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Original Research Article

Differential biomass accumulation among African leafy vegetables as affected by wastewater irrigation in Kutui county, Kenya.

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7 Abstract

8 Water scarcity of fresh water in Sub-Saharan has led to utilization of the wastewater in home gardening and also in commercial production of vegetables. Wastewater is associated with 9 various substances including nutrients and heavy metals hence it is pertinent to evaluate its 10 effects on growth and vield of vegetables. An experiment was conducted to evaluate the effect 11 of waste water released from the municipal council on the biomass accumulation in African leafy 12 vegetables. Field experiments were carried out in two seasons and one greenhouse experiment. 13 The field trial was laid out in a Randomized Complete Block Design (RCBD) and in the 14 greenhouse the treatments were arranged in Complete Randomized Design (RCD) replicated 15 three times. Four leafy vegetables were the treatments replicated three times. The vegetables 16 were irrigated with waste water. The findings revealed differences in biomass accumulation into 17 various organs. Black nightshade depicted the highest leaf dry matter in the greenhouse at both 6 18 weeks after plant (WAP) and 12 WAP (24.62 g and 81.12g respectively). Cowpea showed the 19 highest increment (7 folds) in leaf weight between 6 to 12 WAP as compared to was paltry 3.6 20 folds. The highest stem dry weight was obtained in the amaranth species at 6 WAP and 12 WAP 21 22 both in the greenhouse; recording 32.59 g and 90.12g respectively. A similar trend was noted in root dry weight and root: shoot ratio. Cowpea had the least biomass accumulation potential 23 across all the parameters in both seasons and in the greenhouse. The increased biomass growth is 24 25 an indication sufficient availability of nutrient that promoted vibrant plant growth and also less toxicity from the heavy metals. Therefore, waste water can be put into use to enhance improved 26 27 productivity of African leafy vegetables.

28 Keywords: Nutrient benefits, plant growth, heavy metals

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30 Introduction

African leafy vegetables are essential in meeting the constraints of malnourished people, particularly children and expectant mothers since many of these vegetables contain high nutritional value, and they also do well in range of soil types and thrive well in a number of environments (1, 2). With the current rising human population coupled with the climate change that has hit agricultural farming system, there is need to focus on diverse ways of growing African leaf vegetables as way of mitigating the dire consequences that come along with these current scenarios. Due to water scarcity in most of the regions in the country, most of the vegetable farmers have opted to use waste water from the municipal council as source of irrigation for African leafy vegetables (3). Specifically, this takes place in urban area as way of reducing transport, handling and production costs, making the food products readily available to the urban poor (4). This is based on the fact that the shorter the time of vegetables between harvest and consumption, the greater the nutritional quality.

In many developing countries, as a result of rapid urbanisation and the absence of wastewater 43 44 treatment facilities, urban farmers often use wastewater either directly from sewage drains or indirectly through wastewater polluted irrigation water. Wastewater use in agriculture is 45 common practice and is on an increasing trend as a result of the rising water scarcity worldwide 46 (5). The volume of wastewater has been increasing with the increasing population, improved 47 living standards, urbanization and economic development (6). The continued disposal of waste-48 water into land and water courses reduces the quality of water available for crop growth. 49 Domestic sewage and refuse find their way into the water from settlements, municipal wastes or 50 institution drainages through leaching, direct discharge and runoff (7). Human faeces contain 51 high concentrations of toxic elements from the normal dietary matter presenting principal input 52 of toxic metals to domestic waste water and sludge of domestic origin (8). Due to nutrient 53 accumulation in waste water it is considered beneficial to plant growth hence its extensive use 54 for irrigation of crops. 55

Waste water is associated to have high accumulations of plant growth-promoting and metaltolerant bacteria increase plant growth and reduce metal uptake or translocation to above-ground tissues of plants by reducing the availability of soil heavy metal (9). This makes it quite reliable 59 for the above ground growth which is a good measure of yield in vegetables. Wastewater thus serves as source of fertilizer, especially in low income regions where farmers have the inability 60 of acquiring farm inputs (9). However, over-supply of these nutrients to the vegetables, 61 particularly leafy ones, may cause detrimental effects and also could negatively affected the 62 yield returns. There is need for new and alternative approaches to ensuring food and nutrition 63 security in these developing countries. Such solutions should be sustainable, resilient and of 64 practical solutions to challenges due to climate change and variability. This need has led to 65 renewed focus on identifying and improving underutilized indigenous and traditional crops. The 66 waste-water use is such alternative to improving the productivity of indigenous vegetables(10). 67 Having much being known on the benefits of waste water, this study focused at evaluating the 68 influence of the wastewater on the accumulation of biomass in the selected African leady 69 vegetables 70

71 Materials and Methods

72 **2.1 Site Description**

The field experiment was carried out in Kitui County which is located in Eastern part 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.

78 2.2 Experimental Design, Treatments and Data Collection

The field experiment was laid out in a Randomized Complete Block Design (RCBD). The treatments were four vegetable species replicated three times and irrigated with waste water. In the greenhouse the treatments were arranged in complete Randomized Design (CRD).

The land was cultivated using hand hoe tools and the plot later harrowed using rakes into finer tilth. The seeds of different crop species were drilled and later thinned to recommended spacing two weeks after emergence. The plot since was 3 by 2 m². 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. This was done in week 6 and week 12 of the vegetables growth cycles. The samples were washed in tap water, and were separated into root, stem and leaves and dried in a forced air oven. At 72^oC for 48 hours, the samples were fully dried such that no significant changes occurred before the tests were done. The partitioned plant parts, shoot, leaves, and the roots were weighed using the electronic balance model 6354.

93 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

98 **3. RESULTS AND DISCUSSION**

99 **3.1 Leaf dry weight**

100	Vegetable species exhibited significant differences ($P \le 0.05$) in leaf biomass accumulation under
101	the effect of waste water in both seasons and greenhouse experiment. Black nightshade was the
102	most superior in biomass accumulation in both week 6 and week 12 for both field experiments.
103	In week 6 and 12 the highest shoot biomass for black nightshade was recorded for the
104	greenhouse experiment, recording 24.62 g and 81.12 g respectively. The cowpea vegetable
105	species showed the least biomass accumulation across the experiment recording 6.43 in season 1
106	and 24.42 g in the greenhouse (Table 1). All the species showed an increase in leaf biomass
107	between week 6 and 12, an implication that waste water did not have negative impact (due to
108	heavy metals) on their growth. However, cowpeas showed a greater increment of 7 folds
109	between 6 and 12 WAP in season 1 from 6.43 g (6WAP) to 46.46 g (12 WAP). On the other
110	hand, biomass accumulation between this period was 3.7 folds for black nightshade. This could
111	be as result proper utilization of the available plant nutrients that enhance growth in cowpea or it
112	implies that cowpea is more tolerant to heavy metals that is mostly associated with waste water.

Table 1: Influence of waste water on leaf biomass accumulation in in two seasons and
 green house

		L	eaves dry wei	ght (g)		
	Week 6			Week 12		
Species	S 1	S2	GH	S 1	S2	GH
Cowpea	6.43 ^c	8.43 ^c	6.80 ^d	46.42 ^b	51.62 ^b	24.42 ^c
Amaranthus	10.41 ^b	12.57 ^b	15.31 ^c	62.47^{a}	68.63 ^a	63.48 ^b
Kale	11.38 ^b	12.58 ^b	20.00^{b}	47.53 ^b	51.08 ^b	64.08 ^b
Black	16.93 ^a	19.68 ^a	24.62 ^a	62.63 ^a	68.42^{a}	81.12 ^a
nightshade						
LSD	1.16	2.01	3.18	7.37	7.46	5.48

115 Means followed by the same letter within the same column are not significantly different 116 ($P \le 0.05$). S1- season 1, S2- season 2, GH-greenhouse,

117 According to Anwar et al. (11), irrigation with waste water leads to an increase in biomass

118 formation as a result of increased growth rate which agrees with the findings of this study. In

another study by Zu *et al.* (12), waste water has the potential of stimulating vibrant growth and

consequent biomass accumulation on the above ground as result of adequate concentration for nitrogen and phosphorus that have promoted an increase in the growth. An increase in leaf biomass and leaf area was also noted in the green grams grown in waste water as compared to the control which agrees with the current findings (13). The results of this study also agree with those of previous reported work (14), which reported improved fruit fresh weight and yield as a result of waste water applications as source of irrigation.

126 **3.2 Stem dry weight**

Significant differences (P≤0.05) were observed in stem dry weights in all vegetables specie in 127 two seasons and greenhouse experiments. In week 6 and 12 amaranthus species was superior in 128 biomass accumulation in both two seasons and greenhouse with the highest dry weight being 129 recorded in the greenhouse with 32.59g and 90.12 g in 6 and 12 WAP respectively (Table 2). 130 This could imply that amaranthus may be a phytoaccumulators, hence not significantly affected 131 by the availability of heavy metals in waste water. The lowest biomass was revealed in the 132 cowpea species in all the experiments with season 1 in week 6 recording the least figure of 8.28 133 g (Table 2). However, in 6 WAP and 12 WAP there was a noticeable increase in the biomass 134 accumulation. Similar to leaf dry weight (Table 1), cowpeas consistently showed the greatest 135 increment; with stem dry weight being four in season 1 from 8.28 g at 6 WAP to 33.96 g (12 136 WAP). This could be as result of improved growth rate as result of nutrients available in the 137 138 waste water.

Table 2: Influence of waste water on stem biomass accumulation in in two seasons and
 green house

Stem dry weight (g)						
	Week 6			Week 12		
Species	S 1	S2	GH	S 1	S2	GH
Cowpeas	8.28 ^d	10.65 ^c	9.17 ^d	33.96 ^c	37.72 ^c	28.48 ^c

LSD	1.8	3.36	2.21	7.63	6.63	18.55
Amaranthus	26.27^{a}	31.07 ^a	32.59 ^a	82.05 ^a	89.21 ^a	90.12 ^a
Kale	18.85 ^b	21.35 ^b	29.33 ^b	56.06 ^b	50.67 ^b	66.64 ^{ab}
Black	12.77 ^b	15.03 ^c	10.03 ^c	49.42 ^b	53.72 ^c	61.47 ^b

141 Means followed by the same letter within the same column are not significantly different 142 ($P \le 0.05$). S1- season 1, S2- season 2, GH-greenhouse,

As reported by Hussein, (15) an increase of vegetable species stem dry weight as a result of 143 treated sewage water irrigation may be due to the increase in organic matter, macro-and 144 micronutrients in the sewage water where beneficial nutrients improved the metabolic activities 145 and hence the vegetative growth which is confirmed from this study. Bedbabis et al. (16) found 146 that waste water irrigation of olive trees resulted in significant yield increase when compared to 147 yields from plot using well water. Availability of nutrients in waste water enables the plant to 148 develop strong organs such as the stem to enhance proper growth and support maximization of 149 yield (17). An increase in plant biomass is also an indication that the vegetable species were not 150 affected by the heavy metals such as lead and cadmium that are evident in waste water have the 151 potential of limiting crop growth (18). 152

153 **3.3 Root dry weight**

Root dry weight showed significance differences (P≤0.05) in vegetable species in 6 and 12 WAP 154 during the two seasons and greenhouse experiment. Amaranthus recorded the highest root dry 155 156 weights with the greenhouse being more superior with 43.26 g and 124 .34g in 6WAP and 12 WAP respectively. Cowpea also was less superior in accumulation of the root biomass recording 157 (8.92g) in season one as revealed in table 3. An increase in the root biomass was noted across all 158 the experiments by the vegetable species which is an indication of a vibrant root growth as a 159 result of available nutrient such as phosphorus and potassium in the sewage water that enhance 160 growth. Although cowpea had less root biomass accumulation compared to other species, there 161

was a noticeable increase from week 6 to week 12 during season by 10 folds increasing from

163 8.92 g to 85.05g (table 3).

164	Table 3: Influence of waste water on vegetable species root biomass accumulation in in two
165	seasons and green house

		Ro	oot dry weig	ht (g)		
	Week 6			Week 12		
Species	S1	S2	GH	S1	S2	GH
Cowpeas	8.92 ^b	11.49 ^c	9.48 ^c	85.05 ^b	93.49 ^{ab}	41.23 ^d
Black	12.77 ^b	15.15 ^{bc}	19.05 ^{bc}	79.70^{b}	86.83 ^b	99.20 ^b
nightshade						
Kale	17.00^{b}	20.88^{b}	26.72 ^b	54.13 ^c	59.81 ^c	69.90 ^c
Amaranthus	28.91 ^a	34.44 ^a	43.26 ^a	100.48 ^a	109.82 ^a	124.24 ^a
LSD	6.93	4.65	8.12	8.82	13.53	12.75

166 Means followed by the same letter within the same column are not significantly different 167 ($P \le 0.05$). S1- season 1, S2- season 2, GH-greenhouse,

The results of this study does not agree with the result of Uzma et al. (19) who reported that 168 waste water used in irrigation of vegetables resulted in a decrease of the root biomass. According 169 to the authors, this was as a result of high levels of heavy metals that are thought to inhibit 170 further growth of the root, limiting supply and access of nutrient required for plant growth. 171 Also, in another study carried out in Pakistan, waste water reduced root biomass root growth, 172 leading to inhibition of nutrient uptake and consequently stunted root systems in vegetables 173 (20). The finding which does not conform with the current study. Therefore, based on the 174 175 current findings it could be the heavy metals in the waste water had not reached toxic limit for 176 interfere with the growth of these particular vegetable species. According to Faizan et al. (21) 177 waste water contains significant amount of nutrients that are known to accelerate root 178 proliferation enhancing them to extract more nutrients near the root zone and consequently leading to a higher dry matter; which agrees with the findings of this study. Another study by 179 180 Parveen et al. (22) agrees with this study that waste water increases root biomass as a result of 181 proper mineral nutrition.

182 **3.4 Root: Shoot ratio**

183	There were varied significance differences ($P \le 0.05$) observed in root: shoot ratios of vegetable
184	species during the two seasons and greenhouse experiment. Amaranthus exhibited the highest
185	root: shoot ratio as influenced by waste water, with the highest being in the greenhouse at 6
186	WAP recording 0.94g (table 4). The least root: shoot ratio was noted in black nightshade in
187	6WAP recording 0.44 g in season 1, season 2 and greenhouse experiments. At 12 WAP kales
188	had the least root: shoot ratio of 0.51, 0.58 and 0.53 in season 1, 2 and greenhouse respectively.
189	Root to shoot ratio increased across the weeks, however at 6WAP and 12 WAP in season two
190	cowpea recorded an increment by 77 %. (Table 4)

Table 4: Influence of waste water on vegetable species root biomass accumulation in in two
 seasons and green house

			Root: Shoot 1	ratio			
	Week 6			Week 12			
Species	S1	S2	GH	S1	S2	GH	
Black	0.44 ^b	0.44 ^b	0.44 ^b	0.71 ^b	0.71 ^b	0.70^{a}	
nightshade							
Kale	0.60^{ab}	0.64 ^{ab}	0.57 ^b	0.51 ^b	0.58^{b}	0.53 ^a	
Cowpea	0.60^{ab}	0.60^{ab}	0.60^{b}	1.07^{a}	1.06 ^a	0.89^{a}	
Amaranthus	0.78 ^a	0.78 ^a	0.94 ^a	0.71 ^b	0.71 ^b	0.83 ^a	
LSD	0.21	0.18	0.2	0.14	0.18	0.37	

193 Means followed by the same letter within the same column are not significantly different 194 (P \leq 0.05). S1- season 1, S2- season 2, GH-greenhouse,

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An increase in root to shoot ratio is an indication of plant response to available moisture and nutrition supplied by waste water during irrigation. The higher the root: shot ratio the healthier the plants due to increased absorption of the nutrients by the roots and a higher absorption of energy transformed in yield making in the plants (23). This is an indication that waste water supplied in this study provided the plants with sufficient requirements for maximum plant growth for the case the case of black nightshade, and cowpea. However, for kale and Amaranthus, there was a decrease in root to shoot ratio which could be as a result of toxicity of the heavy metal present in the soils hence limited the growth of plant. The findings of this study agrees with those of Singh and Agrawal, (24) who reported an increase in root to shoot ratios in crops grown in waste water. Similar findings were also reported by (25) working with vegetables in calcareous clay soils.

207 **4.0 Conclusion**

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Wastewater used in irrigation of African leafy vegetables increased biomass in all vegetable 208 species. This was noted from the week 6 and week 12 of planting where an increase in root, stem 209 leaf and root: shoot ratio was revealed in season 1, season 2 and the greenhouse experiments. 210 Biomass accumulation being a measure of yield in vegetables implied that waste water is rich in 211 mineral capable of nourishing and supporting faster growth rate. Also, it can be concluded that 212 the water water did not contain toxic heavy metals such as lead and cadmium that would inhibit 213 maximum plant growth. Therefore, use of this waste water from the municipal council can be 214 recommended for improved vegetable farming. 215

agriculture in developing countries: Results from a global assessment (Vol. 127). IWMI.

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217		References
218		
219	1.	Graham, R. D., Welch, R. M., and Bouis, H. E. (2001). Addressing micronutrient
220		malnutrition through enhancing the nutritional quality of staple foods: principles,
221		perspectives and knowledge gaps.
222	2.	Dube, P., Heijman, W. J., Ihle, R., and Ochieng, J. (2018). The Potential of Traditional
223		Leafy Vegetables for Improving Food Security in Africa. In Establishing Food Security
224		and Alternatives to International Trade in Emerging Economies (pp. 220-243). IGI
225		Global.
226	3.	Raschid-Sally, L., and Jayakody, P. (2009). Drivers and characteristics of wastewater

- Gweyi-Onyango J P and Osei-Kwarteng M (2011). Safe Vegetable Production with
 Wastewater in Developing Countries: Demystifying Negative notions: African Journal of
 Horticultural . Sci. 5:70-83
- 5. Ensink, J. H., Mahmood, T., and Dalsgaard, A. (2007). Wastewater irrigated vegetables:
 market handling versus irrigation water quality. *Tropical Medicine & International Health*, 12, 2-7
- 6. Khaled, S. B. and Muhammad A. S. (2015). Field accumulation risks of heavy metals in
 soil and vegetable crop irrigated with sewage water in Western Region of Saudi Arabia. *Saudi Journal of Biological Sciences*, 23, 32-44.
- 7. Mireri, C., Atekyereza, P., Kyessi, A., and Mushi, N. (2007). Environmental risks of
 urban agriculture in the Lake Victoria drainage basin: A case of Kisumu municipality,
 Kenya. *Habitat International*, *31*(3-4), 375-386.
- 8. Mohammed, S. A., and Folorunsho, J. O. (2015). Heavy metals concentration in soil and
 Amaranthus retroflexus grown on irrigated farmlands in the Makera Area, Kaduna,
 Nigeria. *Journal of Geography and Regional Planning*, 8(8), 210-217
- 243
- Stoltz, E., and Greger, M. (2002). Accumulation properties of As, Cd, Cu, Pb and Zn by
 four wetland plant species growing on submerged mine tailings. *Environmental and experimental botany*, 47(3), 271-280.
- 247 10. Wallace, J. S. (2000). Increasing agricultural water use efficiency to meet future food
 248 production. *Agriculture, ecosystems & environment*, 82(1-3), 105-119.
- 11. Anwar, S., Nawaz, M. F., Gul, S., Rizwan, M., Ali, S., and Kareem, A. (2016). Uptake
 and distribution of minerals and heavy metals in commonly grown leafy vegetable
 species irrigated with sewage water. *Environmental monitoring and assessment*, 188(9),
 541.
- 253 12. Zu, Y. Q., Sun, J. J., He, Y. M., Wu, J., Feng, G. Q., and Li, Y. (2016). Effects of arsenic
 254 on growth, photosynthesis and some antioxidant parameters of Panax notoginseng
 255 growing in shaded conditions. *Int J Adv Agric Res*, *4*, 78-88.
- 256

- 13. Nagajyothi, P. C., Dinakar, N., Suresh, S., Udaykiran, Y., Suresh, C., and Damodharam,
 T. (2009). Effect of industrial effluent on the morphological parameters and chlorophyll
 content of green gram (Phaseolus aureus Roxb). *J. Environ. Biol*, *30*(3), 385-388.
- 14. Torabian, A. (2010). Effect of urban treated sewage on yield and yield components of
 sweet pepper. *Plant Ecophysioly*, 2, 97-102.
- 15. Hussein, A. H. A. (2009). Impact of sewage sludge as organic manure on some soil
 properties, growth, yield and nutrient contents of cucumber crop. *Journal of Applied Sciences*, 9(8), 1401-1411.
- 16. Bedbabis S., Trigui D., Ahmed C. B., Clodoveo ML, Camposeo S., Vivaldi G. A.,
 Rouina BB (2015). Long-term effects of irrigation with treated municipal waste water on
 soil, yield and olive oil quality. Agricultural Water Management. 160: 14-21.
- 268 17. Aronsson, P., and Perttu, K. (2001). Willow vegetation filters for wastewater treatment
 269 and soil remediation combined with biomass production. *The Forestry Chronicle*, 77(2),
 270 293-299.
- 18. Smith, S. R. (2009). A critical review of the bioavailability and impacts of heavy metals
 in municipal solid waste composts compared to sewage sludge. *Environment international*, 35(1), 142-156
- 274
- 19. Uzma, S., Azizullah, A., Bibi, R., Nabeela, F., Muhammad, U., Ali, I., and Häder, D. P.
 (2016). Effects of industrial wastewater on growth and biomass production in commonly
 grown vegetables. *Environmental monitoring and assessment*, 188(6), 328.
- 278 20. Shakoor, S. A. B., and Farooq, M. A. (2013). Effects of irrigation with waste water from
 279 different industries on vegetables grown in vicinity of Faisalabad, Pakistan.
- 280 21. Faizan, S., Kausar, S., and Akhtar, N. (2014). Influence of wastewater application and
 281 fertilizer use on growth, photosynthesis, nutrient homeostatis, yield and heavy metal
 282 accumulation in okra (Abelmoschus esculentus L. Moench). *Pakistan Journal of* 283 *Biological Sciences*, 17(5), 630-640.
- 284 22. Parveen, T., Mehrotra, I., and Rao, M. S. (2014). Impact of treated municipal wastewater
 285 irrigation on turnip (Brassica rapa). *Journal of plant interactions*, 9(1), 200-211.

- 286 23. Hu, X., and Ding, Z. (2009). Lead/cadmium contamination and lead isotopic ratios in
 vegetables grown in peri-urban and mining/smelting contaminated sites in Nanjing,
 288 China. *Bulletin of environmental contamination and toxicology*, 82(1), 80-84.
- 24. Singh, R. P., and Agrawal, M. (2009). Use of sewage sludge as fertiliser supplement for
 Abelmoschus esculentus plants: physiological, biochemical and growth responses.
 International Journal of Environment and Waste Management, *3*(1-2), 91-106.
- 292 25. Heitholt, J. J., and Sloan, J. J. (2016). Enhancement of vegetable crop growth with
 biosolids and yard-waste compost on a calcareous clay soil. *Texas Journal of Agriculture* 294 *and Natural Resources*, 19, 80-92.
- 295

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