

PATHOGENIC BACTERIA IN LAKE VICTORIA SHORE WATER AT KASENYI LANDING SITE IN ENTEBBE MUNICIPALITY, UGANDA: A CROSS-SECTIONAL PILOT SURVEY ON OCCURRENCE PROFILES AND ASSOCIATED FACTORS.

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Abstract

Background:

Goal six (6) of the 2030 agenda and sustainable development goals highlights the need to "ensure availability and sustainable management of water and sanitation for all." Over the years; Lake Victoria, which supports nearly one-third of the total East African population has been facing pollution-related effects and thus risking water-borne infections from pathogenic bacteria for example; *Citrobacter freundii*, *Klebsiella pneumoniae*, *Staphylococcus* species, *Enterococcus* species, etc.; with some of the associated factors including wastewater treatment, agricultural activities, soil surface runoffs, bathing, rubbish dumping among others.

Methods:

A two-month cross-sectional pilot survey was done in which thirty-one (n=31) Lake Victoria surface water samples were collected at the Kasenyi landing site and bacteriologically examined through culturing water samples. Additionally; a one-time field observation accompanied by eleven (n=11) short interviews with key respondents was undertaken to identify the factors potentially associated with the bacterial contamination of the Lake Victoria shore surface water. Data were analyzed using Microsoft Excel 2013.

Results:

Citrobacter freundii accounted for the highest occurrence at 58% (46/79) while *Klebsiella pneumoniae* was the least occurring at 3% (2/79). "Soil surface run-off" emerged as the most occurring factor related to possible bacterial contamination of the shore surface waters (73%; 8/11). One-time field observation revealed the presence of "bathing practices, rubbish dumping, and soil surface runoff."

Conclusion:

Citrobacter freundii was the most occurring bacterial pathogen; soil surface run-off emerged as the most occurring factor; there is a low chance of encountering *Klebsiella pneumoniae* and a high chance of encountering *Citrobacter freundii*, *Staphylococcus* species, and *Enterococcus* species.

Recommendation:

Establishment of control mechanisms (i.e. physical barriers) for soil run-off into the lake shore.

Keywords: Pathogenic bacteria, Lake Victoria, *Citrobacter freundii*, *Staphylococcus* species
Enterococcus species, Submitted: 2023-06-10 Accepted: 2023-06-19

1. Introduction:

Goal six (6) of the 2030 agenda and sustainable development goals highlights the need to "ensure availability and sustainable management of water and sanitation for all" (UN and UNEP, 2015). In past years; the world's second-largest lake; "Lake Victoria," which supports nearly one-third of the total East African population through the provision of employment to up to thirty million people have been facing pollution-related effects such as a decline in fish species as a result of shore-based undertakings including; intensified land use, increased human and livestock populations, urban wastewater and industrial discharges, soil erosion, and agricultural run-off (Arsène, 2022). As such; bacterial caused water-borne diseases such as; typhoid, dysentery, and cholera among others may result among the respective shore-based human population following exposure to an unsafe lake water source thus potentially affecting the still existing 2 billion people lacking access to unsafe water (Prüss-Ustün et al. 2014; UNICEF, 2019; and UN, 2022).

Over the years; the occurrence of pathogenic bacteria in lake water sources has always been a public health concern as was justified by Youn-Joo et al. (2002), Edge et al. (2013), Jin et al. (2018), who identified *Escherichia coli*, Gul-tepe et al. (2017) who isolated *Staphylococcus aureus* and *Enterococcus* species and still is a serious public health problem as studied by Bisimwa et al. (2022) and Rondón-Espinoza et al., (2022), who identified bacterial pathogens such as *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus* species, *Vibrio cholerae*, *Citrobacter freundii*, *Proteus vulgaris*, *Shigella dysenteriae* among others. Such has usually been a result of lake shore-based factors such as; human population increase, increased urbanization, wastewater treatment, agricultural activities, for example, the establishment of crop/farmlands, increased number of livestock, increased surface runoffs as a result of increased

precipitation rates, and transportation activities (Islam et al., 2018); bathing along and/or within the water body; laundry activities; dishwashing; wash off from dumped rubbish; and market effluents runoff into a water body (Bisimwa et al., 2022); open defecation, and presence of animal excreta (Bedane et al.; 2022).

To the best of the researcher's knowledge; there has never been a study conducted and published from the Kasenyi landing site to determine the occurrence profiles of the bacterial pathogens in Lake Victoria shore waters and/or the potentially associated factors, thus a foundational aim for this survey.

2. Methods:

2.1. Study design:

A two-month cross-sectional pilot survey was done in February and March 2023 in which thirty-one (n=31) Lake Victoria surface water samples were collected from the shores at Kasenyi landing site was transported on a cold chain to the microbiology laboratory at the University of Kisubi for bacteriological analysis. Additionally; a one-time field observation accompanied by eleven (n=11) short interviews with key respondents was undertaken to identify the factors potentially associated with the bacterial contamination of the Lake Victoria shore surface water.

2.2. Study area:

Kasenyi landing site is located 8km off Abaita Ababiri trading center along the Entebbe-Kampala highway in Entebbe Municipality, Katabi sub-county, Wakiso District in Uganda.

2.3. Unit of analysis and bacteria of interest:

The survey used Lake Victoria's surface shore water samples as the unit of analysis. These were then subjected to bacteriological analysis for the isolation and identification of the bacterial pathogens of interest i.e. *Staphylococcus* species, *Enterococcus* species, and *Enterobacteria* species.

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2.4. Sample size determination:

The sample size for this particular survey was determined based on the central limit theorem (Arsham, 2005) which considers an amount of, $n > 30$, to be a sufficiently large sample to represent a large population. Here; “n” represents the sample amount obtained at a particular moment in time.

2.5. Water sampling and bacteriological analysis:

2.5.1. Water sampling in the field:

While roaming on the surface of Lake Victoria; a 200ml capacity wide-mouthed stainless steel metallic water sampling cup was dipped into the Lake water and used to collect the water samples at a randomly selected point. The water would then be transferred from the metallic cup into a 100ml capacity wide-mouthed screw-capped plastic container which would then be chilled on ice in a courier for transportation to the microbiology laboratory.

2.5.2. Bacteriological analysis of water samples:

The following methods were undertaken based on Cheesbrough, (2006);

Lab Day 1

On reception of the water samples; unique identifiers would be assigned to them for purposes of laboratory identification during specimen processing and analysis. Primary inoculation was done by the pour plate method where a portion (approximately 10mls) would be poured to cover the surface of the selective agar medium depending on the target bacterial species and/or genera. The inoculated agar plates would then be cultured through incubation at 37°C overnight (i.e. 14 to 18 hours). The selective media that was used in this study included; Mannitol salt agar (Condalab Chapman medium, Batch number: 206042) for the Staphylococcus species group; Bile esculin azide agar (Conda pronadisa, Batch number: 702142) for the Enterococcus species group; and MacConkey bile salts agar (Oxoid limited, Lot number: 2966689) for the Enterobacteriaceae group.

Lab Day 2

Following the first overnight incubation of the water samples; plate reading was the next step where the colony characteristics of the bacterial growth that was obtained on the selective media would be investigated. Bacterial colonies of varying characteristics would then be subcultured by streaking from the selective media onto basic media i.e. nutrient agar (Condalab Chapman medium). The subsequent basic agar plate preparation would then be cultured through incubation at 37°C overnight.

Lab Day 3

Following the second overnight incubation; a plate reading was done to identify the colony characteristics of the bacterial growth that had been obtained on a basic medium (nutrient agar). The bacterial colonies would then be stained through the Gram staining technique to microscopically study and classify them under the two main groups i.e. Gram positive and Gram negative groups.

Based on the Gram staining result; an appropriate biochemical test was then performed to facilitate the confirmation of particular bacterial species and/or genera in the Enterobacteriaceae group i.e. Carbohydrate sugar fermentation and hydrogen sulphide gas production: this was carried out with the aid of triple sugar iron agar medium (Oxoid limited, Lot:1155946); Citrate utilization test: this was carried out with the aid of Simmon's citrate agar medium (Becton, Lot: 3105010); Hydrogen sulphide gas production, indole production, and motility test: this was carried out with the aid of SIM medium and Kovac's reagent (Conda pronadisa, Batch: 802012).

2.6. Investigation for factors potentially associated with bacterial contamination of the Lake Victoria shore water:

The factors implicated in the potential contamination of the Lake Victoria surface shore waters were assessed with the aid of a questionnaire checklist. Random individuals residing and/or working along the Lake Victoria shore at the Kasenyi landing site were identified for the

provision of such information through a short one-on-one one-time interview session. Additionally; a one-time field observation session was also carried out to collect data on the factors implicated in the potential contamination of the Lake Victoria surface shore waters.

2.7. Data analysis:

A descriptive and inferential nature of data analysis was undertaken with the aid of Microsoft Excel 2013 computer office application. The qualitative data about the factors associated with bacterial contamination of water was summarized and compiled into tables while the quantitative data from the laboratory bacteriological analysis of water was summarized and compiled into bar graphs. Inferential data analysis was performed using the chi-square and the p-value tests to infer an association between the independent variables and the dependent variables.

3. Results:

3.1. Occurrence of pathogenic bacteria in Lake Victoria shore water:

The overall occurrence of the bacterial isolates in Lake Victoria shore water

A total of seventy-nine bacterial isolates across four categories i.e. Citrobacter freundii, Klebsiella pneumonia, Staphylococcus species, and Enterococcus species were obtained [Figure 1]. Citrobacter freundii accounted for the highest occurrence at 58% (46/79) while Klebsiella pneumoniae was the least occurring at 3% (2/79).

Occurrence of individual bacterial isolates in Lake Victoria shore water

From the thirty-one (n=31) Lakeshore water samples; 71% (22/31) samples contained Citrobacter freundii while only 6% (2/31) samples contained Klebsiella pneumoniae as shown in Figure 2.

Factors associated with pathogenic bacteria in Lake Victoria shore water

3.1.1. Factors associated with pathogenic bacteria in Lake Victoria shore water

3.1.2. Demographic characteristics of the key respondents

The survey was carried out among eleven (n=11) random key informants for whom all turned out male [Table 1]; their mean age was thirty-seven years (mean = 36.6 ± 15.7 years) whereby the youngest was twenty years (minimum = 20 years) and oldest sixty-three years (maximum = 63 years) [Table 2]. Most informants (73%; 8/11) reported to be residents along the shore [Table 3]. Additionally; most (36%; 4/11) were involved in fish trading as their occupation [Table 4].

3.1.3. Frequency distribution profiles of the factors

A total of eight different factors were assessed among the key informants, from these; eighty-eight responses were obtained for which 34% (30/88) corresponded to a "YES" while 66% (58/88) corresponded to a "NO" [Table 5]. "Soil surface run-off" emerged as the most occurring factor related to possible bacterial contamination of the shore surface waters (73%; 8/11) followed by "rubbish dumping along the shore" (55%; 6/11) while "sewage release into the lake" emerged as the least occurring factor (0%; 0/11) [Table 5].

Further, still; one-time field observation revealed the presence of "bathing practices, rubbish dumping, and soil surface runoff" as factors related to the bacterial contamination of the shore surface waters [Table 5].

3.1.4. Association profiles

Following chi-square (X^2) and p-value analysis as shown in Table 6; a statistically significant association was obtained between all the eight factors for the potential bacterial contamination of the Lake shore water and the occurrence of Klebsiella pneumoniae there-in (i.e. $X^2 > 7.815$; $p < .05$) while there was no statistically significant association between all the eight factors for the potential bacterial contamination of the Lake shore water and the occurrence of Citrobacter freundii,

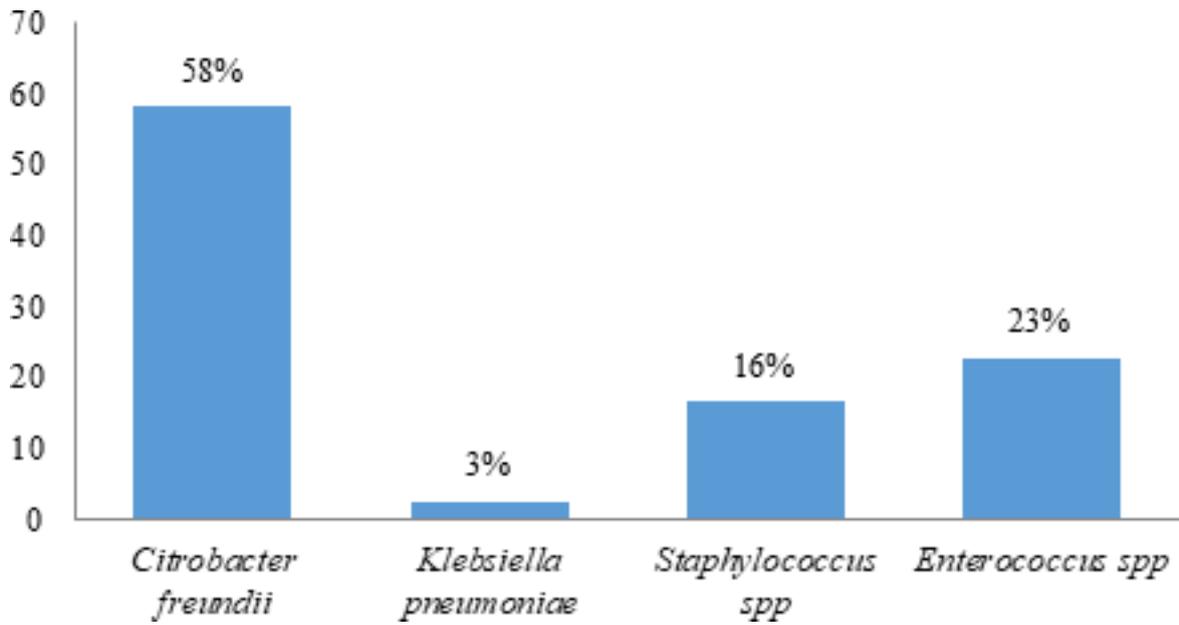


Figure 1: Overall occurrence of bacterial isolates from the Lake Victoria shore water

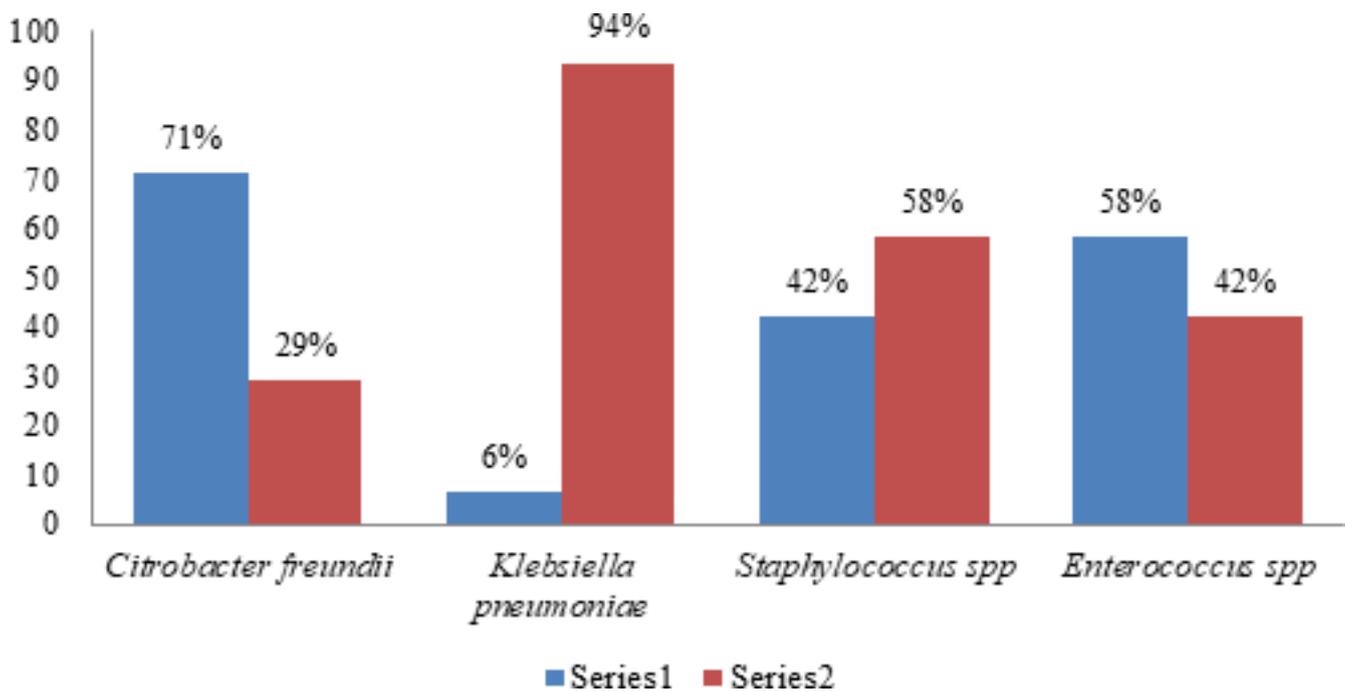


Figure 2: Water sample-based occurrence of individual bacterial isolates Key:series 1 = positive samples; series 2 = negative samples

Table 1: Gender characteristics of the key informants

GENDER NUMBER	Male	Female	Total
	11	0	11

Table 2: Age characteristics of the key informants

ID	AGE
001	50
002	43
003	20
004	24
005	21
006	44
007	48
008	63
009	49
010	21
011	20
Mean	36.6
SD	15.7
Range	43
Minimum	20
Maximum	63

Key: SD – Standard deviation; ID – participant's identification

Table 3: Shore residence profiles among the key informants

SHORE RESIDENCE		%
Yes	8	72.7
No	3	27.3
Total	11	100

Key: % - Percentage

Table 4: Occupation profiles of the key informants

OCCUPATION	FREQUENCY	%
Fisheries education officer	1	9.1
Fish trader	4	36.4
Beach attendant	1	9.1
Fish processor	1	9.1
General shore worker	2	18.2
Fish handler	2	18.2
TOTAL	11	100

Key: % - Percentage

Table 5: Potential factors for shore water bacterial contamination

#	POTENTIAL FACTOR FOR SHORE WATER BACTERIAL CONTAMINATION	FREQUENCY		OBSERVATIONS
		YES (%)	NO (%)	
1	Bathing practices along the lakeshore	5 (45.5)	6 (54.5)	Yes
2	Dishwashing along the lakeshore	4 (36.4)	7 (63.6)	No
3	Laundry practices along the lakeshore	5 (45.5)	6 (54.5)	No
4	Rubbish/dumping grounds along the lakeshore	6 (54.5)	5 (45.5)	Yes
5	Animal rearing along the lakeshore	1 (9.1)	10 (90.9)	No
6	Sewage release along the lakeshore	0 (0)	11 (100)	No
7	Soil surface runoff in the rainy season	8 (72.7)	3 (27.3)	Yes
8	Release or dumping of market effluent/waste into or along the lake	1 (9.1)	10 (90.9)	No
	TOTAL OUTCOME	30	58	
	%Total outcome	34.1	65.9	

Key: % - Percentage

Staphylococcus species, and Enterococcus species (i.e. $X^2 < 7.815$; $p > .05$).

Key: p-value = probability value; X^2 = chi-square value; CV = critical value; α = level of statistical significance; df = degrees of freedom

4. Discussion:

4.1. Occurrence of pathogenic bacteria in Lake Victoria shore water at Kasenyi landing site:

In this study; *Citrobacter freundii* was the most occurring bacterial pathogen at 71% (22/31) while *Klebsiella pneumoniae* was the least occurring at 6% (2/31).

This has laid a similarity to Banciu et al. (2021), a study in which *Citrobacter freundii* was determined as a surface water contaminant. This could be a result of soil surface run-off being that soil is a natural habitat for *Citrobacter freundii*. However; soil surface run-off was not statistically evidenced as attributed to the occurrence of *Citrobacter freundii* in the Lake surface shore water.

On the other hand; Rondón-Espinoza et al., (2022), Gultepe et al. (2017), and Bisimwa et al. (2022) never identified *Citrobacter freundii* and/or *Klebsiella pneumoniae* in their study thus rendering a contradiction here-along. The reason for this difference has been linked to the

difference in the bacteriological research scope between the former study and the current.

4.2. Factors associated with pathogenic bacteria in Lake Victoria shore water:

From this study; “Soil surface run-off” emerged as the most occurring factor related to possible bacterial contamination of the shore surface waters (72.7%; 8/11) followed by “rubbish dumping along the shore” (54.5%; 6/11) while “sewage release into the lake” emerged as the least occurring factor (0%; 0/11).

These findings are similar to those of Islam et al. (2018) who identified “increased surface runoffs” as a factor too; Bisimwa et al. (2022) who identified “wash off from rubbish dumped along the water body;” and Bedane et al. (2022) who also identified “run-off of water into the water bodies during the rainy seasons.” This could be related to the fact that landing sites are mostly not designed with physical barriers between shore waters and the bare land neighboring the shore as is the case at Kasenyi landing site.

On the other hand; the findings from this study contradict those of Islam et al. (2018) who identified “wastewater treatment close to the water body;” Bisimwa et al. (2022) who identified “market effluents runoff into the water body;” and Bedane et al. (2022) who identified “open accessibility of animals to the lake water shores, occasional

Table 6: Chi-square and p-values for the factors for Lake water contamination and the bacterial occurrence in Lake water.

#	POTENTIAL FACTOR FOR SHORE WATER BACTERIAL CONTAMINATION	Citrobacter freundii p-value (X ²) CV=7.815, α=0.05, df=3	Klebsiella pneumoniae p-value (X ²) CV=7.815, α=0.05, df=3	Staphylococcus species p-value (X ²) CV=7.815, α=0.05, df=3	Enterococcus species p-value (X ²) CV=7.815, α=0.05, df=3
1	Bathing practices along the lakeshore	.259 (4.026)	.001 (17.367)	.897 (0.596)	.897 (0.596)
2	Dish washing along the lakeshore	.257 (4.044)	.001 (17.446)	.897 (0.598)	.897 (0.598)
3	Laundry practices along the lakeshore	.259 (4.026)	.001 (17.367)	.897 (0.596)	.897 (0.596)
4	Rubbish/dumping grounds along the lakeshore	.259 (4.026)	.001 (17.367)	.897 (0.596)	.897 (0.596)
5	Animal rearing along the lakeshore	.239 (4.217)	.000 (18.193)	.891 (0.624)	.891 (0.624)
6	Sewage release along the lakeshore	.229 (4.320)	.000 (18.635)	.887 (0.639)	.887 (0.639)
7	Soil surface runoff in the rainy season	.253 (4.082)	.000 (17.607)	.896 (0.604)	.896 (0.604)
8	Release or dumping of market effluent/waste into or along the lake	.239 (4.217)	.000 (18.193)	.891 (0.624)	.891 (0.624)

open defecation by people living and/or working along the lake shores and presence of animal excreta along the lake shores.” This could be a result of the absence of any form of industry near the landing site; the presence of a public waste management site; restrictions imposed on rearing animals near the shore; and the presence of public toilets at the landing site as was revealed by the field observation.

4.3. Association profiles between the factors and the bacteria occurrence rates: From this study; a statistically significant association was obtained between all eight factors for the potential bacterial contamination of the Lakeshore water and the occurrence of Klebsiella pneumoniae while there was no statistically significant association between all the eight factors for the potential bacterial contamination of the

Lakeshore water and the occurrence of Citrobacter freundii, Staphylococcus species, and Enterococcus species.

This research has thus established that there is a low chance or probability (i.e. ≤ 1%) for the occurrence of Klebsiella pneumoniae in the Lake Victoria surface shore water at the Kasenyi landing site. At the same time; there is a high likelihood or probability (i.e. ≥ 23%) for the occurrence of Citrobacter freundii, Staphylococcus species, and Enterococcus species in the Lake Victoria surface shore water at the Kasenyi landing site.

5. Conclusion:

Citrobacter freundii was the most occurring human bacterial contaminant in the shore surface waters; soil surface run-off emerged as the most

occurring factor related to possible bacterial contamination of the shore surface waters; there is a low chance of encountering *Klebsiella pneumoniae* and a high chance of encountering *Citrobacter freundii*, *Staphylococcus* species, and *Enterococcus* species in the Lake Victoria surface shore water at Kasenyi landing site.

6. Recommendation:

The researcher recommends the establishment of control mechanisms (i.e. physical barriers) for soil run-off into the lake shore water to minimize water contamination by *Citrobacter freundii* whose primary source is soil. The researcher also recommends further restriction towards any form of industry establishment near the landing site as this will help keep the lake shore waters safer.

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8. List of Abbreviations And Acronyms:

etc. - And others
n - Sample size
% - Percentage
UN - United Nations
UNEP - United Nations Environment Protection Agency
UNICEF- United Nations Children's Fund
et al - And others
Km - Kilo meters
ml – Milliliters
°C - Degrees Celsius
p-value- Probability value
ID - Identification number
SD - Standard Deviation
X² - Chi-square
CV - Critical Value

α - Level of statistical significance
df - Degrees of freedom
 \leq - Less than or equal to
 \geq - Greater than or equal to
i.e. - Such as
UgSh. - Ugandan Shillings

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10. Conflicts Of Interest:

There are no conflicts of interest associated with this work.

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