1	Original Research Article
2	Analysis of Fecal Coliform Levels at Watering Points along the Upper Reaches of River Isiukhu in Kakamega
3	County, Kenya
4	

### 6 Abstract

7 Diarrheal diseases often attributable to poor sanitary conditions and fecal contamination of drinking water remain a 8 leading cause of mortality for children younger than five years. Water contaminated with human faeces, for example 9 from municipal sewage, septic tanks and latrines, is of particular concern. Animal faeces also contain 10 microorganisms that can cause diarrhea. Kakamega County in Kenya has a diarrhea prevalence rate of 20.2%, which 11 is the highest in the country; a good proportion of these cases are believed to be water borne. This study was 12 designed to determine fecal coliform levels in water samples collected from watering points along the upper reaches 13 of River Isiukhu in Kakamega County, Kenya. Fifty-four samples were collected between August and October 2015 14 from nine sampling points, comprising springs and watering points along the river. Water samples were filtered on 15 nitrocellulose filters by vacuum filtration; fecal coliform counts were conducted using membrane filters cultured on 16 mFC agar to establish contamination levels. The results indicated that counts ranged from 200cfu/100ml -17 1450cfu/100ml in river sampling points and ranged from 0cfu/100ml - 44cfu/100ml in springs sampling points. The 18 fecal coliform counts for River Isiukhu and most springs surrounding it exceeded the WHO recommended drinking 19 water coliform(or *E.coli*) count value of 0cfu/100ml indicating that water from the upper reaches of River Isiukhu 20 and springs is not fit for drinking before treatment, especially during the wet seasons, based on WHO drinking water 21 standards.

22 Keywords: Fecal-coliform, Contamination, River-water, Spring-water, Season

23

# 24 Introduction

25 Water has been classified as a natural resource and is important in sustaining life. Ashbolt et al., (2001) reported that 26 the accessibility and availability of clean drinking water not only plays a vital role in economic development and 27 social welfare, but is also an important component in health, food production and poverty alleviation. Despite its 28 significance, WHO (2006) revealed that safe potable water is not accessible by about 1.1 billion people in the world, 29 and the hourly toll from biological contamination of drinking water is 400 deaths of children below the age of five. 30 In most developing countries, Kenya being one of them, the demand for clean drinking water supply is growing 31 rapidly (Gelover et al., 2006). In addition, a small percentage of people in these countries access piped water. 32 Therefore, those who do not have access to safe drinking water, as well as those who have access but cannot afford 33 it; rely on other sources of water of questionable quality (Gadgil, 1998; Odonkor & Ampofo, 2013). It is often 34 assumed that spring water emerges from the ground clean and free of contaminants, especially in rural areas where 35 industrial contamination is not present (Wampler et al., 2010). Many rural Kenyans know that drinking untreated 36 water from surface streams and rivers is not safe but, they often assume that water emerging from the ground at a 37 spring is clean and safe to drink.

39 Although not absolute, several pathogenic microorganisms have been recommended as indices of fecal pollution and 40 act as indicators of microbiological quality of domestic water (WHO, 2003; Kabler and Clark, 1960; Abera et al., 41 2011).

- 42 Besides anthropogenic activities, natural phenomena are also believed to be contributing to the reduction of water 43 quality worldwide. For example, increasingly unpredictable seasonal discharge of storm-waters into lotic 44 ecosystems due to climate variability synergistically compounds river discharges and the water quality (Onyando et 45 al., 2013). Exhaustion of the natural soil nutrients has forced farmers to use organic and inorganic fertilizers in agro-46 ecosystems leading to high influx of run-off waters, rich in nutrients in rivers and streams. This has, in many 47 occasions resulted into eutrophication which has both health and ecological repercussions in aquatic ecosystems. 48 According to WHO (2006), drinking water should contain zero fecal coliform and coliform organisms per 100 ml. 49 However, human activities, particularly urbanization, and waste disposal and agricultural practices have greatly 50 increased inputs of microbial and other pollutants to terrestrial and aquatic habitats (Smol, 2009). Therefore, the
- present study sought to establish the level of fecal coliform in sampling points in the upper reaches of River Isiukhu. 52 The study was carried out in Kakamega County (Latitude: 0° 16' 60.00" N Longitude: 34° 45' 0.00" E). The average annual rainfall of Kakamega County is 1800mm per annum and is bimodally distributed with peaks in April-May 53 54 and August-September. The driest months are from December-February. Temperatures range from a minimum of 55 10.3°C to a maximum of 30.8°C with an average of 20.5°C. The area is covered by Kakamega phonolites and also 56 tertiary volcanic rocks-olivene basalts and nepheline. Kakamega County has a population of 1.66 million people and 57 an area of 3,033.8km<sup>2</sup>. The Isiukhu River originates from Nandi hills and flows through Kakamega forest, a tropical 58 rain forest. River Isiukhu drains land use, land cover of forest, sugarcane plantation, mixed agriculture and periurban
- 59 from upstream to downstream before joining River Nzoia which empties into Lake Victoria. The river flows in an 60 east-west direction passing about 2 km from Kakamega town in the south side. Anthropogenic activities are done 61 along the river such as agriculture, livestock watering, laundry and bathing and sewage disposal.
- 62

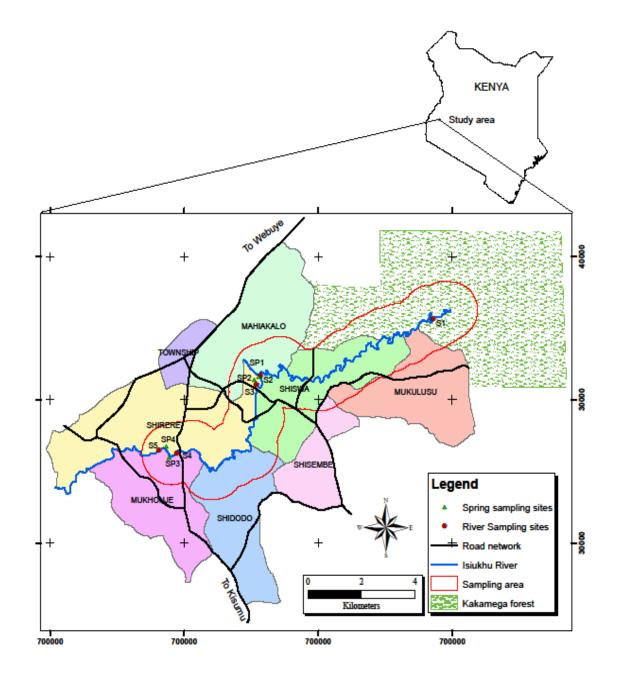
51

#### 63 **Materials and Methods**

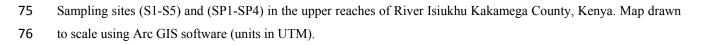
#### 64 Sample collection

65 The sampling sites were River Isiukhu and springs along it. The sites were randomly chosen and they represented 66 the upper reaches of the river. In the study, samples were collected in three successive months (August, September 67 and October). Approximately 250ml of water samples were aseptically collected in duplicates from watering points 68 using sterile containers and immediately stored in an ice box before being transported to the laboratory for analysis. 69 Water samples were collected from a depth of about 10-20 cm below the water surface at each site to avoid potential 70 contamination from surface water.

71



74 Figure 1



#### 80 Research Design

The present study adopted randomized sampling design, where Fifty four duplicate samples were collected between the month of August and October 2015 from nine sampling points along the river and springs that represented the upper reaches of River Isiukhu. The forest area (S1) provides reference data, land cover of forest, human activities likedeforestation. Confluence of Lianila stream and River Isiukhu (S2) assess bacterial load of Lianila stream that drains the treated sewage effluent and farm lands. Activities such as fishing, bathing,

86 livestock watering, and laundry are also done. Savona resort area (S3) quantifies the bacterial contribution from
87 the recreation area

- Amalemba area(S4) is highly populated; determines the bacterial contribution of the small scale mixed agriculture and periurban area activities eg waste disposal of the households, municipal wastes,hotel and dispensaries wastes etc bathing, livestock watering, laundry are done. The drainage of this region is skewed. Waste water entry point (S5) establish the bacterial load from treated sewer waters from municipal sewage treatment plant in the area and the slaughter house drains. All springs (SP1- SP4) are located along River Isiukhu and the study is to establish their bacterial contamination and any variation in season's i.e during dry and wet season. Contamination due to pollution likely from sewage as well as solid waste discharge during run-
- 95 offs.

# 96 Determination of fecal coliform levels at watering points and springs along the upper reaches of River97 Isiukhu.

98

99 Fecal coliform levels in water samples were determined by the membrane filter procedure using mFC agar (Dufour 100 et al., 1981). The samples collected from the river were diluted to 1:100. The biomass from both spring and the 101 diluted river-water samples (100ml) was concentrated via subsequent filtration on nitrocellulose filters, 47mm 102 diameter with pore size of 0.45µm by vacuum filtration. The vacuum pressure for filtration was between 50mm to 103 70mm Hg in order to avoid rupture of bacterial cells that has been observed at pressures above 80 mm Hg (Kepner 104 and Pratt 1994). Following the method recommended by the American Public Health Association in 2006 (Wehr et al., 2004; Britton and Greeson 1987) filters were then aseptically placed with grid side up onto the surface of the 105 106 plates of mFC. All plates were incubated inverted in watertight plastic bags submerged in a 44.5°C water bath for 107 22-24 hours. Fecal coliform colonies that were observed in any shade of blue color were counted using a Quebec 108 colony counter and recorded as colony-forming units per 100ml. The formula used was:

109

$$CFU/100mL = \frac{Colonies \ counted}{mL \ filtered} X \ 100$$

110

111

112 Data analysis

- 113 Data obtained on coliform counts was subjected to one-way ANOVA followed by Turkey's post hoc test at 95%
- 114 confidence level using Winks software version 7.
- 115 Results
- 116 Table 1 shows the mean counts of fecal coliforms from five different sampling points in River Isiukhu for three
- successive months. There was a significantly high (p < 0.05) fecal coliform concentration between sampling point, S5 and all other sampling points in the river in the month of August. Sampling point, S2 also recorded significantly
- higher (p<0.05) coliform counts when compared to S1 in the month of October. In the month of September, S2 and
- 120 S5 had visibly higher coliform counts per 100ml, though they were not significantly different from the rest of the
- samples. Generally, fecal coliform counts were lowest at S1 and highest at S5in all the three month of the study.
- 122

### 123 Table 1: Means of Fecal coliform count given in colony forming units /100mls for five sampling points from

124 River Isiukhu for three consecutive months

Sample code	Environment	Aug.	Sept.	Oct.
S1	Forest (site 1)	200±0.00 <sup>a</sup>	600±141.42 <sup>a</sup>	600±424.26 <sup>a</sup>
S2	Confluence of Lianila stream and	$300 \pm 141.42^{ab}$	$1050{\pm}1343.50^{a}$	1700±424.26 <sup>b</sup>
	River Isiukhu			
<b>S</b> 3	Savona resort area	550±494.97 <sup>ab</sup>	750±636.40 <sup>a</sup>	950±353.55 <sup>ab</sup>
<b>S4</b>	Amalemba area	450±353.55 <sup>ab</sup>	900±848.53 <sup>a</sup>	$1050 \pm 636.40^{ab}$
S5	Waste water entry point	1450±212.13 <sup>c</sup>	1050±353.55 <sup>a</sup>	950±212.13 <sup>ab</sup>

## \*Means followed by the same letter within the same column are not significantly different at P < 0.05

126

# 127 Fecal coliform count for four sampling points from springs along River Isiukhu

Table 2 shows the mean counts for fecal coliform from four different springs for three consecutive months. Generally, the month of October recorded higher coliform count compared to September and August. The month of August recorded few counts across all the springs, in the three months studied. However, it is important to note that there was no significant difference in the counts for all the months studied. In the month of August, spring SP1 recorded no coliform counts, however, increasing counts were recorded in the month of september and October.

# Table 2: Means of Fecal coliform count given in colony forming units /100mlfor four sampling points from spring for six sessions

136

Sample code	Environment	Aug.	Sept.	Oct.
SP1	Confluence of Lianila stream and	$0\pm 0.00^{a}$	5±7.07 <sup>a</sup>	6.5±4.95 <sup>a</sup>
	River Isiukhu			
SP2	Savona resort area	3±1.41 <sup>a</sup>	3±2.83 <sup>a</sup>	44±46.67 <sup>a</sup>
SP3	Amalemba area	2.5±3.54 <sup>a</sup>	6.5±4.95 <sup>a</sup>	6.5±4.95 <sup>a</sup>

<b>SP4</b> Waste water entry point $5.5 \pm 4.95^{a}$ $6.5 \pm 2.12^{a}$ $36.5 \pm 43.13^{a}$
---

\*Means followed by the same letter within the same column are not significantly different at P < 0.05

139 Discussion

140 Generally, coliforms are the most common group of indicator organisms used in water quality monitoring (Sibanda 141 et al., 2013). Furthermore, Alotaibi (2009) elaborated that the presence of fecal coliform indicates the presence of 142 potential fecal contamination and the presence of possible pathogenic microorganisms and to determine the health 143 risk to the consumers. In the present study, significant variation of fecal coliform counts in River Isiukhu was 144 reported during the three months of the study. This difference is attributed to rainfall variation within the three 145 months. Lower rainfalls are normally experienced in the month of August than in the month of October. Similar 146 observations have been made elsewhere, for instance, Wolf (1999) reported that in the dry season, there were fewer 147 incidences of fecal pollution in the water supply system but high fecal contamination of drinking water increases 148 during wet season.

149

Results in the present study further indicated significant differences in means for fecal coliforms in samples taken from the different sampling points in River Isiukhu. This finding agrees with a previous study carried out on River Danube which showed lower bacterial pollution on upstream of the River and higher levels of fecal pollution in the middle part and downstream of the River (Kavka *et al.*, 2002). However, the finding in this study disagrees with that of Uwimpuhwe (2014) where total and fecal coliform from different sampling points in River Nyabarongo showed insignificant differences. This difference could be attributed to the fact that the current study of River Isiukhu included survey of sampling points with different anthropogenic activities taking place at every point.

157

158 According to the WHO guideline values for bacteriological parameters, the total and fecal coliform bacteria should 159 be 0 cfu/100ml in water intended for drinking. However, in this study the fecal coliform counts for River Isiukhu, 160 exceeded the WHO recommended drinking water guideline value. These results are supported by previous studies 161 conducted in rural areas (Abera et al., 2011; Chigor et al., 2011). The studies found that microbiological parameters 162 counts for river water in rural areas were above the permissible limits and were a potential hazard to public health 163 (Chigor, 2011). Even though, WHO recognizes that these targets would be difficult to achieve in some cases, 164 especially in rural communities with untreated water provisions, and recommends that in these settings, the 165 guidelines values would be seen as goals for the future, but not an immediate prerequisite (WHO/UNICEF, 2008)

166

167 Although findings from the study reported insignificant difference in fecal coliform counts from different springs 168 during the three months studied, the water was unsafe to drink based on the World Health Organization drinking 169 water standards (WHO, 2015). Both protected and unprotected springs had bacterial counts in excess of the WHO

- 170 standard, suggesting that water treatment from all sources is necessary to ensure their cleanliness and safety. The
- spring water contamination could be due to what Narain Rai and Sharma (1995) termed as lack of sanitation or

improper waste disposal. The researchers further explained that 40% or more of the disease out breaks were attributed to consumption of polluted ground water. Furthermore, presence of coliforms in drinking water sources indicates need for treatment and proper sanitation which is necessary for drinking (Christine *et al.*, 2006). Further the destruction of microbial ecosystems through deforestation, high spring water temperatures, averaging 24.4°C,

176 may be contributing to the observed bacterial abundance.

177

Findings from the study indicate that springs have more fecal coliform contaminants during wet season. This finding 178 179 is not different from that of Ofoma et al., 2005) where spring water was highly contaminated with fecal coliform 180 during wet seasons compared to dry seasons. They suggested that the contamination was due to pollution; most likely from sewage as well as solid waste discharge during run-offs. This is non-point sources typically wet-weather 181 182 where they diffuse in nature, in that they do not enter water bodies from any single point (e.g. urban litter, 183 contaminated refuse, domestic pet/wildlife excrement and failing sewer lines). This suggests that emphasis on points 184 of use (POU) treatment methods, decontamination of protected and unprotected springs, and behavioral 185 interventions to improve sanitation practices are needed.

186

## 187 Conclusion

188 Results obtained from the study indicated significant contamination of River and spring water at different sites. The 189 levels of fecal coliforms were higher than the accepted levels. In addition, higher fecal coliform levels were recorded 190 during wet than dry seasons, concluding that water from the upper reaches of River Isiukhu and spring is not fit for 191 drinking before treatment especially during wet seasons based on the WHO drinking water standards and the water 192 quality. It is therefore recommended that water from both the stream and springs in the upper reaches of River 193 Isiukhu should be treated before use.

195

#### 196 **References**

197

- Abera S, Zeyinudin A, Kebede B, Deribew A, Ali S, Zemene E (2011). Bacteriological analysis of drinking water
   sources. Afr. J. Microbiol. Res. 5(18): 2638-2641.
- Alotaibi EL (2009). Bacteriological assessment of urban water sources in KhamisMushait Governorate,
   southwestern Saudi Arabia. Int J Health Geogr. 8(1): 1.
- Ashbolt NJ, Grabow WO, Snozzi M (2001). Indicators of microbial water quality. IWA Publishing. 289-316.
- Britton LJ, Greeson PE (1987). Methods for collection and analysis of aquatic biological and microbiological
   samples. U.S. Geological Survey Techniques of Water Resources Investigations. 5 (A4): 37-40.

- Chigor VN, Umoh VJ, Okuofu CA, Ameh JB, Igbinosa EO, Okoh AI (2012). Water quality assessment. surface
   water sources used for drinking and irrigation in Zaria, Nigeria are a public health hazard. Environ. Monit.
   Assess. 184(5). 3389-3400.
- Christine L, Moe, Richard D, Rheingans (2006). Global challenges in water, sanitation and health. Journal of Water
   and Health. 04.IWA Publishing 2006.
- Gadgil A (1998). Drinking water in developing countries. Annual Review of Energy and the Environment
   Resources. 23(1): 253-286.
- Gelover S, Gomez LA, Reyes K, Leal MT (2006). A practical demonstration of water disinfection using TiO 2 films
   and sunlight. Water Res. 40(17): 3274-3280.
- Kabler PW, Clark HF (1960). Coliform group and fecal coliform organisms as indicators of pollution in drinking
  water. J Am Water Works Assoc. 52(12): 1577-1579.
- Kavka GG, Kasimir GD, Farnleitner AH (2006). Microbiological water quality of the River Danube (km 2581-km
   15). Longitudinal variation of pollution as determined by standard parameters. Eigenverlag ICPDR: 415 –
   421.
- Kepner RL, Pratt JR (1994). Use of fluorochromes for direct enumeration of total bacteria in environmental samples.
  past and present. Microbiol Rev. 58(4): 603-615.
- Narain Rai, J.P. and Sharma, H.C.(1995) Bacterial Contamination of Ground Water in Rural Areas of North
   Uttarpradesh. Indian Journal Environmental Health. 37(1):37-41.
- Ofoma AE, Omologbe DA, Aigberua P (2005). Physico-chemical quality of groundwater in parts of Port Harcourt
   City, eastern Niger Delta, Nigeria. Water Resour. Res. 16: 18-24.
- 225 Sibanda T, Chigor VN, Okoh AI. Environ Monit Assess (2013) 185: 6579. <u>https://doi.org/10.1007/s10661-012-</u>
   226 <u>3048-4</u>
- 227 Smol JP (2009). Pollution of lakes and rivers: a paleoenvironmental perspective. John Wiley & Sons.
- Uwimpuhwe M (2014). Microbiological assessment of water quality and prevalence of waterborne diseases in rural
   areas of Masaka, Rwanda (Doctoral dissertation).
- Wampler PJ, Sisson AJ(2011). Spring flow, bacterial contamination, and water resources in rural Haiti.
   Environmental Earth Sciences. 62(8): 1619–1628.http://doi.org/10.1007/s12665-010-0645-9
- Wehr HM, Frank JF, American Public Health Association (Eds.) (2004). Standard methods for the examination of
   dairy products (pp. 327-404). Washington, DC: American Public Health Association.
- WHO (2006). Microbial aspects. In Guidelines for drinking water quality. World Health Organisation 3<sup>rd</sup> ed. Vol. 1,
   Geneva:

- WHO/UNICEF Joint Water Supply, Sanitation Monitoring Programme, World Health Organization, UNICEF
   (2008). Progress on drinking water and sanitation: Special focus on sanitation. World Health Organization.
- 238 Wolf AT (1999). The transboundary freshwater dispute database project. WATER INT. 24(2): 160-163.
- World Health Organization (2003). Water, sanitation and health. Geneva, World Health Organization. World Health
   Organization. (2006). Guidelines for drinking-water quality: First addendum to volume 1, Recommendations
- 241 (Vol. 1). World Health Organization.
- 242
- 243 Dufour, A. P., Strickland, E. R., & Cabelli, V. J. (1981). Membrane filter method for enumerating Escherichia
  244 coli. *Applied and environmental microbiology*, 41(5), 1152-1158.
- World Health Organization. (2015). *Progress on sanitation and drinking water: 2015 update and MDG assessment.*World Health Organization.
- Onyando, Z. A., Shivoga, W. A., Lung'ayia, H., Ochieno, D. W., Agevi, H., & Kigen, C. (2013). The influence of
  land use on nutrient regime in a tropical stream. *Elixir Pollut*, 2013(64), 19290-19294.
- Odonkor, S. T., & Ampofo, J. K. (2013). Escherichia coli as an indicator of bacteriological quality of water: an
   overview. *Microbiology research*, 4(1), 2.