



Effects of Land Use Types on the Levels of Microbial Contamination Based on Total Coliform and *Escherichia coli* counts on the Mara River, East Africa

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Abstract

The effects of land use types on levels of microbial contamination based on total coliforms and *E. coli* (faecal coliform) levels was investigated in the Mara River system, Kenya and Tanzania. Water samples were taken from five sampling sites with different land uses and the Most Probable Number (MPN) method used to determine the total coliforms. A biochemical test was done and the proportions of *E. coli* bacteria given per study site. The mean concentration of total coliforms was lowest at Silibwet Bridge and highest at Kirumi Bridge. However, counts of *E. coli* were highest at an urban site (Bomet Bridge) and lowest at a swamp site (Kirumi Bridge). Overall, the proportion (40.9%) of Eosin Methylene Blue (EMB) broth plates that produced *E. coli* in the study sites was higher than the WHO recommended standards of 0% per 100 ml in potable water. The results show that sections of the river with most human activity and inappropriate types of land-use contributed to high levels of coliform bacteria, particularly *E. coli*. This may indicate the existence of point sources of faecal contamination along the Mara River and corrective measures should be taken to control them.

Keywords: *E. coli*, faecal coliform bacteria, land use type, most probable number, Mara River.

Introduction

Aquatic ecosystems are major subdivisions of the biosphere, and cover almost 71% of the earth's surface area (UNDP, 2006). Continuous changes in land use, both under the influence of human activities and nature, have various impacts on water quality (Gereta *et al.*, 2002). The pressure of population growth, coupled with the demand for natural resources and heightened development is jeopardising catchment areas (Mati *et al.*, 2005; Mutie *et al.*, 2006). Changes in the biological, chemical and physical characteristics of any river system result from different kinds of pollutants such as pesticides, heavy metals, oils, petroleum products, synthetic organics, radioactive isotopes and a large number of inorganic and organic compounds (Adenkule and Eniola, 2008). These originate in the watershed but later find their way into the aquatic system through surface runoff.

Land use changes often have a direct effect on soil degradation through a decline in its productive capacity, loss

of nutrients and organic matter, poor soil structure, concentration of electrolytes and toxic chemicals (Emadi *et al.*, 2008). Recent research findings in the Mara River system revealed an accelerated loss of vegetation cover in the upper catchments associated with changes in land use (Aboud *et al.*, 2002; Dwasi, 2002; Mati *et al.*, 2005). These have led to increased erosion and discharge of pollutants which have a cumulative effect along the river and contribute to the deterioration of water quality through increased conductivity, turbidity, total dissolved solids, and microbial contamination (ICRAF, 2002). These changes in land use have had a significant influence on coliform abundance and other water borne disease vectors in the river (Muyodi *et al.*, 2009).

There is always an upstream-downstream linkage in river basins caused by the water from precipitation and/or head waters within a catchment area. This relatively tight cause-effect relationships between upstream land and water use or flow scenarios and downstream situations leads to variation

in water quality and have environmental consequences for aquatic organisms and human health (USAID EA, 2010). Furthermore, environmental flow and water quality are intimately linked and if the flow is altered, water quality will also change (Malan and Day, 2002). One of the most obvious reasons for this is that the dilution capacity of the system is likely to be altered.

Eroded catchments are vulnerable to torrential rainfall events which in some instances lead to unprecedented mass movements of soil from the uplands into the river channel which bring with them nutrients and other pollutants such as animal and human waste from the catchment area (Kistemann *et al.*, 2002). Such mismanagement of land degrades water quality (Molden *et al.*, 2003). Changes in land use such as the removal of trees and vegetation cover for urban development along the river channel and on catchment areas reduces the water retention capacity, accelerates the deposition of water pollutants such as human and animal waste into the river channel thereby contributing to high coliform levels (Garcia-Armisen and Servais, 2007). Wildlife and domestic animals also contribute coliform bacteria to water bodies through defecation either on the banks or in the river channel (Strauch, 2011).

While urban development is the main source of faecal coliforms, pasturelands and rangelands also have the potential to contribute high levels of coliform bacteria to the water body (Collins and Rutherford, 2004; Nobler *et al.*, 2003). Sewage remains the largest single source of surface water contamination (Azizullah *et al.*, 2011), while diarrhoeal disease outbreaks are more prevalent in overcrowded informal settlements such as slums which lack adequate means to dispose of faecal matter, practice poor hygiene and often lack safe water (Edberg *et al.*, 2000; Craig *et al.*, 2004; Servais *et al.*, 2005). The degradation of natural waters by faecal matter is therefore increasingly becoming a source of concern as pathogens are regarded as pollutants of greatest concern to human health (McBride *et al.*, 2002). Kussi *et al.* (2004) reported an outbreak of gastroenteritis among rural populations in southern Finland after drinking contaminated water that had not been boiled.

The Mara River Basin supports a wide range of human and animal activities such as supplying water for humans and livestock, fishing, animal watering points, large scale or subsistence farming, pastoralism, mining and urban development (Mati *et al.*, 2005). In the past, priority was given to improved agricultural production but there is growing concern over an increase in pollution of the Mara River as a result of population growth and changes in land use in the basin. The increased population in the river basin has led to destructive activities such as deforestation, overutilization of water resources, overfishing, overgrazing, and unplanned disposal of domestic waste.

Knowledge of changes in the water quality of the Mara River is an important component of monitoring and management of activities in the basin. Of particular importance is the influence of such activities on the

abundance of *Escherichia coli* and non-faecal coliforms in relation to different types of land use, such as forestry, agropastoralism, urban settlement, conservancy and natural swamps. Although there are major changes in land usage in the basin, such as the removal of trees and vegetation cover for urban development and irrigation their contribution to water pollution and the abundance of faecal coliforms has not been established. Also, the relationship between physico-chemical conditions and faecal coliform abundance in the basin needs to be determined. Diarrhoeal and other waterborne diseases in the Mara River basin have attracted the attention of many researchers but studies on the levels of contamination of its surface waters are still lacking. The aim of this study therefore was to determine the effect of land use types on microbial contamination by assessing the total coliform and *E. coli* levels along the Mara River. The specific objective of this study was to determine the effect of land usage on the levels of microbial contamination in the river.

Material and Methods

Study area

The Mara River basin covers 13,834 km² and is located across the border of Kenya and Tanzania with about 65% of the area within Kenyan territory. The basin lies between longitudes 33°47' E and 35°47'E and latitudes 0°28' S and 1°52' S (Mutie *et al.*, 2006). Rainfall peaks are bi-modal, falling between April-September and between November-December and the mean annual rainfall ranges from 1,000-1,750 mm in the Mau Escarpment, to 900-1,000 mm in the middle rangelands, and 700-850 mm in the lower Loita hills and around Musoma (USAID EA, 2010). The main perennial tributaries of the Mara are the Amala and Nyangores, which drain the upper catchment of the Mau escarpment forming the Mara River at their confluence in the middle range lands before flowing downstream into Mosirori/ Kirumi swamp, and finally draining into Mara bay on Lake Victoria at Musoma in Tanzania. Rainfall varies with altitude with mean annual rainfall ranging from 1,000 - 1,750 mm in the Mau Escarpment, to 900 - 1,000 mm in the middle rangelands to 700 - 850 mm in the lower Loita hills and around Musoma. The dominant land use types within the basin are agriculture, pastoralism and game reserve/conservancy.

Sampling sites

This cross-sectional survey was carried out between July and August 2011 and five sampling sites were selected on the basis of their main land use characteristics (Figure 1) and their locations plotted with a Global Positioning System. They were:

1. Silibwet Bridge, altitude 1958 m, on the Nyangores River in a forested area with slight human interference.
2. Kapkimolwa Bridge, altitude 1853 m, on the Amara River in a sparsely forested, rocky area with open fields, with agropastoralism being the main land use type.

3. Bomet Town Bridge, altitude 1903 m, on the Nyangores River, an urban area with many different human activities.
4. Ngerende Point, altitude 1678 m, on the Mara River (Kenya) in a conservancy with wild animal watering points but little human settlement; some domestic animals present.
5. Kirumi Bridge, altitude 1139, on the Mara River (Tanzania) in a natural swamp with peripheral aquatic plants; the flow rate of the river is low and the surrounding area is sparsely populated and there is relatively little human interference.

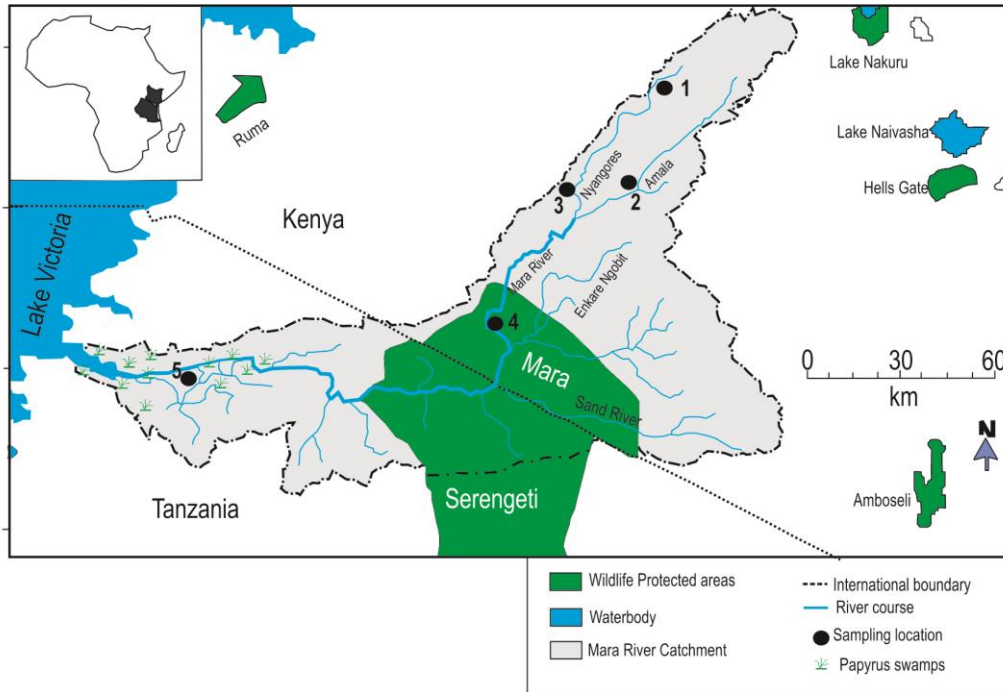


Figure 1: The study area showing the location of sampling sites: 1= Silibwet bridge, 2 = Kapkimolwa Bridge, 3 = Bomet Town Bridge; 4 = Ngerende Point, 5 = Kirumi Bridge.

Sample collection

A land use verification form was used to record the various types of land use, which at each study site was further characterised and classified through observation of the entire study area. Observations on land use and vegetation type, human activities, and presence of domestic and wild animals were recorded. Water samples for bacteria analysis were collected in 10 replicates from each site, using sterile 250 ml glass bottles inverted downwards. Sampling was done against the current and the hand kept downstream from the neck of the bottle to avoid contamination. The replicates were collected from each site at intervals of 10 metres along a 100 m section of the river. The water samples were preserved in a cold ice-packed box pending microbial analysis.

The coliform concentration in the water samples was estimated by the most probable number (MPN) procedure or the multiple tube fermentation test which detects coliform bacteria as indicators of faecal contamination (APHA, 1998). This was followed by a biochemical assay for species

identification. Multiple tube techniques utilized selective and differential liquid media into which multiple aliquots of serial dilutions of samples were inoculated. The technique involved three successive steps, namely, presumptive test, confirmed test and complete test (Tharannum *et al.*, 2009). In the presumptive test, 10mls of McConkey G broth purple (Fluka Sigma-Aldrich) was added into each of 3 sets of 25ml tubes (with inverted Durham's tube inserts). Each set contained three tubes (making 9 tubes in total). The loaded tubes were sterilized by autoclaving, allowed to cool and then inoculated with a ten-fold difference of water sample inoculum volumes, i.e., 0.1ml, 1ml, and 10ml per tube and incubated at 37^o C. After 24 hours, the tubes were examined for acid and gas production. Change from purple to yellow (Khaki colour) indicated that acid production had occurred (APHA, 1998). Each set was scored for the number of positive tubes and the score of all the three sets were recorded and used with the standard MPN table to determine the probable number of coliforms in the water samples.

The presumptive test was then followed by the confirmative test, the complete test and IMViC tests. The confirmative test was performed by streaking a sample from a presumptive positive tube onto eosin methylene blue agar (Fluka Sigma-Aldrich). This agar contains lactose and the dyes eosine Y and methylene blue. When *E. coli* grows on EMB, it ferments so much acid that the two dyes precipitate out in the colony, producing a metallic green sheen appearance, while non-faecal coliforms are pale pink mucoid and red colonies. A positive confirmative test is when green sheen colonies are present on EMB streaked from a presumptive positive test.

The complete test was performed by inoculating a tube of McConkey G purple broth with green sheen colonies from positive confirmative tests. A loop of colony was streaked onto a slant of nutrient agar in two tubes and both tubes were incubated at 37°C for 24hrs. The culture on the nutrient agar was analyzed by Gram staining. The presumptive positive samples from the above tests were subjected to further biochemical assessment including indole production, methyl red, Vogues-Proskauer test, citrate test, oxidase production and catalase production. These biochemical tests were performed according to standard microbiological methods (Cappuccino and Sherman, 2007). These procedures were followed in order to differentiate the Enterobacteriaceae on the basis of their biochemical properties and enzymatic reactions in the presence of specific substrates. The colonies from EMB agar plates were sub cultured on EC broth and incubated in water bath at 44.5°C for 24hrs. Growth was observed by turbidity on the EC broth and these were the *E. coli*, faecal coliform.

Statistical Analysis

The effect of land use type on microbial contamination by coliform and *E. coli* was shown descriptively by relating the land use types in the different sites to actual coliform levels at the same sites. Differences in mean *E. coli*

abundance among the five study sites were compared using one-way ANOVA, followed by Duncan Multiple Range Test to establish specific significant differences in means between the sites. Mean total coliform and their standard deviations were calculated for each site. The differences in proportions of *E. coli* from each site were compared using Chi-square statistics. All the statistical analyses were done using the SAS V9 software package.

Results

The mean concentration of total coliforms in the water samples was lowest (147.8±176.5 MPN/100 ml) at Silibwet bridge located in a forested terrain on the upper Nyangores tributary (Figure 2). The highest concentration, (746.2±386.9 MPN/100l) was recorded at Kirumi bridge in a natural wetland of the main Mara River before Musoma in Tanzania, followed by Bomet Town Bridge (705.0±426.3 MPN/100mls) located in an urban centre. Kapkimolwa recorded coliform levels of 460.4±215.2 MPN/100mls, while Ngerende sampling site had a mean total coliform of 448.2±236.1 MPN/100mls of water.

Analysis of variance showed significant differences in total coliform levels between different land use types (sites) (one-way ANOVA, $F_{(4,49)} = 4.65$, $P=0.0032$). The Duncan Multiple Range Test showed that total coliform levels recorded at Kirumi and Bomet sites differed significantly from Kapkimolwa and Silibwet but no significant difference was observed at Ngerende.

The results of the ANOVA further showed that land use along Mara River basin could explain a 95% of the variability in faecal coliform abundance along the basin. Faecal coliform abundance was found to be influenced by and connected to various land-use types throughout the basin.

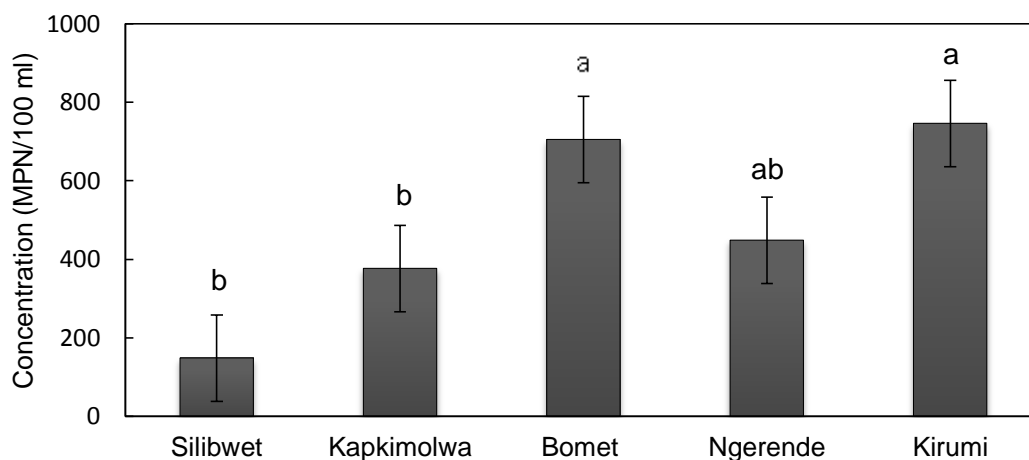


Figure 2: The concentration (MPN/100 ml) of total coliform bacteria in water samples from the Mara River. Means with different superscripts are significantly different at $P < 0.05$.

The highest percentage of *E. coli* (82.9%) was recorded at Bomet Town Bridge while Kirumi bridge sampling site had the lowest (12.8%) percentage of *E. coli* compared to all other sampling sites along the Mara River (Figure 3). The proportions of *E. coli* at Kapkimolwa Bridge, Ngerende

Point and Silibwet Bridge were 68.2%, 47.8% and 24.1% respectively. Chi square test indicated a significant difference in *E. coli* levels among the sites ($\chi^2 = 137.8$, DF = 4, $p < 0.001$).

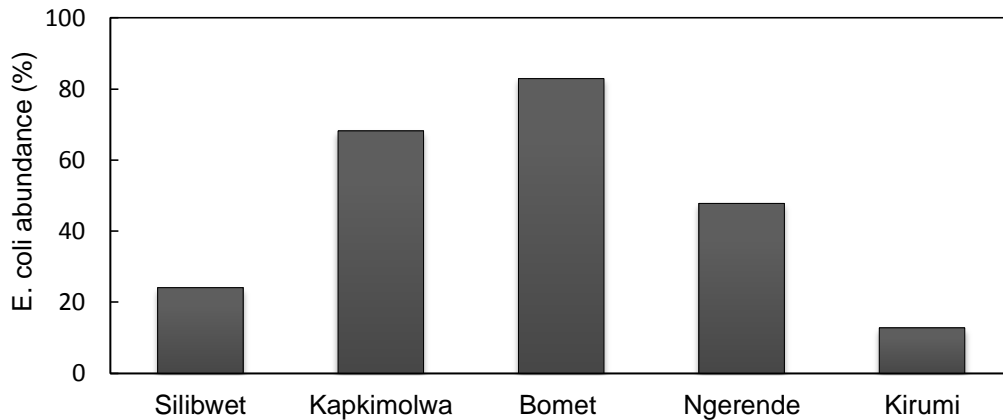


Figure 3: Proportion of faecal coliform (*E. coli*) levels at each site along the Mara River.

Discussion

The concentrations of total coliform varied in water samples from different sites in the Mara River basin with significant differences between the study sites. For instance, the mean total coliform concentration was lowest at Silibwet Bridge, located in a forested terrain on the upper Nyangores tributary and highest at Kirumi bridge, located at a natural wetland of the main Mara River before Musoma in Tanzania, and at Bomet Town Bridge located in an urban centre, characterised by low and high levels of human activities, respectively.

Interestingly, the percentage of *E. coli* was highest at Bomet Town Bridge while it was lowest at Kirumi Bridge even though the latter had the highest concentration of total coliforms. Kapkimolwa Bridge, Ngerende Point and Silibwet followed in that order. These results concurred with the findings of Kasangaki *et al.* (2008), who reported that forest clearance was endangering freshwater ecosystems in East Africa. The fact that the proportion of *E. coli* was lowest at Kirumi Bridge may reflect the natural ability of swamps to purify water.

The concentrations of faecal coliform bacteria were far above the required WHO standards of nil or 0 detection of *E. coli* in any 100ml sample for portable water (WHO, 2008), at sites in areas disturbed by human activities and intensive land use. This suggests that land use and human activities have a considerable impact on the quality of water in the Mara River. The contamination of water by human wastes has been associated across the world with outbreaks of water-borne diseases such as diarrhoea, typhoid and hepatitis

(Servais *et al.*, 2005). High coliform populations in all the water samples are an indication that poor sanitary conditions are prevalent in the Mara River basin.

The high concentrations of total coliforms and *E. coli* at Bomet Town Bridge undoubtedly reflected the high level of human activities along the river banks here. The lack of sewage treatment facilities, defecation along the banks of the river, inadequate and unhygienic handling of solid wastes in riverine towns such as Bomet, are likely to be the main sources of *E. coli* in this area. In addition, surface waters in urban areas are also polluted by domestic animals. The high coliform in urban streams or rivers which flow through urban centres are consistent with the findings of Young and Thackston (1999) who noted that faecal bacteria were more numerous in urban tributaries compared to those in forested basins.

Very low mean *E. coli* proportions were reported from Silibwet and Kirumi Bridges, located in forested and swampy areas, respectively, which is consistent with reports that the levels of coliform bacteria in surface waters were in close proximity to human settlement, pasture lands and agricultural lands, were higher than in forested regions (Servais *et al.*, 2005). Although agropastoralism was the dominant land use around the Kapkimolwa site, it still recorded a high proportion of *E. coli*. This may be explained by local activities such as grazing and watering of cattle in the river and along its banks. Studies have shown that a significant amount of faecal coliform released in wastes within pastureland is mainly produced by animals (Strauch, 2011). This can be a serious problem in waters near cattle

grazing fields, watering areas and cattle tracks where animal waste is not properly contained and thus finds its way into the river. This was the case at Kapkimolwa, where relatively high bacteria levels in water samples were recorded, probably resulting from both human activities along the river and domestic animals.

At the Ngerende Point site, where there was little human influence and wild and domestic animals predominated, there was a slightly lower proportion of *E. coli*. These possibly originated from a combination of human activities, domestic animals and wildlife. In conservancy areas and those sections of the catchment area used for pastoralism, large herds of animals graze and their waste is likely to find its way into the river and therefore contribute to elevated levels of total coliforms as was also reported by Strauch, (2011) in a study of faecal bacteria of semiarid rivers in the Serengeti National Park, Tanzania. The data from Kirumi Bridge represented the part of total coliform pollution brought to rivers by surface runoff, probably originating from wastes from humans, wild animals and livestock, as well from cattle manure spread on cultivated areas upstream, and other sources in the environment.

Contamination of surface waters with faecal-derived pathogens poses a significant threat to human health and represents an important barrier to utilization of the river water for domestic purposes among other uses. Based on the WHO standards which prohibit the detection of *E. coli* in any 100ml sample of potable water, the levels of faecal bacteria in the Mara River makes the water unfit for human consumption, watering animals or irrigation of crops that are eaten raw (WHO, 2008).

To protect human health, water bodies should meet these water quality standards for faecal coliform or *E. coli* concentrations. Faecal coliforms, like other bacteria are usually killed by boiling water or by treating with chlorine while washing thoroughly with soap after contact with contaminated water can also help prevent infections. The local communities can therefore be encouraged to practice these simple methods to prevent waterborne diseases. Controlling microbial levels at the source should be encouraged amongst all the riverine inhabitants as it is the key to providing microbiologically and chemically safe drinking water for all. Also, water sources should be protected from contamination while more effective water treatment strategies should be established in the communities in the Mara River basin. Local authorities, who have seemingly abdicated their duties and responsibilities, must enforce the legislation and regulations for protecting water and catchment areas. Overgrazing should be controlled and agriculture, especially in the upper catchment of the Mara River, should be better managed to reduce its contribution to water pollution.

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