Effect of treated sewage sludge on the quality of okra fruit

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Abstract: Sewage sludge (treated or untreated) if applied to the soil has been identified to increase heavy metals in the soil which are in turn transferred to plants. In view of this, the study aimed at assessing the effects of treated sewage sludge on the quality parameters of okra fruit. The randomized block design was employed in raising okra plants with three treatments (0 t ha^{-1}, 10 t ha^{-1}, and 20 t ha^{-1} sewage sludge amendments) over a land area of 56.3 m^2. Soil analysis was used to determine the level of nitrogen, phosphorus, and potassium in the soil. After two months, the okra fruit was harvested by cutting the pods off, slicing, oven drying, milling prior to laboratory analysis. The heavy metal contents in the okra fruit were analysed using atomic absorption spectrometer. The parameters analyzed were Cd, Pb, Cu, Fe, and Zn. The concentration of the selected heavy metals was subjected to descriptive statistics and one-way analysis of variance (ANOVA). The transfer factor was also determined. Most soils in the okra field was sand. Study found out that the soil was deficient in phosphorus and potassium. Bioaccumulation of heavy metals were not found in the okra fruit since Cu, Zn and Fe have transfer factors of less than one. In addition, transfer factor order in okra fruit was Zn>Cu>Fe>Cd>Pb. The study revealed that the application of sewage sludge at 10 t ha^{-1} and 20 t ha^{-1} resulted in high uptake of Zinc (Zn) and Iron (Fe) whereas cadmium and lead were not detected. The study concluded that consumption of the okra fruit grown on 10 t ha^{-1} and 20 t ha^{-1} were very safe since Cu, Fe, and Zn in the okra fruit were less than desirable limit as recommended by FAO and WHO. There is need to determine hazard quotient, the health risk index, morbidity status the enrichment factor and degree of contamination in okra fruit. Effect of sewage sludge at different application rates between 25 t ha^{-1} and 60 t ha^{-1} should be seriously investigated so as to determine the optimal level of heavy metal in the okra fruit.

Keywords: soil, water, sewage sludge, heavy metals, okra fruit

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1 Introduction

Annually, high volume of sewage is produced due to rapid population and urbanization (Balkhair and Ashraf, 2016). Sludge is a residual solid derived from the treatment of waste water or sewage. Sewage constituent and characteristic depends on its source (George et al., 2003). There are various methods of treating sewage and wastewater and these include digestion, composting, dewatering, thermal drying and activated sludge process (Ahansazan et al., 2014). Sewage sludge produced from activated sludge process is used to improve physical, biological, and chemical properties of the deficit soil (Sort, 1999; Lavado, 2006). It is less costly and easier to use compared to conventional fertilizers because of its availability. Treated sewage sludge contains organic matter, nutrient and heavy metals such as zinc (Zn), copper (Cu) and iron (Fe) for soil and plants improvement (Usman et al., 2012; Ali et al., 2013). However, studies have shown that application of sewage sludge to soil aggravated the accumulation of heavy metals, which in turns transfer them to the edible part of the crops/vegetables (Singh and Agrawal, 2007; Bourioug et al., 2014). In addition, Balkhair and Ashraf (2016)

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reported that vegetables grown on sewage plot have high risk of heavy metals present in the edible part of the crop. Tangahu et al. (2011) defined heavy metals as elements with metallic properties and an atomic number greater than 20. These metals are biodegradable which are toxic at low concentration and their relative density higher than that of water (Amo-Asare, 2012). Ali et al. (2013) classified heavy metals as useful (Fe, Cu, Zn, Manganese, and Nickel) and non-useful (Cadmium, Lead, Arsenic, Mercury and Chromium) element. They also reported that heavy metals accumulation in soil poses serious risk/hazard to the environment which in turn affects human health.

The heavy metals in sewage sludge require numerous hazard assessment such as hazard quotient, health risk index, morbidity status or enrichment factor, degree of contamination and uptake/transfer factor (Balkhair and Ashraf, 2016). Qasim et al. (2001) showed that application of sewage sludge between 10 and 30 t ha^{-1} increase the heavy metals in shoot length of maize. Mehmet (2013) reported that application of sewage sludge increases crop yield. Furthermore, Lato et al. (2012) discovered that Fe has the highest value of transfer factor followed by Cu and Lead (Pb) when different application rate of sewage sludge was added to the soil. Based on these view, this study aims to determine the effects of treated sewage sludge on the quality of Okra fruit to investigate the safety of their human consumption. In addition, the transfer factor of heavy metals in okra plant was evaluated.

2 Materials and methods

2.1 Description of the study area

The experiment was carried out in Federal University of Technology, Minna, Nigeria. The area lies on Latitude 9.5373202 and Longitude 6.4647157, with altitude 262.5 m (Figure 1). The climate of Gidan Kwano is the same as the climate obtainable in Minna as they both belong to the same climatic zone. The minimum and maximum temperatures of the study were 19.3°C and 37.1°C respectively (Galadima, 2014). Gidan Kwano is characterized by two broad seasons: the rainy season occurs normally, between the month of April and October while the dry season follows after up till March. The average rainfall around the study area amounts to about 328 mm (Galadima, 2014).

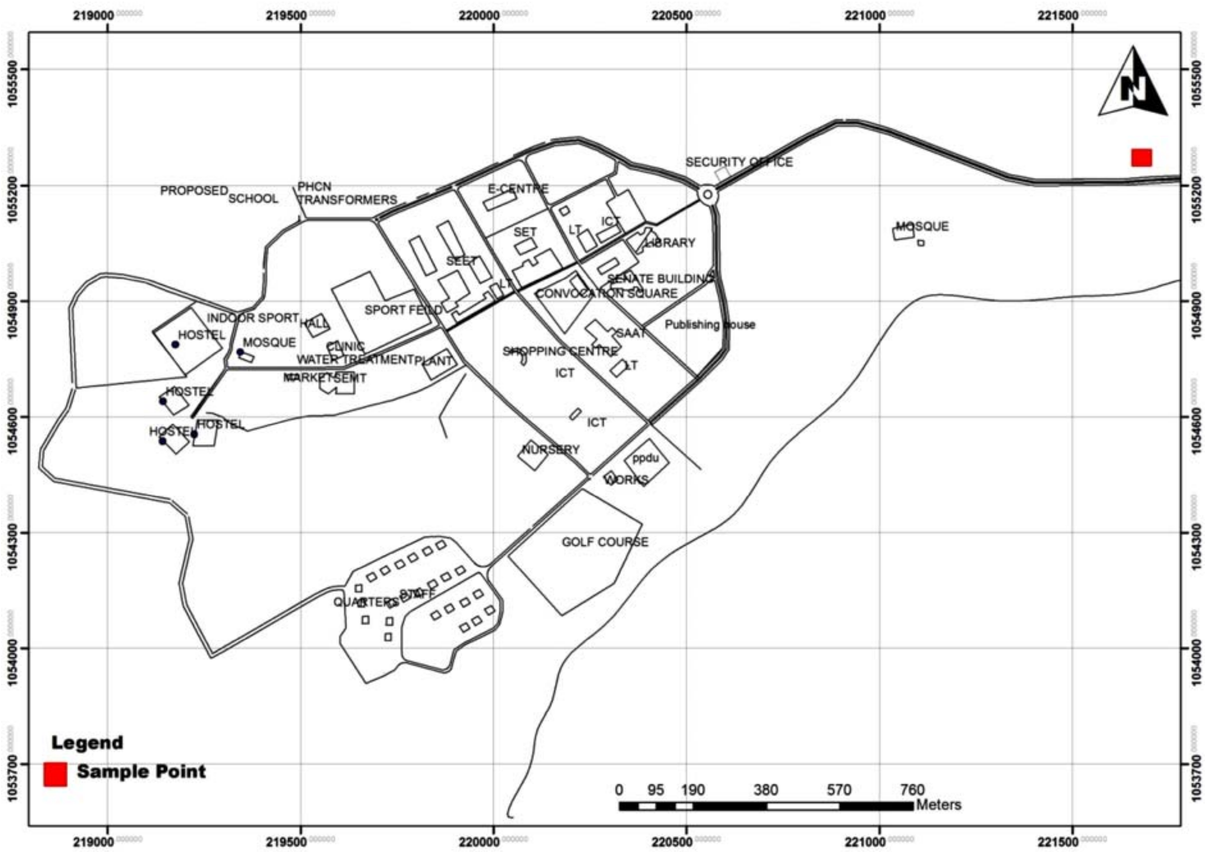

Figure 1 Map of the Gidan Kwano showing the location of Okra Field

2.2 Materials

The materials used for the experiment were as follows:

(i) Caked sewage sludge: This material was used as a fertilizer for soil amendment. Sewage sludge was collected from Municipal Sewage Treatment Plant Abuja. The sample was obtained through activated sludge process where microorganisms were fed on organic contaminants in wastewater that generates a high-quality effluent.

(ii) Okra seeds: Seeds were obtained from the Minna market (commercial) and used for sowing. Local okra seed was used for this study.

(iii) Paper envelopes: They were used for identification of okra fruit of different treatments.

2.2.1 Equipment

The following equipment was used for this study:

(i) Weighing balance: It was used for taking standard weights and measuring sludge application rates.

(ii) Oven: Oven (model number: PBS118SF and serial number: 94L234) was used to dry the milled substance after 24 hours.

(iii) Knife: it was used for harvesting and cutting okra pods

(iv) Food processor: food processor known as CYCLOTEC 1093SM was used for milling dried okra pods

(v) Soil auger: It was used for collecting soil samples at a depth of 30 cm.

(vi) Shovel: It was used for excavating sludge from storage lagoon into carriage bag.

(vii) Atomic Absorption Spectrometer: It was used to determine cations, anions and trace metals in the Laboratory.

2.2 Soil analysis

Soil samples were sampled at a depth of 30 cm using a soil auger. A total of 10 cores randomly sampled from each plot. Soil samples collected from each core were mixed in a bag vigorously and the combined sample was analysed in accordance with 1987 International Institute of Tropical Agriculture. The soil was oven dried for 24 hours at 105ºC due epileptic power supply. The soil parameters analyzed in the laboratory were nitrogen, phosphorus, potassium levels, soil exchangeable bases and organic carbon. In addition, heavy metals such Cadmium (Cd), Pb, Cu, Zn and Fe were also analyzed.

2.3 Experimental design

The okra field trial had an area of 56.25 m^2. The sewage sludge was equally distributed just one time on the okra field and it was manually ploughed (at 30 cm top soil) for soil uniformity. The study chose three treatments (0 t ha^{-1}, 10 t ha^{-1}, and 20 t ha^{-1}). These limits were set lower than 40-60 t ha^{-1}, based on thresholds described by Laţo et al. (2012) so as to avoid the accumulation of heavy metals in the edible part of okra plant. These treatments were replicated thrice randomly over a plot. Each block was a square measuring 2 m×2 m with spacing of 0.5 m between the blocks.

2.4 Raising of plants

Okra seeds were soaked for 15 hours in water prior to planting to aid early germination. Okra seeds were sown on 8th July, 2016 with a spacing of about 0.6 m. Sludge was applied by side dressing four weeks after crop emergence. Weeding was done between the crop period but there was no agricultural practice (such as irrigation, crop rotation, fertilizer regarding NPK and pesticide) carried out on the plot. The plants were then left to grow under rain fed conditions and the pods harvested on 19th August, 2016.

2.5 Crop sampling and laboratory analysis

The okra was harvested by cutting pods off the stem using a knife, and the samples were placed in a bag and properly labeled. The harvested pods were sliced and oven dried for 48 hours at 75ºC and milled. The milled sample was sealed in labeled bags and taken to the laboratory for analysis. The method employed was acid digestion and dry ashing. Five grams of okra samples were weighed into a conical flask and 10 mL of concentrated HNO_3 was added to the flask. This flask was heated at 110ºC and allowed to cool at room temperature. 3 mL of concentrated H_2SO_4 was added to the flask and further heated at 90ºC to release light orange (Idera et al., 2015). This diluted substance was allowed to cool at room temperature. Also, 2 mL of concentrated $HCIO_4$ was added to it and heated at 100ºC until the solution reduced to 5 mL (Idera et al., 2015). Each digested sample was filtered and made up to 50 mL with deionized water in a volumetric flask and analysed for heavy metals (Cd, Pb, Zn, Cu, and Fe) by an atomic absorption spectrometry.

2.6 Heavy metals transfer in the okra fruit

The study calculated transfer coefficient as follows (Mirecki, 2015; Balkhair and Ashraf, 2016):

$$Translocation\ factor(TF) = \frac{Heavy\ metal\ concentration\ in\ the\ harvested\ edible\ part\ of\ okro\ plant}{Heavy\ metal\ concentration\ in\ the\ soil}$$

where, *TF*>1 indicates that okra plant has accumulated element; *TF*=1 shows that okra plant is not influenced by the element; *TF*<1 indicates that okra plant excludes the element from the uptake. If TF have higher values, then edible part of okra plants phytoremediate the heavy metals in the soil.

2.7 Data analysis

The results obtained from the laboratory were presented using chart and tables. These data were subjected to descriptive statistics and one-way analysis of variance (ANOVA).

3 Results and discussion

3.1 Physicochemical properties and heavy metals in the soil

Figure 2 shows the soil types of the field. The soil composition was 81% sandy in nature, while silt and clay contents were 11% and 8% respectively. The soil has a pH value of 6.42. The study infers that soil is majorly sandy.

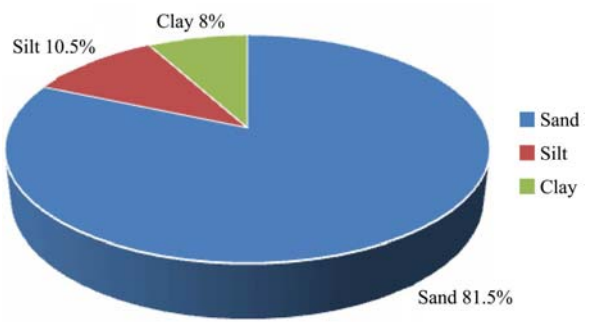

Figure 2 Soil textural classes

The mean concentration of nitrogen N was 1540 g kg^{-1} while potassium and phosphorus were 33.15 mg kg^{-1} and 9.5 mg kg^{-1} respectively (Table 1). Based on Allan (2010) classification, nitrogen had medium concentration in the soil whereas potassium and phosphorus were either very low or low. This may be attributed to nitrogen imbalance from previous soil improvement materials. Organic manure like poultry wastes are richer in nitrogen but deficit in potassium and phosphorus, there is a need to amend the soil to boost the potassium and phosphorus component (Sangodoyin, 1996). Therefore, the fertility of the soil was deficient and there is need to amend the soil with fertilizer.

Table 1 Fertility Parameter of the soil

Fertility content of the soil	Mean concentration, mg kg^{-1}	Allan (2010) Soil classification
Total Nitrogen	1540	Medium
Potassium	33.15	Very Low
Phosphorus	9.5	low

Figure 3 shows heavy metals concentration in the okra field. Fe had the highest concentration value followed by Zn and Cu. In addition, Cd and Pb have the least concentration values in the soil. This implies that Fe and Zn were more on the Okra field.

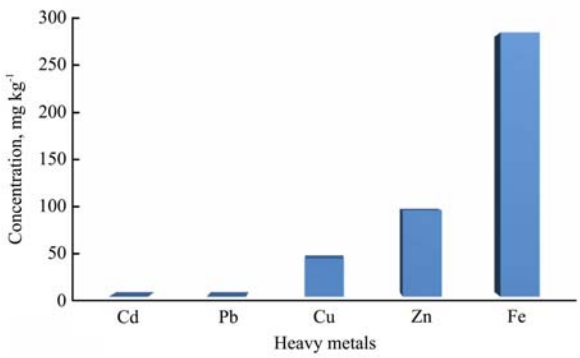

Figure 3 Heavy metals concentration in the soil sample

3.2 Sewage sludge properties

Figure 4 shows the chemical composition of sewage sludge. The sludge had a relatively acidic pH value (5.64). The total nitrogen was 250 mg kg^{-1} in the dry solid. Nitrate and nitrite nitrogen were also determined to be 60 mg kg^{-1} and 1.7 mg kg^{-1} respectively.

Table 2 shows some selected heavy metal content in the sewage sludge such as Cd, Pb, Cu, Zn and Fe. Fe had the highest concentration followed by Cu and Zn (Fe>Cu>Zn) whereas Cd and Pb were not detected. This analysis showed that the concentration of heavy metals in the sewage sludge were below the pollutant limit. The heavy metals concentration in the sewage was lower than that reported by Hussein (2009). This author affirms that the activated sludge process used for the sewage treatment was satisfactory.

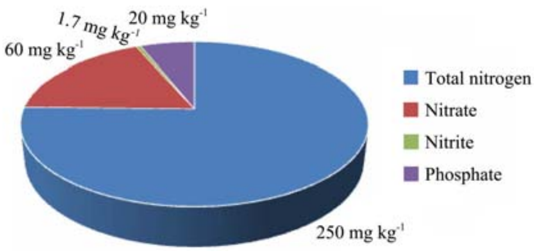

Figure 4 Chemical composition of sewage sludge

Table 2 Heavy metals concentration in the sewage sludge

Heavy metals	Concentration, mg kg^{-1}	United States Environmental Protection Agency, 1994, mg kg^{-1}
Cd	0.00	39
Pb	0.00	300
Zn	0.29	2800
Cu	0.72	1500
Fe	25	nil

3.3 Heavy metals in okra fruit

Cd and Pb were not detected in the tested okra fruit because the soil and sewage sludge showed 0 mg kg^{-1} (Figure 5). Copper (Cu) concentration at different sludge application rates (0, 10 and 20 t ha^{-1}) were 11.2, 7.62 and 7.08 mg kg^{-1} respectively. 43% of Cu concentration was found at 0 t ha^{-1} while 29% and 27% of Cu concentration were found at 10 and 20 t ha^{-1} respectively.

Zn concentration at different sludge application rates (0, 10 and 20 t ha^{-1}) were 19.48, 22.71 and 28.25 mg kg^{-1} respectively. 28% of Zn concentration was detected at 0 t ha^{-1} while 32% and 40% of Zn) concentration were found at 10 and 20 t ha^{-1} respectively. Fe concentration at different sludge application rates (0, 10 and 20 t ha^{-1}) were 24.35, 31.91 and 51.63 mg kg^{-1} respectively. 23% of Fe concentration was discovered at 0 t ha^{-1} while 30% and 49% of Fe concentration were found at 10 and 20 t ha^{-1} respectively.

At 10t ha^{-1}, Cu concentration in okra fruit reduces by 7% whereas Zn and Fe concentration in the okra fruit increased by 20% and 24% respectively when compared with control field (0 t ha^{-1}) as shown in Figure 5. This means that the concentration of Copper (Cu) in the soil (40 mg kg^{-1}). At 20 t ha^{-1}, Cu concentration further reduces in the okra fruit by 7.6% whereas Zn and Fe concentration further increased by 20% and 38% respectively when compared to 10 t ha^{-1} in the okra fruit as shown in Figure 5. The study shows that Cu concentration further reduced by 58% whereas Zn and Fe concentration increased by 31 % and 52 % respectively in the okra fruit when compared to 0 t ha^{-1} field.

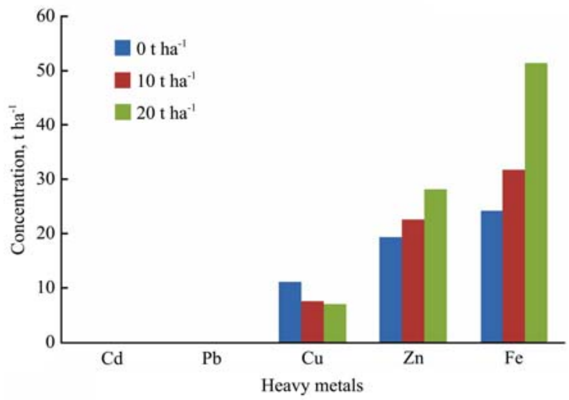

Figure 5 Heavy metals in the okra fruit grown with different sewage sludge rate

Based on Duncan test, application of 10 t ha^{-1} of sewage sludge showed statistically significant differences (1.0>0.05) in the mean concentration of Cu as compared to the control. However, application of 10 t ha^{-1} and 20 t ha^{-1} are not significantly different (Table 3). In addition, there was significant difference in mean concentration of Fe at both treatment (10 t ha^{-1} and 20 t ha^{-1}) compared to the control as shown in Table 3. Furthermore, mean concentration of Zn in the okra fruit was significantly different at both treatments (10 t ha and 20 t ha^{-1}) compared to the control. The application of sewage sludge at 10 t ha^{-1} and 20 t ha^{-1} reduces Cu concentration but the mean is not significantly different. The study implies that application of sewage sludge at 10 t ha^{-1} and 20 t ha^{-1} increases Zn and Fe concentration in okra fruit.

Table 3 Mean concentration of heavy metals in okra fruit

	Cd, mg kg^{-1}	Pb, mg kg^{-1}	Cu, mg kg^{-1}	Zn, mg kg^{-1}	Fe, mg kg^{-1}
Control	0.00	0.00	11.2^{b}±0.0058	19.48^{a}±0.012	24.35^{a}±0.0058
10 t ha^{-1} SSA	0.00	0.00	7.62^{a}±0.012	22.71^{b}±0.0058	31.91^{b}±0.0058
20 t ha^{-1} SSA	0.00	0.00	7.08^{a}±0.58	28.25^{c}±0.058	51.63^{c}±0.17

Note: SSA- Sewage Sludge Amendment. Means with the same superscript are not significantly different (Appendix I).

Table 4 shows the mean concentration of heavy metals in okra fruit with different sewage sludge rate and the allowable concentration limits as recommended by FAO and WHO (2011). The concentration of the selected heavy metals i.e. Cd, Pb, Cu, Zn and Fe in the okra fruit were less than the maximum allowable limits. This means that consumption of the fruit grown on 10 t ha^{-1} and 20 t ha^{-1} sludge treatment will not effect on the human.

Table 4 Mean concentration of heavy metals in okra fruit with different sewage sludge rate

Metals	Concentration in fruit, mg kg^{-1}		FAO/WHO (2001) allowable limit in fruit and vegetables, mg kg^{-1}
	T_1	T_2	
Cd	0.00	0.00	0.05
Cu	7.62	7.08	73.00
Fe	31.91	51.63	425.00
Pb	0.00	0.00	0.30
Zn	22.71	28.25	99.40

Note: T_1- 10 t ha^{-1} sewage sludge treated; T_2- 20 t ha^{-1} sewage sludge treated.

The transfer factor of heavy metals from soil to okra fruit was estimated as shown in Figure 6. Cu, Zn and Fe have transfer factors less than 1 in okra fruit which denotes that bioaccumulation were not found in the okra fruit. The transfer factor order in okra fruit were Zn>Cu>Fe>Cd>Pb. These results do not agree with okra planted on abandon dumpsite (Akpofure, 2012).

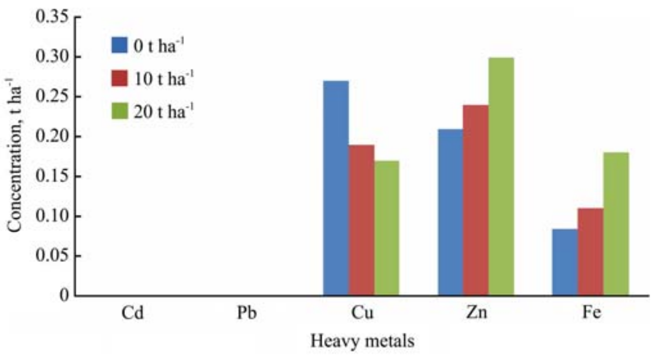

Figure 6 Transfer factors of heavy metals in okra fruit grown with different sewage sludge application rate

4 Conclusion

Effect of different sewage sludge application rate on the quality of okra fruit was assessed with the aim of ascertaining the safety of okra fruit consumption and examining their transfer factor in the crop. The major part of the sandy soil was deficient in potassium and phosphorus. Therefore, the okra field required soil amendment. This study inferred that soil amendment with 10 and 20 t ha^{-1} of dried sewage sludge improved soil fertility without causing no adverse effect on the heavy metal concentration in the crop. The study revealed that application of treated sewage sludge in 10 and 20 t ha^{-1} field resulted in increased uptake of Zn and Fe in okra fruit. At 10 and 20 t ha^{-1}, the Cu concentration decreases in the okra fruit. The Cd and Pb concentration were not detected in okra fruit. The study also revealed that concentration of heavy metals found in okra fruit was lower than the WHO and FAO maximum allowable limits. This means that consumption of the okra fruit grown on 10 and 20 t ha^{-1} were safe for human consumption. The results of transfer factor further affirmed that bioaccumulation was not found in the okra fruit. The study concluded that consumption of the okra fruit grown on 10 and 20 t ha^{-1} were very safe since there was no bioaccumulation of heavy metal in the okra fruit. In addition, Zn concentration was found at 20 t ha^{-1} which is useful for human. There is a need to vary the sewage application between 25 and 60 t ha^{-1} in order to determine more suitable application rates of sewage sludge in the area.

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Appendix I

Analysis of variance for heavy metals in okra fruit

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence		Minimum	Maximum
						Lower Bound	Upper Bound		
Cd	Control	3	0.0000	0.00000	0.00000	0.0000	0.0000	0.00	0.00
	10 t ha^{-1}	3	0.0000	0.00000	0.00000	0.0000	0.0000	0.00	0.00
	20 t ha^{-1}	3	0.0000	0.00000	0.00000	0.0000	0.0000	0.00	0.00
	Total	9	0.0000	0.00000	0.00000	0.0000	0.0000	0.00	0.00
Pb	Control	3	0.0000	0.00000	0.00000	0.0000	0.0000	0.00	0.00
	10 t ha^{-1}	3	0.0000	0.00000	0.00000	0.0000	0.0000	0.00	0.00
	20 t ha^{-1}	3	0.0000	0.00000	0.00000	0.0000	0.0000	0.00	0.00
	Total	9	0.0000	0.00000	0.00000	0.0000	0.0000	0.00	0.00
Cu	Control	3	7.0800	0.01000	0.00577	7.0552	7.1048	7.07	7.09
	10 t ha^{-1}	3	7.6200	0.02000	0.01155	7.5703	7.6697	7.60	7.64
	20 t ha^{-1}	3	11.2000	1.00000	0.57735	8.7159	13.6841	10.20	12.20
	Total	9	8.6333	2.00260	0.66753	7.0940	10.1727	7.07	12.20
Zn	Control	3	19.4800	0.02000	0.01155	19.4303	19.5297	19.46	19.50
	10 t ha^{-1}	3	22.7100	0.01000	0.00577	22.6852	22.7348	22.70	22.72
	20 t ha^{-1}	3	28.2500	0.10000	0.05774	28.0016	28.4984	28.15	28.35
	Total	9	23.4800	3.84152	1.28051	20.5271	26.4329	19.46	28.35
Fe	Control	3	24.3500	0.01000	0.00577	24.3252	24.3748	24.34	24.36
	10 t ha^{-1}	3	31.9100	0.01000	0.00577	31.8852	31.9348	31.90	31.92
	20 t ha^{-1}	3	51.6300	0.30000	0.17321	50.8848	52.3752	51.33	51.93
	Total	9	35.9633	12.19842	4.06614	26.5868	45.3399	24.34	51.93

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Cd		2		
Pb		2		
Cu	3.879	2	6	0.083
Zn	2.781	2	6	0.140
Fe	3.729	2	6	0.089

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Cd	Between Groups	0.000	2	0.000		
	Within Groups	0.000	6	0.000		
	Total	0.000	8			
Pb	Between Groups	0.000	2	0.000		
	Within Groups	0.000	6	0.000		
	Total	0.000	8			
Cu	Between Groups	30.082	2	15.041	45.101	0.000
	Within Groups	2.001	6	0.334		
	Total	32.083	8			
Zn	Between Groups	118.037	2	59.019	16862.486	0.000
	Within Groups	0.021	6	0.004		
	Total	118.058	8			
Fe	Between Groups	1190.230	2	595.115	19793.188	0.000
	Within Groups	0.180	6	0.030		
	Total	1190.411	8			

Post Hoc Tests

Multiple Comparisons

Dependent Variable				Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Cu	LSD	Control	10 t ha^{-1}	–0.54000	.47152	.296	–1.6938	.6138
			20 t ha^{-1}	–4.12000*	.47152	.000	–5.2738	–2.9662
		10 t ha^{-1}	Control	0.54000	.47152	.296	–.6138	1.6938
			20 t ha^{-1}	–3.58000*	.47152	.000	–4.7338	–2.4262
		20 t ha^{-1}	Control	4.12000*	.47152	.000	2.9662	5.2738
			10 t ha^{-1}	3.58000*	.47152	.000	2.4262	4.7338
Zn	LSD	Control	10 t ha^{-1}	–3.23000*	.04830	.000	–3.3482	–3.1118
			20 t ha^{-1}	–8.77000*	.04830	.000	–8.8882	–8.6518
		10 t ha^{-1}	Control	3.23000*	.04830	.000	3.1118	3.3482
			20 t ha^{-1}	–5.54000*	.04830	.000	–5.6582	–5.4218
		20 t ha^{-1}	Control	8.77000*	.04830	.000	8.6518	8.8882
			10 t ha^{-1}	5.54000*	.04830	.000	5.4218	5.6582
Fe	LSD	Control	10 t ha^{-1}	–7.56000*	.14158	.000	–7.9064	–7.2136
			20 t ha^{-1}	–27.28000*	.14158	.000	–27.6264	–26.9336
		10 t ha^{-1}	Control	7.56000*	.14158	.000	7.2136	7.9064
			20 t ha^{-1}	–19.72000*	.14158	.000	–20.0664	–19.3736
		20 t ha^{-1}	Control	27.28000*	.14158	.000	26.9336	27.6264
			10 t ha^{-1}	19.72000*	.14158	.000	19.3736	20.0664

Note: *. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Cu

Control		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	20 t ha^{-1}	3	7.0800	
	10 t ha^{-1}	3	7.6200	
	Control	3		11.2000
	Sig.		0.296	1.000

Note: Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000.

Zn

Control		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	Control	3	19.4800		
	10 t ha^{-1}	3		22.7100	
	20 t ha^{-1}	3			28.2500
	Sig.		1.000	1.000	1.000

Note: Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000.

Fe

Control		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	Control	3	24.3500		
	10 t ha^{-1}	3		31.9100	
	20 t ha^{-1}	3			51.6300
	Sig.		1.000	1.000	1.000

Note: Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000.