

Effects of wastewater on lead and cadmium concentrations in on selected vegetable species indigenous in Kitui County, Kenya

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ABSTRACT

Water scarcity in Sub-Saharan has led to utilization of the wastewater in home gardening and also in commercial production of vegetables. Wastewater is associated with various contaminants, including heavy metals that are toxic and life threatening to humans. It was therefore important to evaluate and quantify the levels of heavy metals in the vegetable tissues supplied with both wastewater and tap water. Field and greenhouse experiments were carried out to evaluate the uptake and accumulation of cadmium and lead in selected indigenous vegetables for two seasons from September to November 2017 and January to March 2018. The field trial was laid out in a Randomized Complete Block Design (RCBD) and in the greenhouse the treatments were arranged in Complete Randomized Design (RCD) replicated three times. Two types of water sources (wastewater and tap water), two types of irrigation (foliar and root-applied) and four vegetables were the main treatments. The findings revealed that Amaranthus accumulated highest amount of lead in its tissue for the field experiments in season 1, season 2 as well as the greenhouse (3.69mg kg⁻¹ and 4.85mgkg⁻¹ respectively). For cadmium Kales had the highest uptake of 3.38mgkg⁻¹, 0.639mgkg⁻¹ and 0.36mgkg⁻¹ in season 1, season 2 and greenhouse. Interaction effects were also reported in the two metals and the respective vegetable species. Due to high levels of contamination in the water used in irrigation could be the reason for high accumulation of the metals in the vegetable species. Proper phytoremediation measures need to be put across to improve the quality of the vegetables in the region to promote healthy living among the consumers.

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1. Introduction

Recently, the reuse of wastewater in agriculture has become a widespread practice in regions where water deficits are most pronounced (Hajjami *et al.*, 2013). In general, this resource contains substantial amounts of beneficial nutrients and toxic pollutants, which are creating opportunities and problems for agricultural production, respectively, particularly to small holder farmers and policy makers (Gweyi-Onyango and Osei-Kwarteng, 2011; Alghobar and Suresha, 2017). The long-term application of treated and untreated wastewater has resulted in a significant buildup of heavy metals in the soil as reported by Ullah *et al.* (2012). Apart from buildup in the soil, there is information pointing towards leachate to groundwater through dumpsites (Oyeku and Eludoyin, 2010). Furthermore, according to Kumar Sharma *et al.* (2007), there is potential risks to the consumer resulting from consumption of vegetables and cereals and their subsequent transfer to the food chain. Report by Sing *et al.* (2010) indicate that heavy metal concentrations in plants grown in wastewater-irrigated soils were significantly higher than in plants grown in the reference soil, and Khan *et al.* (2008) are of the opinion that the use of treated and untreated wastewater for irrigation increased the contamination with Cd, Pb and Ni in the edible portions of vegetables, causing a potential health risk in the long term.

Other researchers have found that the bioaccumulation of Pb and Cr in vegetables was above the critical concentrations for plant growth, while Pb and Cd were above the prescribed limit for animal diets (Sachan *et al.*, 2007; Khan *et al.*, 2012).

Cadmium and its compounds might travel through the soil, but its mobility depends on several factors, such as pH and the amount of organic matter, which varies depending on the local environment. Generally, cadmium binds strongly to organic matter, becoming immobile in the soil and is taken up by plant life, eventually entering the food chain (Balkhair and Ashraf 2016). Heavy metals are one of the important types of contaminants that can be found on the surface and in the tissues of fresh vegetables. The prolonged human consumption of unsafe concentrations of heavy metals in foodstuffs may lead to the disruption of numerous biological and biochemical processes in the human body. Vegetables, especially leafy vegetables grown in heavy metal-contaminated soils, accumulate higher amounts of metals than do those grown in uncontaminated soils because they absorb these metals through their leaves (Al Jassir *et al.*, 2005).

Cadmium (Cd) is relatively rare in the Earth's crust; however, due to electroplating, wastewater irrigation, mining, smelting and the abuse of pesticides and fertilizers, the Cd contamination in soils seems increasingly severe (Edgan *et al.* 2007; Wang *et al.*, 2007).

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According to documented reports by Salt *et al.*, (1995), the regulatory limit of cadmium (Cd) in agricultural soil is 100 mg/kg soil and plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips and finally death (Guo *et al.*, 2008). According to Alcantrara *et al.* (1994), inhibition of root Fe (III) reductase induced by Cd led to Fe (II) deficiency, and it seriously affected photosynthesis. In general, Cd has been shown to interfere with the uptake of other nutrients as well as the metabolism. Metal toxicity, as argued by Costa and Morel (1994), particularly Cd, can affect the plasma membrane permeability, causing a reduction in water content through tight relationship with plant water balance.

Lead toxicity in soil occurs as a result of municipal sewage disposal, lead containing paints, paper or pulp, mining and smelting activities. It has adverse effects on growth, morphology and photosynthetic processes. With high levels of lead in the soil, plants have water imbalance, altered membrane permeability, disturbed mineral nutrition and inhibition of enzyme activities (Yadav, 2010). The heavy metals cumulative effect impacts negatively on ecosystems and plants (Chang *et al.*, 2014). Heavy metal contamination of the soil is a concern about food security and safety globally (Toth *et al.*, 2016) and Lead (Pb) is one of the ubiquitously distributed most abundant toxic elements in the soil. It is known to affect photosynthesis by inhibiting activity of carboxylating enzymes (Stiborova *et al.*, 1987). High level of Pb also causes inhibition of enzyme activities (Sinha *et al.*, 1988), water imbalance, alterations in membrane permeability and disturbs mineral nutrition (Sharma and Dubey 2005). The primary cause of cell growth inhibition arises from a lead-induced simulation of indol-3 acetic acid (IAA) oxidation. Lead is also known to affect photosynthesis by inhibiting activity of carboxylating enzymes (Stiborova *et al.*, 1987). The high accumulation of heavy metals in the consumable vegetables is due to limited access of clean water for cultivation hence growers and farmers are forced to use waste water for irrigation contributing to increased accumulation of the heavy metals in the edible parts of the vegetables (Orsini *et al.*, 2013; UN water, 2006). In African and Asian towns, studies have shown that fifty percent of vegetable production for urban areas relies on agriculture with wastewater with the farmers not worried about the environmental dangers and mainly concerned with maximizing production and their profits (Sardar *et al.*, 2013). The citizens dwelling in towns have to deal with poor diets and limited incomes thus have resulted to farming activities in backyards, open public spaces such as industrial areas, under power lines and along river banks (Carin *et al.*, 2012). Therefore, this study was carried out to determine uptake of the lead and cadmium as the most toxic heavy metals in selected vegetable species grown with wastewater.

2. Materials and methods

2.1 Site Description

The field experiment was carried out in Kitui County is located in the of Kenya and lies within latitudes 0°10' and 3°0' South and longitudes 37°50' and 39°0' East and covers an area of about 30,570 km². 6,369 km² of the total area is under Tsavo National Park, 14,137.2 km² is arable agricultural land and 6,364.4 km² non arable land. The experiment was evaluated for two seasons from September to November 2017 and January to March 2018.

2.2 Experimental Design, Treatments and Data Collection

The field experiment was laid out in a Randomized Complete Block Design (RCBD).

The eight treatments (2 water treatments × 4 vegetable species) were subjected to two irrigation systems replicated three times.

In the greenhouse the treatments were arranged in complete Randomized Design (CRD). All agronomic management practices such as weeding, watering and spraying against pests and diseases were performed uniformly when necessary using recommended fungicides and pesticides respectively. Ten (10) plants were sampled from each plots, stored in a cool box for subsequent measurements of dry weight and laboratory analysis. The samples were washed in tap water, followed by deionized water to remove dust particles (Myung 2008). Plants were separated into root, stem and leaves and dried in a forced air oven. At 60°C for 24 hours, the samples were fully dried such that no significant changes occurred before the tests were done. Atomic Absorption Spectroscopy (AAS) [Shimadzu Model: 6200] was used for determination of heavy metals. Elements Specific wavelengths were provided by passing a lamplight beam through a flame whose cathode is made from the element. A photon multiplier detected the amount of reduction in light intensity due to analyte absorption relating to the amount of element in the sample (Okalebo *et al.*, 2002). Determining heavy metals concentration was based on soil and vegetable dry weight and expressed in milligrams per Kilogram of soil (mgkg⁻¹). The uptake of the respective heavy metals was done by multiplying the total concentration of the metal and total biomass accumulation in the plant.

2.3 Data Analysis

The collected data was managed in the excel spreadsheet and subjected to analysis of variance using GenStat statistical software version 15. Significance differences between means was performed using Fischer's Protected Least Significance Difference (L.S.D) test at 5% level of significance

3. Results and Discussion

3.1 Tissue Lead concentrations in difference vegetables species

Significant differences ($P \leq 0.05$) were observed on the total uptake of Pb in the selected vegetable species during season two and greenhouse experiment, while there were no significant differences in season one (Table 1). During seasons one and two, Amaranthus spp showed the highest concentrations of Pb accumulated in the tissues (3.69mg kg⁻¹ and 4.85mgkg⁻¹) respectively, while kale had the lowest in the two seasons with 2.59 mg kg⁻¹ and 3.19 mgkg⁻¹ (table 1). In the greenhouse, there were differences with black nightshade recording the highest Pb accumulation of 5.66 kg g⁻¹, while cowpea had the lowest uptake of 1.68 mg kg⁻¹.

In season 1, season 2 and greenhouse experiment, wastewater resulted to highest accumulation of Pb compared to clean water with 3.50 mg kg⁻¹, 4.49 mgkg⁻¹ and 4.86 mg kg⁻¹. This could be probably due to high concentration of pb as a heavy metal in the waste water compared to clean water. Similarly, root irrigation showed high levels of Pb accumulation compared to the shoot irrigation recording 3.50 mg kg⁻¹, 4.52mgkg⁻¹ and 4.40 mg kg⁻¹ during season 1, season 2 and greenhouse respectively (Table 1). This could be attributed to by the plant mechanism of the root absorbing more of the Pb form the root and translocated to other parts of the plants compared to when on the shoot.

Interaction effects in season 1 and season 2 were revealed between the type of irrigation in the vegetable species and uptake of Pb. (figure1) Amaranthus irrigated in the root showed the highest uptake of Pb in the tissue

recording 4.48 mgkg⁻¹ and 5.88 mgkg⁻¹ in season I and two respectively.

Cowpea irrigated in the root showed the lowest uptake of Pb in both season one and two recording 1.964mgkg⁻¹ and 2.6 mgkg⁻¹ respectively.

This can be to the reason that most of the absorbed lead in the vegetables especially for home gardeners is retained in the root hence limiting the translocation of the Pb to other parts hence leading to lower uptake of the heavy metal.

Table 1. Lead concentrates in selected vegetable species as affected by quality of water and irrigation type.

Species	Pb (mg/kg)		Conc
	Season 1	Season 2	Greenhouse
Kale	2.60 ^a	3.19 ^b	4.45 ^a
Cowpea	2.75 ^a	3.64 ^{ab}	1.68 ^b
Amaranthus	3.70 ^a	4.85 ^a	4.96 ^a
Black nightshade	3.51 ^a	4.57 ^{ab}	5.67 ^a
LSD	0.926	1.17	1.63
Water Quality			
Clean water	2.77 ^b	3.63 ^a	3.518 ^a
Waste water	3.50 ^a	4.47 ^a	4.86 ^a
LSD	0.67	0.86	1.39
Irrigation type			
Shoot	2.77 ^b	3.61 ^b	3.97 ^a
Root	3.50 ^a	4.52 ^a	4.40 ^a
LSD	0.66	0.86	1.44
SXWQ	NS	NS	NS
SXIT	*	*	NS

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$). S- Species, WQ – water quality, IT-irrigation type.

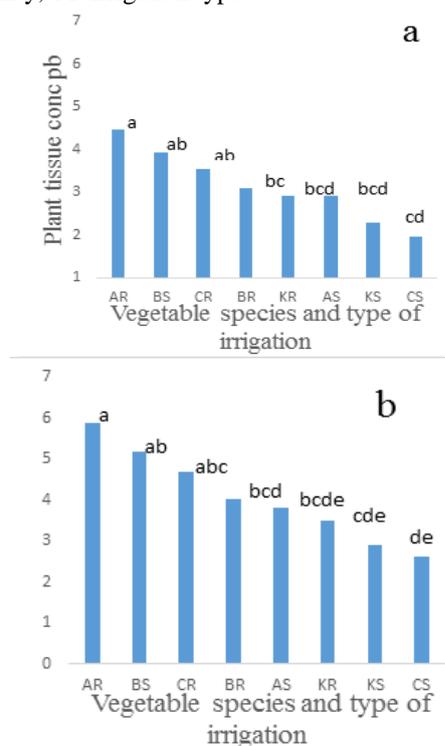


Figure 1. Interactions effects on vegetable species and type of irrigation on Pb uptake. AR-Amaranthus root irrigation, BS-black nightshade shoot irrigation. CR-cowpea root irrigation, BR-Black nightshaderoot irrigation, KR-kale root irrigation, AS-Amaranthus shoot irrigation. Season 1-(a), Season 2-(b).

The findings of this study disagrees with those of Wang *et al.* (2004) who reported that vegetable plants uptake of lead heavy metal is minimal owing to the fact that it binds

with organic matter in the soil hence limiting its distribution to the upper parts of the plant.

According to Tooze, (2006), cultivation of vegetable crops using waste water is great threat to individual life.

This is based on the fact that it results to high accumulation of the bioavailable metal that area toxic to the human at consumption. Therefore, the findings of this study agrees with the fact that waste water lead to higher uptake of pb in the respective vegetable plants. The high accumulation of Pb in the experiments could be due to the reason that they have been document to absorb high concentrations of heavy metal from the surface deposits of the soils as well as on the polluted air. Importantly, the study results also agree with those of Atayese *et al.* (2009), who reported that tissues of amaranth have improve mechanism of absorbing more heavy metal in the soil compared to other vegetables. Importantly, the potential of amaranth to accumulate high levels of heavy metals especially has been marked as one of the phytoremediation technique that can be used to clean contaminated soils (Tu,*et al.*, 2002). Therefore, based on the findings of this study it is clear that the species with the ability of absorbing more of the Pb form either soil or the water t and transporting its to various tissues. Lasat, (2002) also reported that plants that are genetically enhanced have the potential to clean up contaminate soils through accumulating in its tissues.

3.2 Tissue cadmium concentrations of different vegetable species

Significant differences ($P \leq 0.05$) were observed on the total uptake of Cd in the selected vegetable species during season 1, season 2 and greenhouse Table 4.19. Kales had the highest uptake of 3.38mgkg⁻¹, 0.639mgkg⁻¹ and 0.36mgkg⁻¹ in season 1, season 2 and greenhouse respectively (Table 2). Quality of water also showed significant differences ($P \leq 0.05$) on the uptake of Cd with waste water having the highest uptake in season 1, season 2 and greenhouse with 3.31mgkg⁻¹, 0.776mgkg⁻¹ and 0.23mgkg⁻¹ respectively.

Table2. Cadmium uptake in selected vegetable species as affected by quality of water and irrigation type.

Species	Cd conc (mg/kg)		
	Season 1	Season 2	Greenhouse
Kale	3.38 ^a	0.63 ^{ab}	0.37 ^a
Cowpea	2.59 ^{ab}	0.47 ^b	0.05 ^b
Amaranthus	3.13 ^{ab}	0.860 ^a	0.15 ^b
Black nightshade	2.05 ^a	0.53 ^{ab}	0.11 ^b
LSD	0.82	0.26	0.13
Water Quality			
Clean water	2.27 ^b	0.47 ^b	0.10 ^b
Waste water	3.31 ^a	0.77 ^a	0.23 ^a
LSD	0.57	0.17	0.11
Irrigation type			
Shoot	2.49 ^a	0.54 ^a	0.11 ^b
Root	3.09 ^a	0.71 ^a	0.23 ^a
LSD	0.62	0.193	0.11
SXWQ	NS	NS	*
VXIT	*	*	NS

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$). S- Species, WQ – water quality, IT-irrigation type.

The results on irrigation type had significant differences ($P \leq 0.05$) in the green house experiment while in season 1 and 2 no significant differences were observed. The root irrigation was superior in Cd concentration uptake compared to shoot irrigation in both seasons and also the greenhouse experiments as it recorded 3.09 mgkg⁻¹, 0.708 mgkg⁻¹ and 0.225mgkg⁻¹. This could be due to high biocontrol factors

between the soil and the root issue hence enhance a higher uptake.

Interaction effects on vegetable species and type of irrigation were revealed in both season 1 and season 2. In season 1 kales irrigate in the shoot (KS) showed highest uptake of 3.812 mgkg^{-1} followed by Amaranthus in the root irrigation (AR), while black nightshade shoot (BS) had the lowest of 1.261 mgkg^{-1} (Figure 2). Kales having a higher Cd uptake could be due to the increased surface of the leaves hence allowing them to collect deposited additional cadmium from the air in the polluted sources.

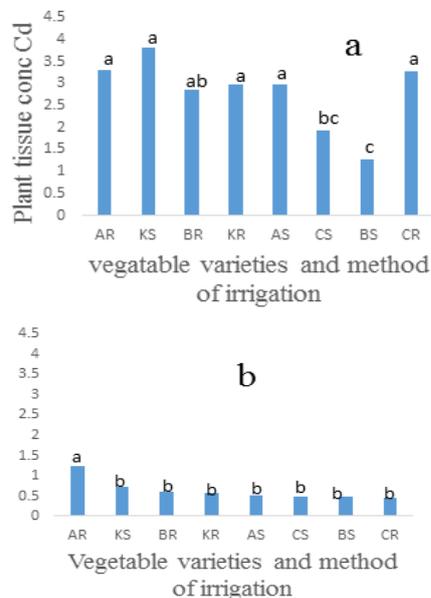


Figure 2. Interactions effects on vegetable species and type of irrigation on Cd uptake. AR-Amaranthus root irrigation, BS-black nightshade shoot irrigation, CR-cowpea root irrigation, BR-Black nightshade root irrigation, KR-kale root irrigation, AS-Amaranthus shoot irrigation, KS-kale shoot irrigation, CS-cowpea shoot irrigation. Season 1-(a), Season 2-(b).

In season two Amaranthus root irrigation (AR) was the highest with 1221.0 mgkg^{-1} . Cowpea root irrigation (CR) also showed the lowest uptake of 458.1 mgkg^{-1} . The results in the greenhouse also revealed an interaction between the qualities of water on uptake of cadmium in the selected vegetable species figure (3). Kales irrigated with waste water (Kww) was superior in the uptake of cadmium recording 0.54 mgkg^{-1} , while cowpea waste water had the least uptake of $0.042.7 \text{ mgkg}^{-1}$. Kales being superior could be due to the large surface area of the leaves as well accumulating higher heavy metal. Also, it could be the waste water used also had a higher concentration of the heavy metal.

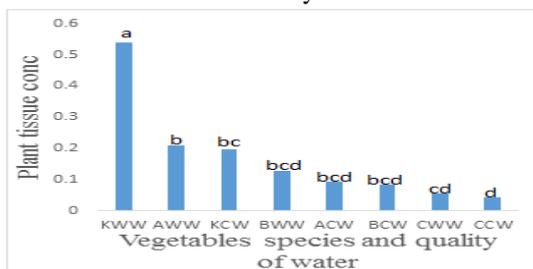


Figure 3. Interactions effects on vegetable species and quality of water on Cd uptake in greenhouse. KWW-kale waste water, AWW-Amaranthus wastewater, KCW-kale clean water, BWW-Black wastewater, ACW-Amaranthus clean water, BCW-black nightshade clean water, CWW-cowpea wastewater, CCW-cowpea clean water in the greenhouse.

According to Hovmand *et al.* (1983), plants with broader leaves have higher chances of accumulating deposited Cd on their aerial parts and is later transported to other parts of the plants hence representing a significant amount in the uptake calculation of the given plant which is in agreement with the findings of this study on the case of kale. Kader and Delseny, (2011) also reported that kale is a hyperaccumulator hence has been used in various studies to act as a phytoremediation and yielded significant results in lowering the levels of cadmium in the contaminated soils due to its high uptake and accumulation which agrees with the current findings. The results are also in agreement with those of Mitra and Gupta (1999) who reported higher uptake of Cadmium in vegetables irrigated with sludge waste water. Mensah *et al.* (2008) also reported that the higher the level of cadmium concentration in the irrigation water, the higher the uptake in the leafy vegetables tissue. Importantly, root solute (cadmium) uptake is coupled by root water uptake which implies that the more the water is uptake by the crop the more is cadmium is absorbed in the tissues (Ingwersen and Streck, 2005). Importantly, the higher uptake of cadmium under root irrigation was enhanced by the increased surface area of absorption compared to the shoot irrigation (Sun *et al.*, 2009). The results also conquer with those of Karanja *et al.* (2009) who reported that kales irrigated with waste water tends to take up high levels of cadmium compared with other vegetables.

4. Conclusion

Wastewater which is used in cultivation of the most consumed leafy vegetables in Kitui County affected the uptake and accumulation of the lead and cadmium heavy metals in the edible parts. The type of the irrigation either shoot or root also influenced the amount of heavy metal accumulate in the vegetables for season 1, season 2 and greenhouse experiments. The high accumulation of the heavy metals on the edible parts of the vegetables is an implication of sources of toxicity to consumers that can result to infections. Therefore, this implies that the available water used locally by the farmers to produce the vegetables need to be treated due to high levels of toxicity revealed from this experiment.

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