Research Paper

Microbiological contamination of water pans in Baringo County

Edith J. Kurui, George M. Ogendi and Wilkister N. Moturi

ABSTRACT

Water pans constitute the main source of rural water supply in Baringo County. This study sought to assess the spatial-temporal variation of total coliforms, *Escherichia coli*, *Fecal streptococcus* and *Salmonella species* in the water pans. A sanitary survey was conducted to observe the potential sources of microbial contamination on the water pans. Water was sampled from one protected and five unprotected water pans ($n = 6$) in the study area for a period of 4 months (June–October 2015). A total of 72 water samples were sampled in triplicate from the water pans for microbial analyses, membrane filtration technique was used in assaying for microbial counts of total coliforms, *E. coli*, *F. streptococcus* and *Salmonella* species in water samples. The results show that there was a significant spatial variation in *F. streptococcus* amongst the protected and the unprotected water pan sampled sites ($p = 0.008$), and there was a statistically temporal significant difference ($p = 0.001$) for total coliforms and *Salmonella* species during the dry seasons, respectively. Given the prevalence of the selected diseases causing pathogens in water above the WHO drinking water quality guidelines, households are advised to treat the water before use.

Key words | Baringo, fecal contamination, microbiological water quality, water pans

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INTRODUCTION

Access to quality water is essential to health, a basic human right and a component of effective policy for health. Safe drinking water is essential to sustain life and a satisfactory (adequate, safe and accessible) supply must be available to all. Safe drinking water, as defined by World Health Organization guidelines, does not represent any significant risk to health over time of consumption (WHO/UNICEF 2008; WHO 2011). Water is a basic requirement in life.

The majority of populations in developing countries are not supplied with potable water and are forced to use water from surface sources such as water pans, boreholes and rivers that make water unsafe for domestic and drinking purposes due to high possibilities of contamination. In Kenya, 80% of the residents live in arid and semi-arid lands (ASALs) and draw their water from unprotected sources such as water pans, boreholes, rivers and lakes. Risks of water related diseases are a major public health concern in Kenya's rural areas. The provision of safe drinking water and sanitation are some of the major challenges the livelihoods in the ASALs face and have been recognized as some of the major developmental challenges the country is facing towards the realization of the vision 2030 (Vision 2007) and in meeting the United Nations Sustainable Development Goals 3 and 6 respectively (WHO 2016).

Baringo County is situated in the water scarce ASALs of Kenya. Central and South Baringo are located mainly in agro ecological zones IV and V. It is made up of Mogotio, Eldama Ravine and Marigat sub-counties of Baringo County. These lands experience erratic rainfall with an annual average rainfall ranging from 150 to 450 mm in

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Marigat sub-county and 500–800 mm in Eldama/Ravine and Mogotio sub-counties, respectively (UNDP 2013).

The National Drought Management Authority (NDMA) January 2014 bulletin on drought monitoring reported that water sources currently in use in Baringo County include water pans, dams, natural rivers, traditional river wells, springs, boreholes and lakes. Wetangula *et al.* (2010) reported that surface water sources such as dams and water pans have been developed in Baringo County to provide water for domestic use and livestock watering. A report on water and sanitation in Kenyan counties revealed that 29.3, 3.4 and 2.0% of the human population in Mogotio, Eldama ravine and Marigat sub-counties respectively depend on ponds and dams for their domestic water uses (KNBS & SID 2013). However, these water sources are categorized as unimproved (WHO 2008). Lack of distinct watering points for human beings fetching water for domestic use and livestock watering presents a risk of microbial contamination. Lack of water pan protection increases the rate of water pan contamination by both human and animal fecal matter.

Fecal contaminated water harbors pathogenic organisms in water that are agents of disease transmission to human beings. The main bacterial microorganism of concern in contaminated water include total coliforms, *Escherichia coli, Vibrio cholera* and *Shigella* species (Gwimbi 2011). The presence of fecal coliforms or *E. coli* has been used as an indicator for the presence of any of the waterborne pathogens. The World Health Organization (WHO) recommends that no fecal coliform should be present in 100 mL of drinking water (WHO 2016). Identification of pathogenic organisms in water is extremely difficult, unreliable and not routinely performed as a laboratory procedure. The presence of the indicator organisms, which may be associated with pathogenic organisms, is usually determined phenotypically.

METHODS

Study design

Case control study design was used in this study. One water pan was protected and was referred to as the control water pan and the unprotected water pans were referred to as cases. It was also a cross-sectional study design since the study was carried out in one point in time (Blumenthal *et al.* 2001). It was an observational study design in the sense that there was no manipulation of the sanitary surveys conducted along the water pans during the entire study period. The sampling sites were purposefully selected as protected and unprotected water pans (Figure 1). The sampling sites were protected (Cheraik) and unprotected (Kinyach, Kaptipsegem, Chepnyorgin, Kapchelukuny and Kures) water pans. The protected water pan (Cheraik) was fully enclosed with a chain link to prevent both humans and livestock from stepping into the water during collection and watering respectively. The water pan was also provided with an animal water trough for animals to drink from and a piped connection for the community members to access their water. The Cheraik water pan was used as the reference site owing to its protection status. The unprotected water pans had no barriers and were therefore susceptible to microbial contamination.

Sampling and analysis of microbiological parameters in water pan samples

Water samples were obtained in triplicate from the protected (Cheraik) and unprotected (Kinyach, Kaptipsegem, Chepnyorgin, Kapchelukuny and Kures) water pans. For all the water pans, sampling was performed twice during the wet season (June–July) and dry season (September–October), respectively. The sampling sites were located using a GPS. Sterilized 250 mL polyethylene bottles were used to collect water samples from 30 cm below the water pans. The sampling bottles were aseptically filled up.

Water temperature and percentage saturation of dissolved oxygen were measured *in situ* using a WTWO microprocessor pH/temperature meter. The meter was calibrated with pH 4 and 7 using standard buffer solutions according to manufacturer's instructions (WTW, Vienna, Austria). The electrode was rinsed with distilled water between samples. Electrical conductivity was measured using a WTWO microprocessor conductivity meter calibrated at 25 °C. All water samples were stored in a cool box with ice and transported to Egerton University, Department of Biological Sciences laboratory, for analysis.

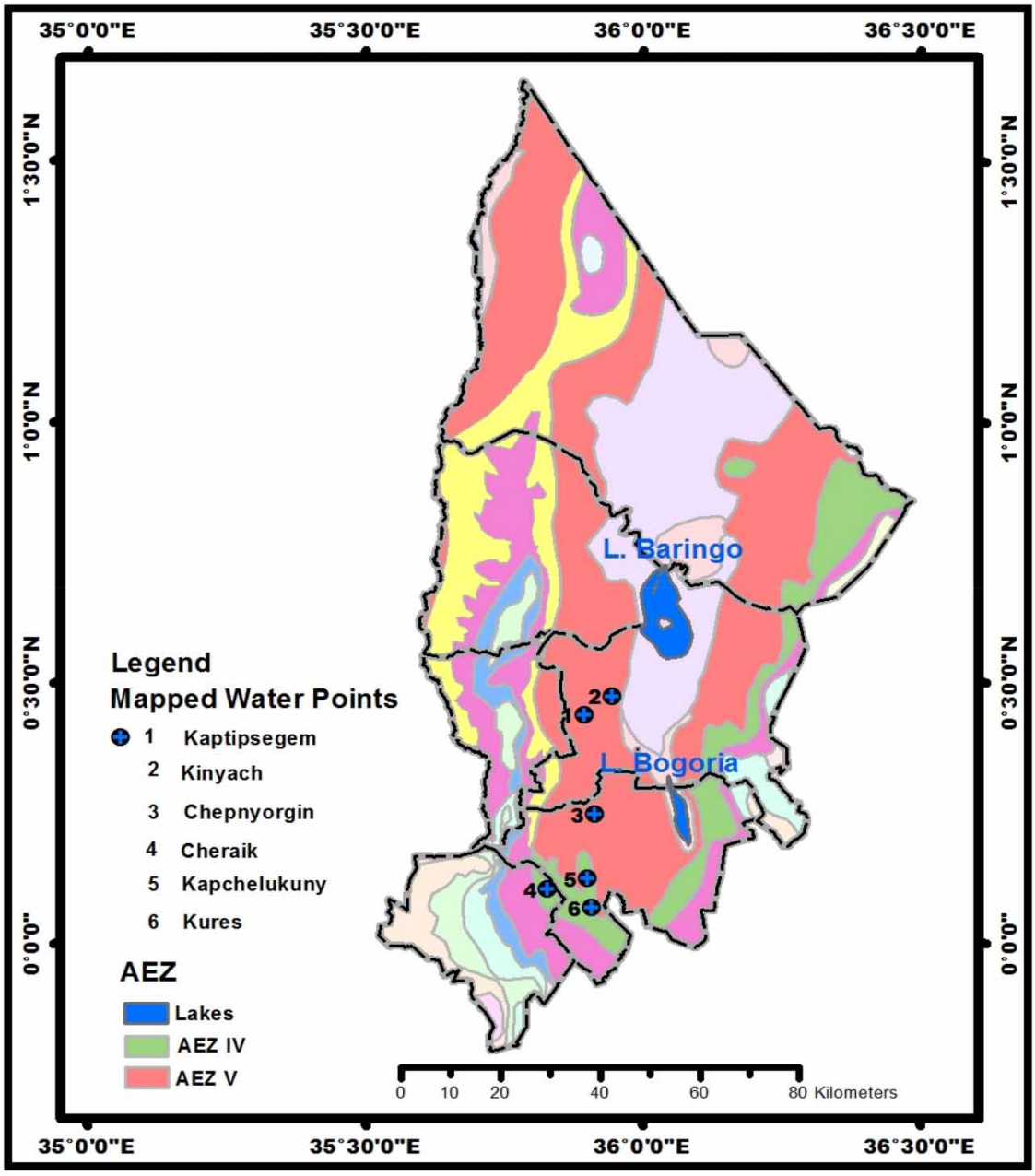

Figure 1 | Map showing the sampling sites.

Bacteriological samples analysis

Analysis of water samples for various types of microbiological indicators of fecal pollution followed guidelines outlined in APHA (2005). This was performed within 6–24 hours after sampling to avoid changes of the bacteria count due to growth or die off. Aseptic techniques were observed in all the water sample analyses. A Membrane Filtration Technique (MFT) was used to assay for the presence of total coliforms, *E. coli*, *F. streptococcus* and *Salmonella* species. The nutrient and selective media was prepared in advance for each procedure as per the manufacturer's instructions. Serial dilutions of samples were made as appropriate for each test.

Membrane filtration technique

During the microbiological analyses period, quality control of the media, apparatus and the target organisms were monitored daily. The media was tested for positive and negative controls according to the manufacturers and approved standards (APHA 2005). Positive control for total coliforms and *E. coli* was the growth of *E. coli* in chromocult media after 24 h. Negative control was the growth of *K. pneumonia* and *S. aureus*. *F. streptococcus* was monitored by using the growth of *Enterococci faecalis* with pink colonies, while the growth of *E. coli* indicated a negative control.

Water samples were diluted serially using 1 mL pipettes and 9 mL sterile physiological saline. Aliquots of 0.1 mL of water sample and water at 10 and 100× dilutions were aseptically filtered separately for each dilution by passing the sample through a membrane filter (47 mm diameter, 0.45 μm pore size) on a filtration unit. The filter was taken off using a pair of forceps and placed on the surface of the corresponding culture media. For total coliforms and *E. coli* counts, filters were placed onto chromocult agar plates and incubated at 37 °C for 18–24 hours. Typical colonies appearing pink and dark blue were counted as total coliforms, *E. coli* were the blue colonies (Figure 2(b)). Numbers of cells were expressed as colony forming units per 100 ml (APHA 2005). For *F. streptococcus* counts, filters were placed onto M-enterococci agar plates and incubated at 35 °C for 24–48 h. Typical colonies appearing pink as in Figure 2(c) were counted as *F. streptococcus* and numbers expressed as CFUs/100 ml (APHA 2005). For *Salmonella* counts, filters were placed onto *Salmonella-Shigella* agar plates and incubated at 35 °C for 24–48 h. Black colonies were identified as *Salmonella* species (Figure 2(d)). Please refer to the online version of this paper to see Figure 2 in colour: http://dx.doi.org/10.2166/washdev.2017.258.

Total bacterial count

The colonies which gave 30–300 colonies per plate were used. The total bacterial count in every 100 ml was calculated using the formula:

$$\frac{\text{Total colony count}}{\text{Volume filtered}} \times 100 \text{ mL}$$

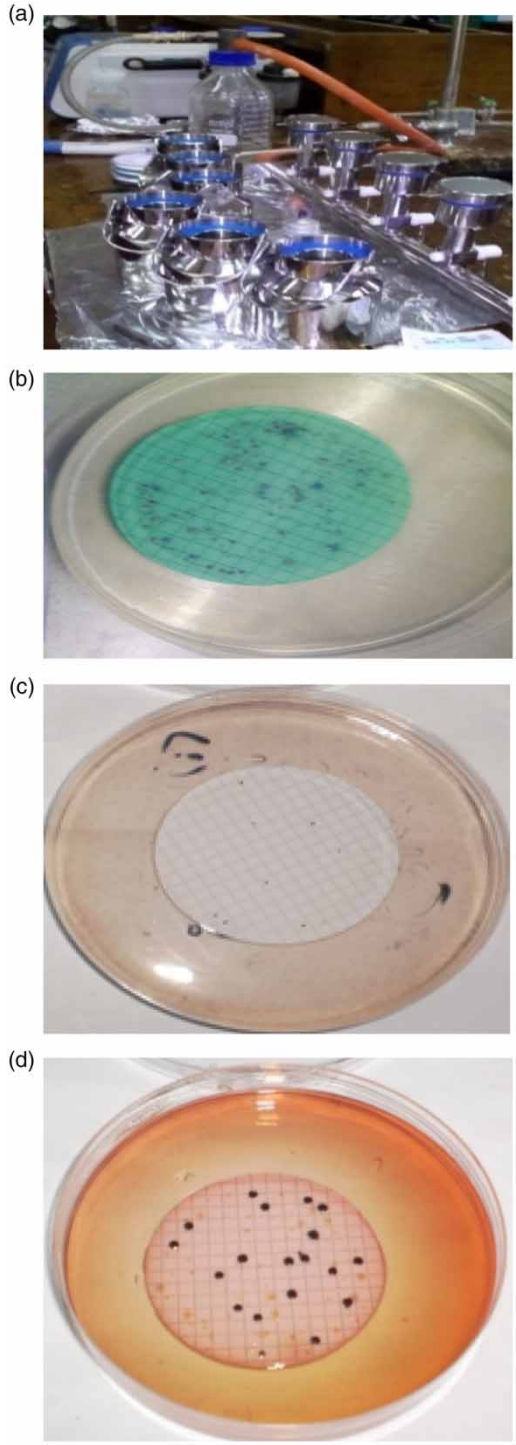

Figure 2 | Plates of MFT showing CFUs. (a) Membrane filtration technique, (b) total coliform and *E. coli*, (c) *F. streptococcus* and (d) *Salmonella* species.

Data analysis

Normality and homogeneity of variance of the data were tested using the Shapiro Wilk test and Levene's test, respectively. The results revealed a normally distributed data ($P > 0.05$) for some of the parameters tested. For the few that did not meet assumptions of normality and homogeneity of variance, the data were $\log_{10}$ transformed. The data were managed using Statistical Package for Social Science (SPSS) statistical software version 20 and Minitab @17. In all analyses, α was pegged at 5 and 95% level of significance. One way analysis of variance (ANOVA) and Kruskal Wallis were used to determine the difference between the spatial and temporal variations using the pooled counts of the total coliforms, *E. coli, Salmonella* species, and *F. streptococcus* species from all sampling sites of the protected and unprotected water pans ($P < 0.05$). Multiple comparison of the protected and unprotected water pans were further performed using a least significant difference (LSD) test.

RESULTS AND DISCUSSION

Sanitary survey on water pans in Baringo County

A sanitary survey was used to assess the sources of microbial contamination of water pans in the study area, as shown in Table 1. The sanitary survey identified point and non-point sources of fecal contaminants to surface water that causes human health impairments (Jung *et al.* 2014). The survey therefore was able to point out some of the sources that may cause microbiological contamination among the sampled water pans. Land uses such as farming produced pesticides, fertilisers, animal manure and livestock access to water pan banks, this may cause a foul smell in the water and accelerate erosion. Storm water running into the water pan may be contaminated with car oil, dust, soil and animal feces containing toxicants and chemicals. These pollutants may harbour the presence of fecal indicator bacteria such as total coliforms, *E. coli*, *Salmonella* species and *F. streptococcus* responsible for causing water related diseases. The protected water pan was observed to contain less of the microbial contaminants on its banks as compared to the unprotected water pans. The survey revealed fewer sources of point source pollution on the protected water pan.

Physical and chemical parameters of water pans in Baringo County

The results on mean values for physical and chemical parameters from the sampled protected and unprotected water pans during the study period as compared to the acceptable NEMA and WHO standards are shown in Table 2. The unprotected water pans were associated with high pollutant load, this was as a result of free access by livestock and humans to the water pans which increases the concentration of dissolved ions and lowers the level of dissolved oxygen in water.

Table 1 | The activities observed around the six water pans during the sanitary survey

	Activities observed around the water pans				
Water pans	**Riparian vegetation cover**	**Agricultural waste**	**Animal and human waste**	**Water source protection**	**Distinct water points for humans and animal**
Cheraik (protected)	Sparse	Yes	None	Yes	Yes
Kapchelukuny (unprotected)	Sparse	Yes	Yes	None	None
Kures (unprotected)	Sparse	Yes	Yes	None	None
Chepnyorgin (unprotected)	Dense	Yes	Yes	None	None
Kaptipsegem (unprotected)	Sparse	Yes	Yes	None	None
Kinyach (unprotected)	Sparse	Yes	Yes	None	None

Table 2 | Physical-chemical parameters of protected and unprotected water pans in comparison to the NEMA and WHO guidelines

	Results mean in seasons					
Physical chemical parameters	**Protected water pan (wet)**	**Protected water pan (dry)**	**Unprotected water pan (wet)**	**Unprotected water pan (dry)**	**NEMA**	**WHO**
pH	7.50	5.11	7.47	5.16	6.5–8.5	6.5–8.5
Temp (°C)	22.10	28.07	22.10	27.27		
DO (mg/L)	53.30	5.68	52.80	5.33	8	
Cond (μs/cm)	76.27	110.43	122.54	165.42	1,500	

Temperatures were within the range recommended for supporting aquatic life forms in both the protected and the unprotected water pans. A similar study in Owena Dam, Nigeria recorded a slightly higher mean temperature value of 28.41 °C (Irenosen *et al.* 2012). According to Ndubi *et al.* (2015), water pans in Narok South sub-county recorded lower temperatures during the rainy season (12.367 °C) and dry season (22.913 °C). The temperatures were adequate in enhancing maximum growth rate in fish contained in the water pans, through increased disease resistance and tolerance to toxins. pH mean values obtained in this study were within the recommended limits of WHO and NEMA during the wet season. This could be associated with the dilution factor as a result of rainfall event, increasing the pH. During the dry season the pH was far below the recommended guideline value. This could be associated with partial decomposition of organic matter in the water, thus producing gases that may alter the pH of water in the water pan and increasing the acidicity of water. This contrasted with Ndubi *et al.* (2015) in their study in Narok South who found the mean pH of water pans during the dry season to be 8.045. pH showed no significant variation amongst the sampling sites ($p > 0.05$) (Table 2). This could be attributed to the soils forming the base of the water pans; clay soils could have high levels of hydrogen ions thus increasing the acidity nature of the water pan. Similar studies recorded higher pH values in water dams in Samburu district and Narok South sub-county (Cheluget 2011; Ndubi *et al.* 2015).

Temperature showed significant variation among the sampling sites ($p < 0.05$) (Table 3). This could be attributed to direct insolation as a result of sparse and less dense riparian vegetation cover observed along the water pans. Other studies reported lower temperatures in water pans due to the presence of riparian vegetation cover along the water pans, therefore the cooling effect lowered the water temperature (Ndubi *et al.* 2015). Dissolved oxygen (DO) showed significant variation between the sampling sites ($p < 0.05$) (Table 2). Similar studies showed a range of 8.720–13.180 mg/L of dissolved oxygen (Ndubi *et al.* 2015). This

Table 3 | Mean ± SE values for physical and chemical parameters for water from the sampled water pans

	pH		Temperature		Dissolved oxygen		Conductivity	
Water pans	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**
Cher	6.30 ± 0.38	4.36–7.66	27.24 ± 0.84	23.60–31.30	6.32 ± 0.80	3.42–10.84	93.35 ± 5.30	71.20–114.80
Chel	6.25 ± 0.40	4.35–7.73	26.21 ± 1.05	22.10–31.80	4.62 ± 0.07	4.22–5.00	104.1 ± 1.21	100.00–114.60
Kur	6.45 ± 0.34	4.56–7.59	28.38 ± 0.92	24.10–32.60	4.43 ± 1.00	1.20–9.00	180.18 ± 16.57	119–241
Chep	6.32 ± 0.36	4.54–7.50	28.02 ± 0.41	26.40–30.20	7.98 ± 0.29	6.89–9.85	168.75 ± 4.25	152.40–202.30
Kap	6.29 ± 0.39	4.33–7.62	27.66 ± 0.43	26.30–30.10	6.37 ± 0.38	4.07–7.87	117.08 ± 9.00	118.00–147.20
Kiny	6.31 ± 0.15	4.33–7.73	27.03 ± 0.33	22.10–32.60	5.84 ± 0.29	1.20–10.84	135.54 ± 5.28	71.20–241.00

Cher: Cheraik; Chel: Kapchelukuny; Kur: Kures; Chep: Chepnyorgin; Kap: Kaptipsegem; Kiny: Kinyach.

could be attributed to high pollutant load in the water pans that could reduce the solubility of oxygen. The value of electrical conductivity gives an indication of the presence of dissolved ions in water. There was a significant variation of electrical conductivity among the sampling sites ($p < 0.05$) (Table 3). This indicated the difference of the electrical conductivity recorded in the sampling sites, and this could be attributed to the varying concentrations of dissolved solids among the unprotected water pans.

Spatial variation of microbiological parameters in protected and unprotected water pans

The results of spatial variation of microbiological parameters (total coliforms, *E. coli*, *F. streptococcus* and *Salmonella* species) among the protected water pan (Cheraik) and the unprotected water pans (Kures, Kapchelukuny, Chepnyorgin, Kaptipsegem and Kinyach) are shown in Figure 2. The results revealed that there was no statistical significant spatial variation in total coliforms, *E. coli* and *Salmonella* species amongst the sampled water pans ($p > 0.05$). The lack of statistical significance on the protected water pan as compared to the protected water pan could be attributed to the location of the protected water pan. During the sanitary survey it was observed that the protected water pan was located in a sloping land. The possibility of contamination as a result of run-off during a rainfall event could carry along with it fecal matter that could increase the growth of the pathogenic organisms in the water.

Bacterial incidences on protected versus unprotected water pans

An LSD test of microbiological parameters between the protected and the unprotected water pans revealed that there was a statistically significant variation of total coliforms and *Salmonella* species between the protected (Cheraik) water pan and the unprotected (Kures) water pan ($p < 0.05$). However, the mean densities of total coliforms and *Salmonella species* were higher in unprotected as compared to protected water pan (Figure 3). This could be associated with the observed animal and human waste, and lack of distinct water points for human and animals in unprotected

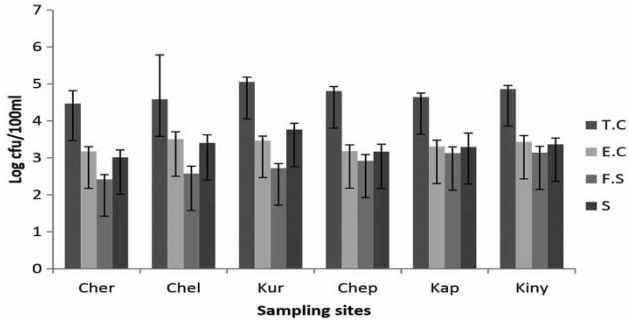

Figure 3 | Mean densities of the microbiological parameters (log cfu/100 mL) per sampling sites in Central and South Baringo. Mean densities: is two sampling periods are averaged to obtain the dry season and two are averaged to obtain the wet season. Legend: T.C (total coliforms), E.C (*Escherichia coli*), F.S (*Fecal streptococcus*) and S (*Salmonella species*). Cher; Cheraik; Chel: Kapchelukuny; Kur: Kures; Chep: Chepnyorgin; Kap: Kaptipsegem; and Kiny: Kinyach.

(Kures) water pan. These activities could be associated with the increase in total coliforms and *Salmonella* species in the water pan.

F. streptococcus showed a statistical significant spatial variation among the sample water pans ($p = 0.008$; $p < 0.05$) (Figure 3). However, an LSD test revealed a statistically significant spatial variation between the protected (Cheraik) and the unprotected (Chepnyorgin, Kaptipsegem and Kinyach) water pans ($p < 0.05$). The unprotected water pans had slightly higher values of *F. streptococcus* than the protected water pan (Figure 3). The results are consistent with a study by Amenu *et al.* (2013) where protected water sources were observed to have very low levels of contamination.

Seasonal variation of microbiological parameters of water pans in Baringo County

The mean densities of the microbiological parameters in months (seasons) are shown in Figure 2. There was a significant temporal variation in total coliforms ($p = 0.001$) and *Salmonella* spp. ($p = 0.001$) between seasons. Total coliforms had higher mean counts during the dry season (September and October), as shown in Figure 2. The presence of total coliforms during the dry season could be associated with high concentrations of coliforms in the soils or the environment. An LSD test showed a significant temporal variation of total coliforms between the protected water pan (Cheraik) and the unprotected water pan (Kures)

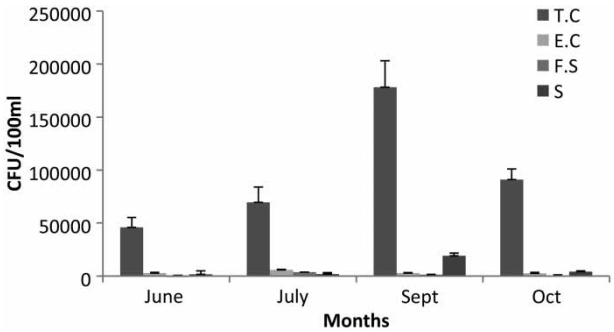

Figure 4 | Temporal variations of the microbiological parameters (cfu/100 mL) against the months. Wet season (June and July); dry season (September and October). T.C: total coliforms; E.C: *E. coli*; F.S: *F. streptococcus*; and S: *Salmonella* species).

($p = 0.011$) during the dry season. Unprotected (Kures) water pan recorded the highest total coliform mean count (3.07×10^5 cfu/100 mL) as compared to the protected (Cheraik) water pan total coliform mean count (1.08×10^5 cfu/100 mL). This could be attributed to high pollutant load in the unprotected water pan as a result of low dilution factor.

This study finding was supported by Mwajuma (2010) in her study in selected water sources in Samburu South, who found the highest number of coliforms in a dam sample. *Salmonella* species recorded a higher mean value during the dry season (Figure 4). The presence of *Salmonella* species during the dry season could be due to high pollution levels in the water pans. Studies reveal that *Salmonella* species thrive in hot weather (Gwimbi 2011). *E. coli* and *F. streptococcus* ($p > 0.05$) did not show a statistically significant temporal variation in mean densities between seasons (Figure 2). This study was in congruence with Omondi *et al.* (2013). The study contradicts with other studies which found higher Enterococci counts during the wet season (Amenu *et al.* 2014; Edokpayi *et al.* 2015).

Correlation between microbiological and physical-chemical parameters of water pans in Baringo County per season

Wet season

Correlation results between the physical-chemical and microbiological parameters measured during the wet season are shown in Table 4. pH and *Salmonella* species showed a significant positive correlation ($r = 0.631$; $p < 0.05$). The pH range of the water during the wet season was favorable for the growth of *Salmonella* species. pH is important for the growth of aquatic organisms in water. Temperature and *Salmonella* species showed significant positive correlation ($r = 0.587$; $p < 0.05$) and the temperature range was favorable for the growth of *Salmonella* species. Dissolved oxygen and *Salmonella* species ($r = 0.582$; $p < 0.05$) showed significant positive correlation. The amount of dissolved oxygen in water provided adequate amounts of oxygen for the survival of *Salmonella* species. Temperature and *F. streptococcus* ($r = 0.470$; $p < 0.05$) showed significant positive correlation. The temperature range recorded in the water pans was favorable for the growth of *F. streptococcus*. Dissolved oxygen and *F. streptococcus* ($r = 0.468$; $p < 0.05$) showed significant positive correlation. Dissolved oxygen in the water pan allowed for the growth of *F. streptococcus* in water.

Table 4 | Correlation of microbiological and physical-chemical parameters during the wet season

	pH	TEMP	DO	CON	T.C	E.C	F.S	S
pH	_	_	_	_	_	_	_	_
Temp.	**0.823****	_	_	_	_	_	_	_
DO	**0.824****	**0.999****	_	_	_	_	_	_
CON	−0.305	−0.135	−0.137	_	_	_	_	_
T.C	−0.226	−0.208	−0.222	0.321	_	_	_	_
E.C	0.076	0.073	0.071	−0.006	0.238	_	_	_
F.S	0.122	**0.470****	**0.468****	0.167	0.029	0.077	_	_
S	**0.631****	**0.587****	**0.582****	−0.207	−0.088	0.197	0.349*	1

*$N = 36$ refers to the number of cases with the non-missing values, where the test of significance used was two-tailed.
**Correlation is significant at $p < 0.05$.

Table 5 | Correlation of microbiological and physical-chemical parameters during the dry season

	pH	Temp.	DO	CON	T.C	E.C	F.S	S
pH	_	_	_	_	_	_	_	_
Temp.	−0.088	_	_	_	_	_	_	_
DO	−0.018	0.305	_	_	_	_	_	_
CON	0.183	−0.067	−0.390*	_	_	_	_	_
T.C	−0.171	0.161	−0.380*	**0.495****	_	_	_	_
E.C	0.268	0.064	−0.158	0.129	0.156	_	_	_
F.S	0.415*	−0.018	0.175	0.114	−0.181	0.389*	_	_
S	0.010	−0.191	−0.211	0.084	−0.183	0.366	0.092	_

*$N = 36$ refers to the number of cases with the non-missing values, where the test of significance used was two-tailed.
**Correlation is significant at $p < 0.05$.

The presence of total coliforms and *E. coli* could be attributed to other external factors, such as fecal contaminants in water, other than rainfall events. Omondi *et al.* (2013) in their study in Lake Naivasha basin did not record temporal variation in the density of fecal contamination.

Dry season

Correlation results between the physical-chemical and microbiological parameters measured during the wet season are shown in Table 5. The results showed that total coliforms and conductivity had a significant positive correlation ($r = 0.495$; $p < 0.05$). This could be attributed to the level of dissolved solids as a result of sedimentation in water pans that enhanced the growth of total coliforms.

CONCLUSIONS

The sources of microbial contamination of water pans in the study area were inadequate protection of water sources, human and animal wastes, agricultural waste, sparse and less dense riparian vegetation cover and lack of distinct watering points among the unprotected water pans. Total coliforms and *Salmonella* species were recorded during the dry season; this was due to high pollutant load. The protected water pan recorded a reduced colony count of the pathogenic organisms in both seasons; this was attributed to the hygienic practices at the protected water pan. Given the prevalence of the selected diseases causing pathogens in water above the WHO drinking water quality guidelines, households are advised to treat the water before use. We also recommend that WASH campaigns be held in the study area with the support of all stakeholders in the water and sanitation sector.

ETHICAL APPROVAL

The study was approved by National Commission of Science and Technology Ref (Nacosti/P/15/0999/7318). Confidentiality was highly maintained while carrying out the study.

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