HEAVY METAL ACCUMULATION IN THE EDIBLE LEAFY PARTS OF AFRICAN NIGHTSHADE *(Solanum Scabrum* Mill) IRRIGATED WITH VARYING WATER QUALITY

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ABSTRACT

This experimental study was carried out to assess how heavy metals accumulates in the edible leafy parts of African nightshade (*Solanum Scabrum* Mill) irrigated with varying water quality. Different types of water used included wastewater, tap water, water from shallow wells and from borehole. The experiment was carried out under randomized complete block design (RCBD) with four replications. From the results, crop samples grown using deep well registered the highest mean value in regard to Zn^{2+}, Mn^{2+} and Pb^{2+} concentration at 37.17 (3.70), 10.63 (0.48) and 8.49 (0.35) ppm respectively. Low concentration of heavy metals Pb^{2+}, Cu^{2+} and Cd^{2+} at 4.36 (0.40), 3.98 (0.20) and 2.09(0.12) respectively was detected in crops samples grown using tap water. This is an indication that tap water possess ability to flush out the toxins in the soils. As compared to the WHO standards, the values of Cd^{2+} and Pb^{2+} were significantly above the recommended limits across all the blocks for the plant samples which will result to bioaccumulation in the long run.

Key terms: *Borehole water, shallow well, Solanum Scabrum, tap water, wastewater, World Health Organization standards*

1.0 INTRODUCTION

The accumulation of toxic elements in the plants and soil remains a primary environmental concern worldwide due to their ability to accumulate in biosystems (Saglam, 2013). As noted by Tangahu *et.al* (2011), plants have evolved specific mechanisms through which they translocate as well as store micronutrients. By using the same mechanism, heavy metals and micro nutrients are taken by the plants as they have chemical properties which simulate those of useful elements. The determination of distribution patterns in the plants in relation to the soils is one of the ways in which one can determine the extent to which the growth media samples contributes to the environmental pollution. However, it is important to note that the rate of accumulation of metals in plants is dependent on the plant species, type of the soil, growth stage, environmental and weather condition (Singh et al 2012; Saglam, 2013).

For instance, this is evident in a study carried out in 2012 to assess the level of iron (Fe), Manganese (Mn), copper (Cu), Zinc (Zn) cadmium (Cd) and lead (Pb) concentration in different vegetables (stems, roots, legumes, leafy and fruits). The study, targeting four urban and major industrial cities (Riyadh, Damamm, Tabouk, and Jazan) in Saudi Arabia, established that leafy vegetables, especially *Petroselinum Crispum* (parsley) had the highest values of mercury at 0.048 µg/g and 543.2 µg/g for iron. High concentration levels of cadmium at 4.13 µg/g were detected in *Spinacia Oleracea* (spinach). On the other hand, *Pisum Sativum* (peas) had the highest concentration of zinc at 71.77 µg/g. The highest concentration of lead was at 6.98 µg/g on dry matter basis was detected in *Cucumis Sativus* (cucumber), and all of these were above the above the recommended levels as proposed by the joint WHO/FAO Expert Committee on Food Additives (Ali & Al-Qahtani, 2012). The World Health Organization limits for cadmium, chromium, manganese, iron, lead, cadmium, copper and zinc in vegetables are 0.1, 10, 100, 500, 2.00, 0.02, 30.00 and 60.00 µg/g respectively. This is supported by another study carried out by Chandran *et al* (2012) to ascertain the level of accumulation of heavy metals such lead, cadmium and chromium in several crops such as *Solanum Melongena* (*eggplant*). The results were as indicated by the table 1.

Table 1: Level of accumulation of heavy metals on crops

S/N	Concentration of heavy metals (mg/kg)	Plant species			WHO/FAO guidelines (mg/kg)
		Panicum Maximum	*Alternanthera sessilis*	*Solanum Melongena*	
1.	Cadmium	1.8	2.65	1.7	0.02
2.	Chromium	0.85	1.15	0.45	0.1-0.2
3.	Lead	1.0	0.2	0.4	0.1

From the table, it was clear that the accumulation of heavy metals in all the three plants species exceed the permissible limits as set by WHO/FAO. Further, Chandran *et al* indicates that availability of cadmium in plants is regulated by redox potential, soil pH as well as other physic-chemical properties of the soil. Carrier proteins which are present in vascular tissue’s membrane help to regulate the uptake of trace elements. The high level of accumulation of cadmium in food crops mostly the leafy vegetables is because the crops are sensitive to cadmium as well as relatively high accumulators of cadmium. On the other hand, the great variation of lead contents in plants is influenced by several environmental factors. This includes pollution, presence of geo-chemical anomalies, seasonal variation as well as genotype ability to accumulate lead. It is notable that lead, unlike other toxic metals, has a low solubility and readily available for uptake by crops, due to the fact that it precipitates as sulfates and phosphates, chemicals mostly found in the plants’ rhizospheres as there are no direct channels of lead uptake by plants . Further, there is the immobilization of lead in the soil when it forms complexes with organic matter (Chandran *et al*, 2012).

It is also worth noting that when the quality of irrigation water is altered the accumulation level of micro and macro elements in the plants is also affected. This is supported by a study done by Kim *et al* (2016) investigating the response of lettuce and Chinese cabbage to various salinity levels of irrigation water in greenhouse cultivation. The study demonstrated that the continuous irrigation of saline water under greenhouse conditions could result to a significant increase in electric conductivity (ECe) level and Na^+

concentration in soil, as well as Na^+ concentration in leaves of crops. Since the concentration of heavy metals in soil directly affects subsequent accumulation of these heavy metals in the plants through soil-crop transfer, it is notable that any treatment to the soil causing change will also affect the plants growing on it. For example, in a study carried out by Al-Dakheel (2010) to investigate the effect of irrigation water quality on heavy metals content in soil of Al-Hassa Oasis, it was found that soil treated with a mixture of groundwater, drainage water and tertiary treated wastewater (GW+DW+TTWW) had adverse effects to the soils as compared to others as indicated on table 2. The other treatments included ground water (GW), mixture of groundwater and drainage water (GW+DW), and mixture of groundwater and tertiary treated wastewater (GW+TTWW).

Table 2: The chemical analysis of soil irrigated by different irrigation water types in Al-Hassa Oasis

Irrigation water	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)	Cd (ppm)	Co (ppm)	Ni (ppm)
GW	2.13	2.94	0.37	1.18	0.10	0.26	0.28
GW+DW	2.95	6.02	0.56	2.31	0.13	0.41	0.39
GW+TTWW	3.95	9.06	0.84	4.48	0.17	0.53	0.48
GW+DW+TTWW	7.40	9.86	1.48	6.00	0.21	0.65	0.67
LSD (0.05)	1.14**	2.36**	0.41**	1.85**	0.01**	0.03**	0.05**

** Significant at 1% significance level

Therefore, any crops planted on soil treated with GW+DW+TTWW had a higher chance of having increased levels of heavy metal accumulation as compared to those in the other treatments. This is a clear indication that the type of quality of irrigation water used affects the chemical concentration of micro and macro elements in the plants (Al-Dakheel, 2010). Based on the above, the study aims at assessing how heavy metal accumulation in the edible leafy parts of African nightshade (Solanum Scabrum Mill) varies depending on the quality of irrigation water used.

2. 0 MATERIALS AND METHODS

2.1 Study area

The study was located at 1°11'59.43''S and 36°56'00.03''E. This area has around 100 farmers mainly growing *S. Scabrum* among other leafy vegetables and preferred due to high demand resulting from a large number of people residing along Thika Super Highway.

2.2 Growth and Collection of Crop Samples

The crop samples were grown in a nursery bed for a period of one month. During this period, the land in the selected experimental area was cultivated in order to mix the soil as well as remove unwanted materials such as root stumps. The crops were randomly selected from the nursery bed at the 30th day after germination and then randomly allocated to various treatments within the blocks in the experimental area. All the individual plants in the various treatments were watered daily at 6.00 pm for a period of 30 days after which they were ready for sampling and laboratory testing (Muthomi and Musyimi, 2009).

As noted by A&L Eastern Laboratories, proper sampling is critical to getting reliable results in plant analysis. For leafy crops such as *S. Scabrum*, only the recently mature leafs should be picked by the use of non-destructive method of harvesting. This is based on the fact that, they are neither shinny green from immaturity nor green from aging. In this regard, between 12-20 leaf samples were randomly taken from each of the four treatments and replications. Further, all the selected leaves were those which were free of diseases or other forms of damages (A&L Eastern Laboratories, 2016). They were placed in brown sugar papers, clearly labeled by the use of a marker pen and transported to the laboratory.

Figure 1: Collection of Crop Samples (Source: Field Trip)

2.3 Laboratory Preparation and Tests for the Crop Samples

In the laboratory, all the samples were first washed under running tap water with the aim of removing dust particles that may interfere with the results from the analysis. Subsequently, the samples were washed with acidified distilled water (1ml Conc. HCL/Liter), and then rinsed thoroughly with distilled water (Masabni, 2015). All the samples were placed in an oven at approximately 70^{o} C for a period of 24-48 hours. Since the water content in each sample varies significantly, their weights were noted throughout. They were regarded as dry when the weight was constant for two consecutive readings. After drying, the plant samples were ground to pass a 1.0-mm screen (20 mesh), as the sample aliquot assayed was >0.5 g (Molina, 2011). The plant samples were then mixed thoroughly and transferred to polythene bags, labeled clearly and stored.

Thereafter, one gram of plant tissues from each sample was carefully weighed and placed in 100 ml volumetric flask. 5 ml of acid mixture (HNO_3 and $HCLO_4$ mixed in the ratio of 2:1 respectively) were added to the each crop sample. The solution was then heated by the use of hot plates for 15 minutes in a fume chamber at 60 ^{o}C until the reaction was complete. Thereafter, the samples were heated at 120 ^{o}C for 75 minutes until the liquid turned colorless. They were then removed from the hot plates, cooled, transferred to a 100 ml volumetric flask and filled to the mark using distilled water and filtered using Whatman No. 1 filter paper. By the use of the Atomic Absorption Spectrophotometer (AAS), the level of concentration of copper, iron, zinc, manganese, lead and cadmium were obtained from the crop samples.

2.4 Data analysis

Wilcoxon signed-rank test was conducted using STATA 14 statistical computer package at a significance level of $P < 0.05$.

3.0 RESULTS AND DISCUSSION

It is clear that using various types of water to irrigate *S. Scabrum* play a critical role as far as the chemical properties of the edible leafy parts is concerned. In the experiment seven elements namely cadmium, manganese, iron, zinc, magnesium, lead and copper were considered as they are often the ones found on the edible parts of vegetables. This is owing to the fact that these elements are strongly absorbed by the soil colloids and pose human and animal health risks at plant tissue concentrations that are generally phytotoxic (Dion, 2010). There is also a high probability of human bioaccumulation through the soil-plant- animal food chain. Uptake of these elements through the roots of the plants depends on a number of factors such like the soluble content of the element in the soil, soil pH, plant growth stages, humidity, and temperature, type of crop, soil and fertilizers used. When in trace quantities, some of these heavy metals are micronutrients. However, in elevated concentrations or after prolonged dietary intake, they can pose a significant health risk to humans, leading to various chronic diseases (Drechsel *et.al*, 2016).

Table 3 gives the mean concentration of ions (Cd^{2+} , Mn^{2+} , Fe^{3+} , Zn^{2+} , Mg^{2+} , Pb^{2+} and Cu^{2+}) in the four water treatments, where 1 gram of *Solanum Scabrum* was used from each water treatment. From the table 3, it is clear that concentration of copper ions was highest in crop samples grown using wastewater and lowest in those grown using tap water at a mean value of 4.77 and 3.98 ppm respectively. This is attributable to farming activities taking place upstream where farmers use chemicals containing copper to spray cash crops which get to the river through floods and leaching activities. On the other hand, crop samples grown using borehole registered the highest mean value in regard to Zn, Mn and Pb concentration at 37.17 (3.70), 10.63 (0.48) and 8.49 (0.35) respectively. This is supported by Kitt & Pater (2011) who indicate that in deep acquifers, minerals which cause hardness in water such as magnesium are more plentiful as compared to ground and tap water. Also the high amount of lead can be attributed to the geological factors where the rocks in deep wells around study area might be having a high level of lead content and other heavy metals elements. For instance, in study carried out by Gichuki & Gichumbi (2012) to evaluate the physical and chemical parameters of four boreholes in Kihara division, Kiambu County, it was found out that though majority of the parameters evaluated were within the permissible limits of WHO, cadmium was not. In this regard, once crop samples are irrigated with water from such sources, they will be transferred to the edible parts.

From the table 3, it is also notable that low concentration of heavy metals Pb^{2+}, Cu^{2+} and Cd^{2+} were detected in crops samples grown using tap water, an indication that tap water possess ability to flush out the toxins in the soils. However, a significant amount of Fe^{3+}, Mn^{2+} and Mg^{2+} were detected in crop samples grown with the use of tap water. This is supported by Ferrante *et.al* (2013) who indicate that in Lithuania 55% of drinking water sources have excess of iron. Also, high doses of Mn^{2+} and Fe^{3+} in tap water are common in Eastern and Central European countries due to lack of efficient technologies for removal of these contaminants that often occur naturally in ground water.

The figure 1 is a graph showing the concentration of Cd^{2+} , Mn^{2+} , Fe^{3+} , Zn^{2+} , Mg^{2+} , Pb^{2+} and Cu^{2+} in the four treatments wastewater, tap water,boreholeand shallow well. From the graph it is clear that high levels of Fe^{3+} was detected in crop samples grown using water from the shallow wells and lowest from those grown using tap water. The figure also indicates that high amount of Mg^{2+} was detected in shallow wells while the lowest was in wastewater although none of the treatments had a significant difference in comparison to the tap water as recorded in table 4. However, there was a significant diffrence between the concentration of Mn^{2+} ($p \leq 0.01$) and Zn^{2+} ($p \leq 0.01$) in crop samples irrigated using borehole in comparison to that of tap water. This is attributable to the high level of Mn^{2+} and Zn^{2+} occuring naturaly in the rocks in deep acquifers.

Results of sample analysis and their comparisons with standard values on the table 5 indicates that majority of chemical elements tested (Mn^{+2} , Fe^{3+}, Zn^{2+} , Mg^{2+}, and Cu^{2+}) were below the standard levels of the World Health Organization. However, for cadmium and lead, the values were significantly above the standard value on all the samples from the four treatments. The mean concentration (ppm) of cadmium ions was 2.96 ±0.33, 2.09 ±0.36, 3.60 ±0.37 and 3.62 ±0.31 in crop samples grown using wastewater, tap water, borehole and shallow well respectively. This was above the WHO accepted limits which is 0.02 ppm in vegetables (Naser *et.al*, 2011). It is important noting that cadmium is widely dispersed into the environment through the air through mining and smelting as well as through human activities. Some of the activities include usage of phosphate fertilizers, presence in sewage sludge, and various industrial uses such as NiCd batteries, plating, and pigments. In vegetables, high concentrations of cadmium are found in the leaves, followed by storage roots and tubers, seeds or grain and fleshy fruits (Stevens, 2003). Due to continuous usage of wastewater to irrigate the farms along the study area, soils are contaminated with cadmium. This is owing to the fact that the river has high levels of plastic wastes collected from the surrounding areas such as Githurai and Kahawa Wendani wards. Since cadmium is used as a stabilizer in plastics, then this becomes a notable point of contamination leading to bioaccumulation (Drechsel *et.al*, 2016).

From the table 5, the level of lead contamination was also above the recommended value of 2.00 ppm by the WHO. The mean concentration (ppm) of lead was 7.79±1.14, 4.36 ±1.98, 8.49±0.98, and 7.65 ±0.88 in crop samples grown using wastewater, tap water, borehole and shallow well respectively. Just like cadmium, soils in farms along the study area are highly contaminated with lead. This is from sources such as lead-based paints from old building in the surrounding areas. As noted by Jen & Chen (2017), lead in vegetables mainly comes from contaminated water and soil. Lead contamination can cause a reduction in transpiration, disruption to the plant water balance, a decrease in chlorophyll production, and can result in adverse effects on photosynthesis, lamellar organization of chloroplast, and cell division.

Table 3: Mean concentration of ions (Cd^{2+} , Mn $^{2+}$, Fe^{3+} , Zn^{2+} , Mg^{2+} , Pb^{2+} and Cu^{2+}) in the four water treatment (wastewater, tap water,borehole and shallow well) (μ±S.E, n=8). 1 gram of *Solanum Scabrum* was used from each water treatment.

Treatment	Cd^{+2} (S.E)	Mn $^{2+}$ (S.E)	Fe^{3+} (S.E)	Zn^{2+} (S.E)	Mg^{2+} (S.E)	Pb^{2+} (S.E)	Cu^{2+} (S.E)
Wastewater	3.96(0.13)	8.92 (0.19)	224.60 (14.59)	32.32 (0.79)	114.31 (10.87)	7.79 (0.70)	4.77 (0.29)
Tap water	2.09(0.12)	8.50 (0.58)	170.69 (8.18)	30.06 (1.42)	119.84 (24.53)	4.36 (0.40)	3.98 (0.20)

Deep well	3.60(0.13)	10.63 (0.48)	221.31 (22.92)	37.17 (3.70)	151.16 (5.72)	8.49 (0.35)	4.05 (0.28)
Shallow well	3.62(0.11)	9.38 (0.0.96)	259.42 (17.58)	26.41 (1.74)	170.87 (8.37)	7.65 (0.31)	4.08(0.25)

N=8

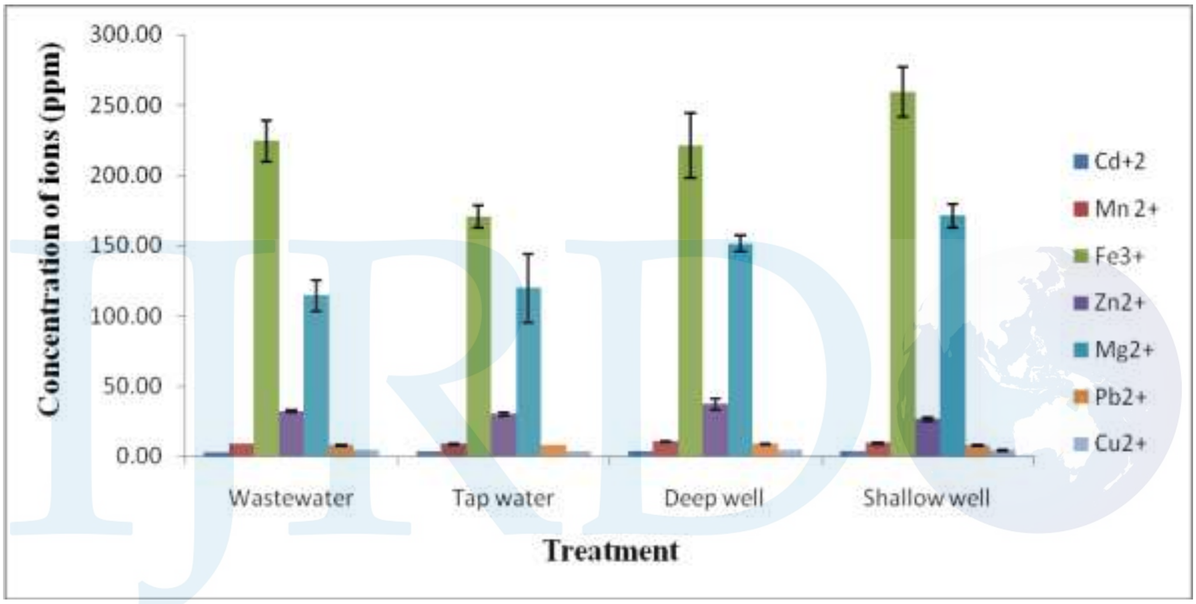

Figure 1: Graph showing the concentration of ions (Cd^{+2} , Mn^{+2} , Fe^{3+} Zn^{2+} , Mg^{2+} , Pb^{2+} and Cu^{2+}) in the four-water treatment. 1 gram of crop residue was used from each water treatment.

Table 4: Significance level (p-value) of concentration of ions (Cd^{+2} , $Mn+^{2}$, Fe^{3+} Zn^{2+} , Mg^{2+} , Pb^{2+} and Cu^{2+}) of 1 gram of crop residues of various treatments (wastewater, borehole and shallow well) as compared to 1 gram of crop residue grown from tap water.

Parameter	Treatment	Z-test	P-value
Cd^{+2}	wastewater	0.7	0.48
	Deep well	-2.1	0.04
	Shallow well	-2.1	0.04
Mn^{2+}	wastewater	-0.98	0.33
	Deep well	-2.51	0.01

	Shallow well	-0.51	0.57
Fe^{3+}	wastewater	-2.51	0.01
	Deep well	-2.51	0.01
	Shallow well	-2.51	0.01
Zn^{2+}	wastewater	-1.54	0.12
	Deep well	-2.51	0.01
	Shallow well	0.98	0.33
Mg^{2+}	wastewater	0.56	0.58
	Deep well	-0.56	0.58
	Shallow well	-0.98	0.33
Pb^{2+}	wastewater	1.26	0.21
	Deep well	0.00	1.00
	Shallow well	1.89	0.06
Cu^{2+}	wastewater	-2.38	0.02
	Deep well	-0.70	0.48
	Shallow well	0.56	0.58

Table 5: Comparison of the mean concentration of ions (µ±S.D, n=8), in various treatments as compared to WHO standards.

Treatment	Variable	Concentration (ppm)± STDEV	WHO Standard (ppm)	Z-test	P-value
Wastewater	Cd^{+2}	2.96 ±0.33	0.02	2.53	0.01
	Mn^{2+}	8.92±1.65	500.00	-2.52	0.01
	Fe^{3+}	224.60±23.13	425.00	-2.52	0.01
	Zn^{2+}	32.32±4.01	60.00	-2.52	0.01
	Mg^{2+}	114.31±69.4	150.00	-2.10	0.06
	Pb^{2+}	7.79±1.14	2.00	2.52	0.01
	Cu^{2+}	4.77±0.57	30.00	-2.52	0.01
Tap water	Cd^{+2}	2.09 ±0.36	0.02	2.83	0.00
	Mn^{2+}	8.55±0.54	500.00	-2.52	0.01
	Fe^{3+}	170.69 ±41.27	425.00	-2.52	0.01
	Zn^{2+}	30.06 ±2.25	60.00	-2.52	0.01

	Mg^{2+}	119.84 ±30.74	150.00	-0.56	0.58
	Pb^{2+}	4.36 ±1.98	2.00	2.52	0.01
	Cu^{2+}	3.98 ±0.82	30.00	-2.52	0.01
Borehole	Cd^{+2}	3.60 ±0.37	0.02	2.52	0.01
	Mn^{2+}	10.63±1.35	500.00	-2.52	0.01
	Fe^{3+}	221.31 ±64.83	425.00	-2.52	0.01
	Zn^{2+}	37.17 ±10.47	60.00	-2.52	0.01
	Mg^{2+}	151.16±16.19	150.00	0.28	0.78
	Pb^{2+}	8.49±0.98	2.00	2.52	0.01
	Cu^{2+}	4.045 ±0.79	30.00	-2.52	0.01
Shallow well	Cd^{+2}	3.62 ±0.31	0.02	2.52	0.01
	Mn^{2+}	9.38 ±0.31	500.00	-2.52	0.01
	Fe^{3+}	259.42 ±2.71	425.00	-2.52	0.01
	Zn^{2+}	26.41 ±49.72	60.00	-2.52	0.01
	Mg^{2+}	170.87±23.67	150.00	1.68	0.09

4.0 CONCLUSION

From the results it is clear that the chemical concentration of all the elements tested in the leaves of *S. Scabrum* varied according to the type of irrigation water used. The concentration of copper ions was highest in crop samples grown using wastewater and lowest in those grown using tap water at a mean value of 4.77 and 3.98 ppm respectively. This was attributable to farming activities taking place upstream where farmers use chemicals containing copper to spray vegetables and excess of it get to the river through floods and leaching activities. Crop samples grown using borehole registered the highest mean value in regard to Zn^{2+}, Mn^{2+} and Pb^{2+} concentration at 37.17 (3.70), 10.63 (0.48) and 8.49 (0.35) ppm respectively. Low concentration of heavy metals Pb^{2+}, Cu^{2+} and Cd^{2+} were detected in crops samples grown using tap water, an indication that tap water may possess ability to flush out the toxins in the soils. There was also a significant diffrence between the concentration of Mn^{2+} ($p\leqq0.01$) and Zn ($p\leqq0.01$) in crop samples irrigated usingboreholein comparison to that of tap water. This is attributable to the high level of Mn^{2+} and Zn^{2+} occuring naturaly in the rocks in deep acquifers.

Results of sample analysis and their comparisons with the standard shows that majority of chemical elements tested were below the standard levels of the World Health Organization, except for Cadmium and Lead on all the crop samples tested across the treatments. The highest mean concentration of Cadmium ions at 3.62 ±0.31 ppm was detected in crop samples from shallow wells while lead ions was the highest in wastewater with a mean concentration of 7.79±1.14 ppm. The recommended WHO limits for Cadmium and Lead ions is 0.02 and 2.00 ppm respectively.

5.0 RECOMMEDATION

Further research need to be carried out to determine the biological quality of *S. Scabrum* irrigated using various types of water. There is also the need to ensure that wastewater is treated prior to irrigation as a way of reducing bioaccumulation.

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