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Microbiological evaluation of water quality from peri-urban watersheds for domestic water Supply Improvement in eastern of Democratic Republic of Congo

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ABSTRACT

***Key words*: *Escherichia coli*, Surface water, Contamination, Cattle keeping, Crop production**

Agricultural and urban runoffs may be major sources of pollution of water bodies and major sources of bacteria affecting the quality of drinking water from surface water bodies. The goals of this study were to determine the distribution, diversity, and antimicrobial resistance of pathogenic *Escherichia coli* isolates from low flowing river water and sediment with inputs from different sources before water is discharged into ground water and to compare microbial contamination in water and sediment at different sampling sites throughout the River Nyabarongo watershed. There was a diversity of *E.coli* populations from different sources throughout the watershed. Agricultural and urban runoffs are the major source of pollution of water bodies (streams, rivers, wells, etc) in rural landscapes of eastern DRCongo.

RESUME

***Mots-clés:* Escherichia coli, Eau de surface, Contamination, Maintien du bovin, Production agricole**

L'eau de ruissellement issue des zones agricoles et urbaines peut être une source principale de la pollution de rivières et autres douces dans les zones rurales et urbaines, bien que très souvent les scientifiques n'y croient vue que l'eau de sources traverse le sol et que le sol est un filtre reconnu. La pollution bactérienne affecte la qualité de l'eau de consommation issue des cours d'eau. L'objectif de cette étude était de déterminer la distribution, la diversité des isolats d' *Eschericia coli*. Les données étaient prélevées sur différent sites de l'amont à l'aval. Ce sont les sédiments déposés a différent sites du bassin versant de la rivière Nyabarongo qui étaient examines pour détecter la présence de *Eschericia coli.* Les résultats de l'étude indiquent les *E.coli* ont été détectés a tous points de déchargés des ruissellements dans la rivière Nyabarongo. Ainsi donc, l'eau de ruissellement (d'érosion) issue des zones urbaines et rurales est une source de contamination vraie des eaux douces et des cours d'eau en milieu rural surtout si le ruissellement traverse les zones d'élevage des bovins, petits ruminants et porcins.

1. INTRODUCTION

The Nyabarongo river is one of the river flowing from Kahuzi Biega national park, crossing various landscapes and habitats and ending on the left side of Lake Kivu after crossing Mushobekwa wa Kalimba farm. This river which is the natural boarder separating Kabare and Kalehe territories (South-Kivu Province, DRCongo). The river is a major source of domestic water supply for over one quarter million people that live Kabare and Kalehe territories. Nyabarongo river is critical for replenishment of the territories groundwater basin since over ¼ million residents in these territories depend on groundwater for 87-90% of their water supply. Thus, any factor in the watershed which degrades the river affects automatically the drinking water supply. The river extends from its headwaters in Mountains of Kahuzi National park into the left side of Lake Kivu Basin.

The river crosses various peri-urban centers with extensive facilities to recharge much of the flows in the River into the underlying groundwater basin. Sources of non-point contaminants into the river may be from peri-urban and village centers wastewater, agricultural waste discharges (via soil erosion on sloppy hills and landslides), peri-urban runoffs, and a combination of the above factors. Currently, the River is impacted by highest concentrations of cattle keeping zones established along the national park. Currently, the watershed is undergoing drastic changes. In general, the varying land uses in the middle watershed include agriculture (small-scale polycultures/monocultures established on sloppy hills), open space, cattle keeping and rapidly growing rural/peri-urban centers. Along the axe of Lemera, there are various several small-scale and to medium scaled dairy farms. Pollutants in the watershed mainly consist of pathogens and nutrients due to the densely populated areas, runoff from agricultural activities on hills and peri-urban (rural development centers) and small-scale industries factories (coffee factories) in the region.

There have been no agencies to monitor fecal bacterial composition in the surface water in rural areas of DRCongo. Nothing has been previously done to determine/ detect the presence of *Escherichia coli* within water bodies flowing in various areas of rural areas of South- Kivu province, province where majority of the population relies on surface flowing water bodies as source of drinking water and as source of water for various needs and domestic utilities.

E. coli are very diverse in rural environment. Most *E. coli* are nonpathogenic, but there are some such as *E. coli* O157:H7 that cause human diseases such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). In addition to *E. coli* O157: H7, there are other *E. coli* pathogroups that causes diseases in human such as enteropathogenic *E. coli* which causes diarrhea in children especially in rural areas of Sub-Sahara Africa, enterotoxigenic *E. coli* which causes traveler's diarrhea and others .

There is an extensive review of sources of pathogenic *E. coli* in the environment , but their distributions in rural waters of DRCongo urban has been limited to very few to almost no studies in different territories of the country (Ishii and Sadowsky 2008, Kaper et al. 2004, Ibekwe and Lyon 2007, Ibekwe and Lyon 2008). Due to the increasing urbanization of rural zones and the large number of soil erosion, small-ruminant, pork and cattle keeping in the studied watersheds, the health risk from pathogenic *E. coli* is a major concern to drinking water quality, particularly since the water from these rivers is used by rural people for most domestic uses. There is virtually no information on the occurrence of pathogenic *E. coli* in rural zones of DRCongo and in the Nyabarongo watershed despite the high concentration of agricultural activities of hilly slopes in the watershed.

Most pathogenic *E. coli* are commonly carried by healthy cattle/ small ruminants/pork in their feces, especially in zones where agriculture is integrated to livestock under the zero grazing schemes. The fecal excretion of these organisms by cattle appears to be seasonal, with excretion rates highest during short and long rainy seasons than during dry seasons (Kaper et al. 2004, Ibekwe and Lyon 2007, Ibekwe and Lyon 2008). This study sought to detect the presence, identify and characterize pathogenic *E. coli* isolates obtained in terms of their virulence profiles. Such information may assist in the epidemiologic tracing of pathogenic *E. coli* isolates of medical concern in the region. Therefore, the goals of this study were to determine the distribution and diversity of pathogenic *E. coli* isolates from low to high flowing zones of Nyabarongo river and sediment with inputs from different sources before water is discharged into ground water. The sub-objective was to compare microbial contamination in water and sediment at different sampling sites. There was also a need to incorporate the evaluation of fecal bacterial contamination of drinking water aquifer sand material at a specific site that receives water from the above sources before discharge into ground water used by the communities of Kabare and Kalehe territories.

2. MATERIALS AND METHODS

a. Study Area and Sample Collection

The study was conducted in the study area as above described .Various sampling points for selected located along the river. Surface water samples and sediment were collected from a natural/open-space location to evaluate bacterial contributions from natural or background source.

Some points were located in National Park of Kahuzi Biega Effluent from three wastewater treatment factories (Lemera, Mabingu, Ihimbi) were also analyzed . All sampling locations and their land use types (cropping patterns) were recorded. Reference samples were taken quarterly for 12 months (from November 2011 to November 2012).

All samples were collected at the water surface in duplicate in sterile recipients, and sediments from the bank of the river, stored at 4 °C, and analyzed within 6–8 hours. Sediment samples were collected in duplicate from the river banks with a stainless steel instrument and analyzed within 24 hours.

The topography in the Nyanbarongo River watershed ranges from hilly, steep lands, rugged mountains with peaks as high as 2,261 m above sea level, to a broad alluvial-filled valley towards Lake Kivu. Nyabarongo river has various tributaries. The bottom of the basin—the effective base of the freshwater aquifer—consists of relatively impermeable sedimentary and igneous bedrock formations that are exposed at the surface in the surrounding mountains and hills. The Nyabrongo River crosses a region that is reach geologically with various basaltic, igneous/ rocks.

Most recharge to the ground-water reservoirs of the Nyabarongo River is from percolation of direct precipitation and infiltration of stream flow within tributaries exiting the surrounding mountains and hills and within Nyanbarongo River. Potential sources of recharge into the river Nyabarongo basin include the following: (i) infiltration of flow within unlined stream channels overlying the basin, (ii) infiltration of rural-urbanized zone wastewater discharges within the channel of the Nyabarongo River, (ii) underflow from the saturated sediments and fractures within bounding mountains and hills, (iv) and recycled water, (v) underflow from seepage across the bounding faults of construction buildings, (vi) intermittent underflow from adjacent basins, and (vii) deep percolation of precipitation and returns from use on sloppy agricultural lands.

The climate of the region is tropical humid. The average annual precipitation ranges from about 1450 to 2150 mm. Most precipitation occurs during the rainy season between September and May. Average precipitation for September to May ranges from about 76 mm to 129 mm; whereas average precipitation from June to August is less than 1 to 23mm across the whole basin (Ishii and Sadowsky 2008). The spatial distribution of average monthly precipitation is similar for most months and is characterized by the topographic effect of Kakuzi Biegea mountainous area. Overall, the average rainy season precipitation is over 90% greater than that for all other months in the dry season.

Air temperatures across the basin are generally cool, with average daily temperatures ranging from about 19 °C to as high as 27°C.

b. Enumeration of E. coli from the basin

Water samples were processed in the laboratory within six hours of sample collection. All water samples were transported on ice to the laboratory and analyzed by adding 100 mL of water sample to a Colilert vessel and processing following the manufacturer's protocol. *E coli* populations were enumerated and expressed as most probable number (MPN/100mL). For isolation of *E. coli* colonies from Colilert vessels, 100 µL liquid sample was removed from positive wells, then spread plated onto Chromagar ECC agar, and was incubated at 37 °C for 24 h. Individual colonies of pure cultures that were isolated were stored at –80 °C for further characterization. Moist sediment samples (10 g) were diluted with 90 mL of phosphate buffered saline (PBS) water (0·0425 g/L KH2PO4 and 0·4055 g/L MgCl2) and shaken for 18 minutes. Ten mL of the suspension was added to Colilert vessel, diluted 1:10 and mixed. One mL from the 1:10 dilution was transferred to another vessel and was further diluted 1:1,000; and an aliquot was added to the Colilert media, mixed, then sealed in QuantiTrays and incubated at 37 °C for 24 h. Samples were processed following the manufacturer's protocol

c. Isolation of Pathogenic E. coli from Chino Basin

One gram or 1 mL of environmental samples was added to 9 mL of PBS, vortexed briefly, serially diluted and plated for the enumeration of *E. coli* O157 on Harlequin cefixime-tellurite sorbitol MacConkey (CT-SMAC) agar with BCIG (5-bromo-4-chloro-3-indoxyl-β-D-glucuronide) containing 0.05 mg of cefixime L−1 and 2.5 mg of tellurite L−1 (LAB M: IDG–Lancashire). The plates were incubated at 37 °C for *E. coli* O157 for 16 h; and later the different isolates of E-coli were identified following classical microbiological procedures.

d. Sampling Collection during Sand Filtration Experiment

Water samples were collected from the Nyabarongo river at different water territory stations. The water consists of source water (water from the river) and filtrate water (water from aquifer sand material after passing through a sand filtration system).This process was repeated three times to determine reliability of data. The experiment was conducted in a 1.1 × 1.1 × 1.8 m filtration tank built with stainless steel outside the field station. Aquifer sand material was heterogeneous native Lake Kivu sediment that had been processed through a sand washing plant to remove the majority of silt and clay particles.

The material was trucked to the station and packed into the tanks. Samples from aquifer material were collected at the end of each experiment. Source water was obtained from the River water. The water ran through a 2cm PVC pipe into the sand filtration unit. The water runs another 1 m through the filtration tank containing aquifer materials and collected at the outlet for analysis of fecal bacteria.

e. ***Enumeration of Heterotrophic Bacteria and E. coli in Water Before and After Sand Filtration***

Water sample was collected in 1-L sterile bottles, transported on ice to the laboratory, and processed within 6 h using standard procedure. Various dilutions and volumes were filtered with the goal of achieving 50–500 colonies per dilution. Surface water samples was vortexed and volumes of 100, 10 and 1 mL were filtered in phosphate buffered saline (PBS) water (0.0425 g/L KH2PO4 and 0.4055 g/L MgCl2) to obtain the best sample conditions. Tenfold and 100-fold dilutions were also prepared in PBS, vortexed, and 1 mL of each dilution was filtered in duplicate. Volumes of 1 mL, 10 mL and 100 mL (via membrane filtration) were plated onto tryptic soy agar (TSA) (for heterotrophic plate counts [HPC]) and sorbitol-MacConkey agar (SMAC-BCIG without cefixime-tellurite) for *E. coli*, and incubated at 37 °C for 24 h and colonies were enumerated. *E.coli* strains are β-glucuronidase positive and/or sorbitol positive, so produce pink/red colonies with a purple center, or green colonies (some may be translucent with a green center).

f. Statistical Analysis

All analyses were performed in triplicate, and the data shown in the graphs are the average of three separate measurements conducted. Thus, an analysis of variance (ANOVA) was conducted with log10-transformed density of *E. coli* bacteria using STAT 11 version 11 to determine statistical significant differences using Tukey's studentized range (HSD) test for mean separation.

3. RESULTS

a. Fecal Indicator Bacterial Concentrations in Nyabarongo Basin

Indicator bacteria in sediment and surface water were determined on various water and sediment samples collected from 22 sites over a 12-month period (2011-2012). Counts ranged from undetectable (detection limit 1 MPN 100m/L) in the surface water to 2.5 × 104 MPN/ 100g in the sediment (Fig. 1).

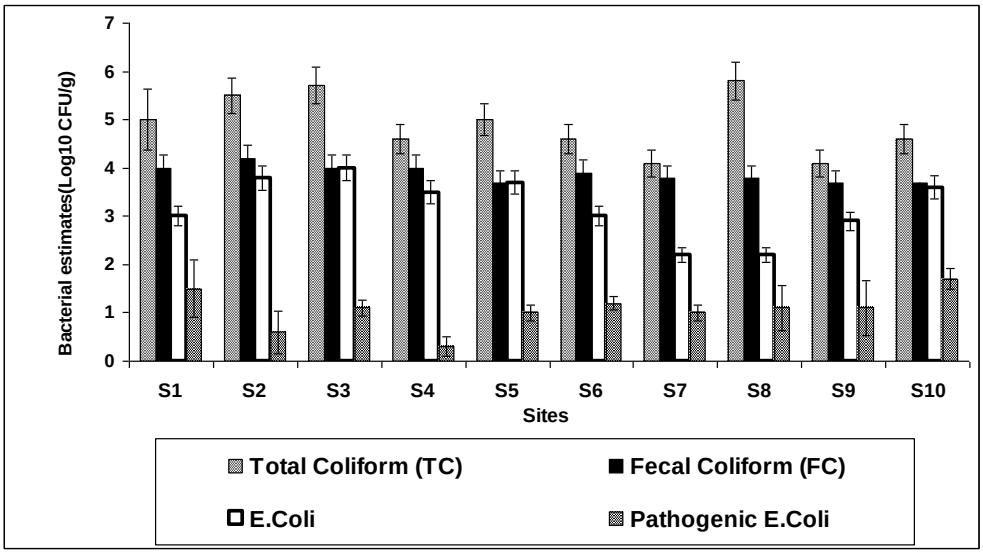

Fig. 1: Concentration of indicator bacteria in sediment on various sampling points along the major sources. Samples S1 to S7 are from per-urban runoff and samples S8 to S10 are from agricultural inputs landscapes crossed by the river. An errors bar represents standard errors of three replicates samples. Only samples with potential Pathogenic *E coli* are shown

Basic univariate summary statistics for pathogenic *E. coli* counts included in Figure 1 are from sediment samples because most of the potentially pathogenic *E. coli* were recovered from sediment samples. The statistics summarized the log10 transformed counts for each indicator bacterial group. Total coliform counts were the highest, and with the greatest variability in concentrations. Presumptive pathogenic *E. coli* were small in numbers and most of the times below 10 cfu/g.

b. Fecal Indicator Bacterial Levels in Source Water and Aquifer Sand Material During Sand Filtration

There were no differences in the levels of heterotrophic bacteria as determined by plate count in the source water (influent) and the filtration (water that has gone through sand filtration (Figure 4). Significantly higher levels ($P < 0.001$) of HPC were found in water samples in late March-May than in September - December. There were significant ($P = 0.05$) higher numbers of *E. coli* in source water in March-April than in October-November. After water has gone through filtration tanks containing aquifer sand material, there was a 1 to 2 log reduction in *E. coli* in aquifer sand tank. This showed that the filtration unit with aquifer material had limited impact on *E. coli* population (Fig. 2).

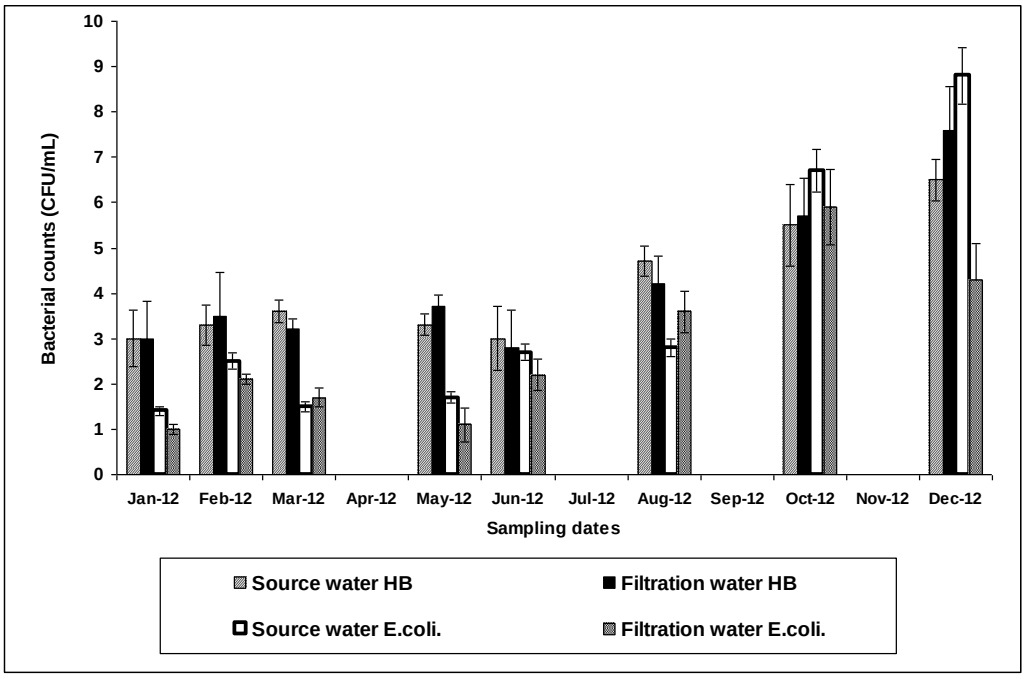

Fig. 2: Levels of heterotropihic (HB) and E.Coli.as determined by plate count in the source water and after filtration through aquifer sand materials. All samples were taken in January through December 2012

4. DISCUSSION

Examination of each site throughout the watershed indicated that indicator bacterial concentrations were high from all point sources. There was a high concentration of fecal coliform in the samples. The major sources of pollution to the watershed by these fecal coliform were cattle keeping and soil erosion running with contaminated water from hills. Peak in concentrations of *E. coli* depended more bacteria loading rates in the sources (tributaries). Heavy rains generated runoff, and thus bacteria wash off, in hilly regions.

Land uses that were assigned the highest bacteria-loading values were agriculture and cattle keeping. These activities had concentrations exceeding 235 cfu/100mL (Ishii and Sadowsky 2008, Kaper et al. 2004, Ibekwe and Lyon 2007, Ibekwe and Lyon 2008) . Thus, land use was the major factor affecting the concentration of *E. coli* in the Nyabarongo river watershed. In contrast, national park of Kahuzi Biega and open space land use areas had a significant decrease in the frequency of bacteria concentrations in waterways.

The microbiological data provided in this study can help water utility companies in their understanding of source water quality and help them in the processing of tertiary treated water that may be subsequently available for domestic use for rural communities. There is an urgent need for combined effort to be made to supplies rural communities with treated water sources rather than living these communities continue using the river water for any kind of domestic use including drinking water.

5. CONCLUSION

In this study E. *coli* was found in the surface water from Nyabarongo river. The study area has high number of agricultural activities being conducted on hilly slopes and by several cattle keeping points. Agricultural activities, land pf pit latrines in the villages surrounding the river and animal grazing are the main reservoirs of pathogenic *E. coli*.

There is a need for developing a monitoring program for all rural freshwater used by communities for different uses. There is an emergent need for humanitarian agencies and government o develop rapidly treated water supply systems.

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