highly polluted irrigation water might have contributed to high counts of FC in the vegetable leaves. In their study, Aiello et al. (2007), reported an increase of $3 \times 10^3$ and $1.2 \times 10^3$ of *E. coli* and FS respectively on the soil surface after the application of wastewater for irrigation. However, the findings in this study are comparable to the report by Cirelli et al. (2012) who reported that low values of *E. coli*, FC and FS were detected in the fruits, for instance, 20 CFU/100 mL. Similarly, Christou et al. (2014) reported no detection of *E. coli* and FC in the tomato fruits irrigated with tertiary treated wastewater (SSF and chlorination) through drip irrigation indicating the importance of the use of tertiary treated wastewater and subsurface irrigation. Mcheik et al. (2018) reported high bacterial load on lettuce leaves and it was thought that it might be due to large surface areas with many folds and fissures providing good shelter for microorganisms and reproduction in the inner tissues. Li and Wen (2016) observed no *E. coli* counts in the stems of lettuce but there were a few counts on the leaves irrigated with secondary wastewater through drip irrigation which differs with the findings of this study where *E. coli* were only detected in the roots. Contamination on leaves was attributed to poor irrigation management practices. The spinach in this study did not have folds and fissures, unlike lettuce; therefore, limiting favourable conditions for bacterial multiplication. The results of this study clearly suggest that secondary treated effluent should be the option for irrigation. This will minimize pathogenic infections to human beings consuming the irrigated vegetables as well as farm workers who are in contact with effluent many times. The results also show the need to analyse the different parts of the vegetables, not only the edible parts so that cross contamination can be avoided or minimized. It was interesting to observe that no bacteriological indicator organisms were detected in plant stems and leaves minimizing the risks of cross contamination. The other observation was that only TC and FS were present in the roots of the vegetable and this could be mainly from the environment rather than irrigation water. It is advisable to separate the roots from aboveground (stem and leaves) during harvesting to avoid cross contamination. Luna-Guevara et al. (2019) reported the presence of *E. coli* in vegetables that included alfalfa sprouts, fresh spinach and raw clover sprouts significantly higher at final postharvest stages compared to earl stages of handling and thus attributing this to direct contamination or multiplication. Hence separating aboveground parts from roots will reduce cross contamination after harvest as well.

## 4. Conclusion

In this study, the accumulation of heavy metals and bacteria in the different parts of spinach vegetable irrigated with secondary wastewater treated through filtration process were investigated. Due to scarcity of water in semi-arid countries such as Botswana, the use of wastewater is now gaining recognition for irrigation as horticulture projects utilizing wastewater are in place. The results of the research show that secondary wastewater treated using a combination of roughing and slow sand filters meets the national standard of irrigation water. The accumulation of heavy metals in the different parts of the spinach varied overtime. The Health Index was used to measure the health risk and it was observed to be >1 during the first month in the stem was consumed for both adults and children and then during the third month in both stems and leaves consumed by children and adults. The main contributors were iron during the early stages of plant growth and then arsenic during the later stages, hence the need to analyse heavy metals during the entire period of irrigation to avoid transfer to human beings during consumption. The effluent used was found to be free from bacteriological contamination suggesting the need to further threat secondary wastewater before irrigation. Besides minimizing public health risks, further treating secondary wastewater can reduce the risk of environmental pollution, including groundwater contamination.

## Declarations

### Author contribution statement

Moatlhodi Wise Letshwenyo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Gobusaone Mokokwe: Contributed reagents, materials, analysis tools or data; Wrote the paper.

### Funding statement

This work was supported by Botswana International University of Science and Technology office of Research and Innovation through research initiation grant, R00019 of 2019.

### Competing interest statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

## Acknowledgements

The author would like to thank department technical staff who assisted in the procurement of research materials and consumables.

## References

Achak, M., Mandi, L., Ouazzani, N., 2009. Removal of organic pollutants and nutrients from olive mill wastewater by a sand filter. J. Environ. Manag. 90, 2771–2779.

Ackah, M., Anim, A.K., Gyamfi, E.T., Zakaria, N., Hanson, J., Tulasi, D., Enti-brown, S., 2014. Uptake of heavy metals by some edible vegetables irrigated using wastewater : a preliminary study in Accra , Ghana. Environ. Monit. Assess. 186 (1), 621–634.

Ahmed, M., Matsumoto, M., Ozaki, A., Van Thinh, N., Kurosawa, K., 2019. Heavy metal contamination of irrigation water, soil, and vegetables and the difference between dry and wet seasons near a multi-industry zone in Bangladesh. Water (Switzerland) 11.

Aiello, R., Cirelli, G.L., Consoli, S., 2007. Effects of reclaimed wastewater irrigation on soil and tomato fruits: a case study in Sicily (Italy). Agric. Water Manag. 93, 65–72.

Alghobar, M.A., Suresha, S., 2017. Evaluation of metal accumulation in soil and tomatoes irrigated with sewage water from Mysore city, Karnataka, India. J. Saudi Soc. Agric. Sci. 16.

Ametepey, S.T., Cobbina, S.J., Akpabey, F.J., Duwiejuah, A.B., Abuntori, Z.N., 2018. Health risk assessment and heavy metal contamination levels in vegetables from Tamale Metropolis , Ghana. Int. J. Food Contam 5, 5, 2018.

Anwar, S., Nawaz, M.F., Gul, S., Rizwan, M., Ali, S., Kareem, A., 2016. Uptake and distribution of minerals and heavy metals in commonly grown leafy vegetable species irrigated with sewage water. Environ. Monit. Assess. 188.

Arora, M., Kiran, B., Rani, S., Rani, A., Kaur, B., Mittal, N., 2008. Heavy metal accumulation in vegetables irrigated with water from different sources. Food Chem. 111, 811–815.

Baldantoni, D., Morra, L., Zaccardelli, M., Alfani, A., 2016. Ecotoxicology and Environmental Safety Cadmium accumulation in leaves of leafy vegetables. Ecotoxicol. Environ. Saf. 123, 89–94.

Balkhair, K.S., 2016. Microbial contamination of vegetable crop and soil profile in arid regions under controlled application of domestic wastewater. Saudi J. Biol. Sci. 23, S83–S92.

Boechat, C.L., Pistóia, V.C., Gianelo, C., 2016. Accumulation and translocation of heavy metal by spontaneous plants growing on multi-metal-contaminated site in the Southeast of Rio Grande do Sul state , Brazil. Environ. Sci. Pollut. Res. 23, 2371–2380.

BOS 463, 2011. Water Quality for Irrigation. Botswana Bureau of Standards.

Chauhan, G., Chauhan, U., 2014. Human health risk assessment of heavy metals via dietary intake of vegetables grown in wastewater. Int. J. Sci. Res. Publ. 4, 1–9.

Chopra, A.K., Pathak, C., 2012. Bioaccumulation and translocation efficiency of heavy metals in vegetables grown on long-term wastewater irrigated soil near bindal river , Dehradun. Agric. Res. 1, 157–164.

Christou, A., Maratheftis, G., Eliadou, E., Michael, C., Hapeshi, E., Fatta-Kassinos, D., 2014. Impact assessment of the reuse of two discrete treated wastewaters for the irrigation of tomato crop on the soil geochemical properties, fruit safety and crop productivity. Agric. Ecosyst. Environ. 192, 105–114.

Cirelli, G.L., Consoli, S., Licciardello, F., Aiello, R., Giuffrida, F., Leonardi, C., 2012. Treated municipal wastewater reuse in vegetable production. Agric. Water Manag. 104, 163–170.

Emurotu, J.E., Onianwa, P.C., 2017. Bioaccumulation of heavy metals in soil and selected food crops cultivated in Kogi State, north central Nigeria. Environ. Syst. Res. 6, 1–9.

Federation, W.E., 1999. Standard Methods for the Examination of Water and Wastewater Part 1000 Standard Methods for the Examination of Water and Wastewater.

Ferniza-García, F., Amaya-Chávez, A., Roa-Morales, G., Barrera-Díaz, C.E., 2017. Removal of Pb , Cu , Cd , and Zn present in aqueous solution using coupled electrocoagulation-phytoremediation treatment. Int. J. Electrochem. 2017.

Gan, Y., Wang, L., Yang, G., Dai, J., Wang, R., Wang, W., 2017. Chemosphere Multiple factors impact the contents of heavy metals in vegetables in high natural background area of China. Chemosphere 184, 1388–1395.

Gupta, N., Khan, D.K., Santra, S.C., 2008. An assessment of heavy metal contamination in vegetables grown in wastewater-irrigated areas of Titagarh, West Bengal, India. Bull. Environ. Contam. Toxicol. 80, 115–118.

Gupta, S., Satpati, S., Nayek, S., Garai, D., 2010. Effect of wastewater irrigation on vegetables in relation to bioaccumulation of heavy metals and biochemical changes. Environ. Monit. Assess. 165, 169–177.

Gupta, N., Khan, D.K., Santra, S.C., 2012. Heavy metal accumulation in vegetables grown in a long-term wastewater-irrigated agricultural land of tropical India. Environ. Monit. Assess. 184, 6673–6682.

Huang, Y., Chen, Q., Deng, M., Japenga, J., Li, T., Yang, X., He, Z., 2018. Heavy metal pollution and health risk assessment of agricultural soils in a typical peri-urban area in southeast China. J. Environ. Manag. 207, 159–168.

Jin, E., Cao, L., Xiang, S., Zhou, W., Ruan, R., Liu, Y., 2020. Feasibility of using pretreated swine wastewater for production of water spinach ( Ipomoea aquatic Forsk .) in a hydroponic system. Agric. Water Manag. 228, 105856.

Khan, S., Cao, Q., Zheng, Y.M., Huang, Y.Z., Zhu, Y.G., 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Environ. Pollut. 152, 686–692.

Letshwenyo, M.W., Lebogang, L., 2019. Assessment of roughing and slow sand filter modified with slag and clinker ash for removal of microorganisms from secondary effluent. Environ. Technol. 1–12.

Li, J., Wen, J., 2016. Effects of water managements on transport of E . coli in soil-plant system for drip irrigation applying secondary sewage effluent. Agric. Water Manag. 178, 12–20.

Lonigro, A., Rubino, P., Lacasella, V., Montemurro, N., 2016. Faecal pollution on vegetables and soil drip irrigated with treated municipal wastewaters. Agric. Water Manag. 174, 66–73.

Luna-Guevara, J.J., Arenas-Hernandez, M.M.P., Martínez De La Peña, C., Silva, J.L., Luna-Guevara, M.L., 2019. The role of pathogenic E. coli in fresh vegetables: behavior, contamination factors, and preventive measures. Int J. Microbiol. 2019.

Mazhari, S.A., Abhari, A., Mazhari, S.N., 2019. Geochemical and environmental investigation of sewage-irrigated soils and crops of Sabzevar, NE of Iran. SN Appl. Sci. 1, 1–15.

Mcheik, A., Awad, A., Fadel, A., Mounzer, C., Nasreddine, S., 2018. Effect of irrigation water quality on the microbial contamination of fresh vegetables in the bekaa valley , Lebanon. AJAF 6, 191–197.

Meng, W., Wang, Zuwei, Hu, B., Wang, Zhongliang, Li, H., Goodman, R.C., 2016. Heavy metals in soil and plants after long-term sewage irrigation at Tianjin China: a case study assessment. Agric. Water Manag. 171, 153–161.

Mi, B., Liu, F., Xie, L., Zhou, H., Wu, F., Dai, X., 2019. Evaluation of the uptake capacities of heavy metals in Chinese cabbage. Ecotoxicol. Environ. Saf. 171, 511–517.

Obb, G.E.P.C., Ands, K.R.S., Aters, M.E.W., Ixson, B.O.G.W., Ing, E.L.D.O., 2000. Accumulation of heavy metals by vegetables grown in mine wastes. Environ. Toxicol. 19, 600–607.

Pajevi, S., Arsenov, D., 2018. Heavy metal accumulation in vegetable species and health risk assessment in Serbia. Environ. Monit. Assess. 190 (8), 459, 11.

Qureshi, A.S., Hussain, M.I., Ismail, S., Khan, Q.M., 2016. Chemosphere Evaluating heavy metal accumulation and potential health risks in vegetables irrigated with treated wastewater. Chemosphere 163, 54–61.

Ramachandra, T.V., Sudarshan, P.B., Mahesh, M.K., Vinay, S., 2018. Spatial patterns of heavy metal accumulation in sediments and macrophytes of Bellandur wetland , Bangalore. J. Environ. Manag. 206, 1204–1210.

Tasrina, R.C., Rowshon, A., Amr, M., Rafiqul, I., Ali, M.P., 2019. Metals contamination in vegetables and its growing soil. Int. J. Environ. Anal. Chem. 2 (3), 2015.

Rehman, Z.U., Khan, S., Shah, M.T., Brusseau, M.L., Khan, S.A., 2018. Transfer of heavy metals from soils to vegetables and associated human health risks at selected sites in Pakistan. Pedosph. An Int. J. 28, 666–679.

Fujimaki, S., Suzui, N., Ishioka, N.S., Kawachi, N., Ito, S., Chino, M., et al., 2010. Tracing cadmium from culture to Spikelet : noninvasive imaging and quantitative characterization of absorption , transport , and accumulation of cadmium. Plant Physiol. 152, 1796–1806.

Sadovski, A.Y., Fattal, B., Goldberg, D., Katzenelson, E., Shuval, H.I., 1978. High levels of microbial contamination of vegetables irrigated with wastewater by the drip method. Appl. Environ. Microbiol. 36, 824–830.

Sridhara Chary, N., Kamala, C.T., Samuel Suman Raj, D., 2008. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. Ecotoxicol. Environ. Saf. 69, 513–524.

Tom, M., Fletcher, T.D., Mccarthy, D.T., 2014. Heavy metal contamination of vegetables irrigated by urban Stormwater : a matter of Time ? PloS One 1–21.

Tripathi, V.K., Rajput, T.B.S., Patel, N., Nain, L., 2019. Impact of municipal wastewater reuse through micro-irrigation system on the incidence of coliforms in selected vegetable crops. J. Environ. Manag. 251, 109532.

Wang, X., Sato, T., Xing, B., Tao, S., 2005. Health risks of heavy metals to the general public in Tianjin , China via consumption of vegetables and fish. Sci. Total Environ. 350, 28–37.

Woldetsadik, D., Drechsel, P., Keraita, B., Itanna, F., Erko, B., Gebrekidan, H., 2017a. Microbiological quality of lettuce ( Lactuca sativa ) irrigated with wastewater in Addis Ababa , Ethiopia and effect of green salads washing methods. Int. J. Food Contam. 4 (30), 2017.

Woldetsadik, D., Drechsel, P., Keraita, B., Itanna, F., Gebrekidan, H., 2017b. Heavy metal accumulation and health risk assessment in wastewater-irrigated urban vegetable farming sites of Addis Ababa, Ethiopia. Int. J. Food Contam. 9, 2017.

Zhou, H., Yang, W.T., Zhou, X., Liu, L., Gu, J.F., Wang, W.L., Zou, J.L., Tian, T., Peng, P.Q., Liao, B.H., 2016. Accumulation of heavy metals in vegetable species planted in contaminated soils and the health risk assessment. Int. J. Environ. Res. Publ. Health 3 (3), 289, 13.