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TRANSFORMATION TOWARDS SUSTAINABLE
AND RESILIENT WASH SERVICES

Antibiotic resistance in the sediments of a second order stream passing through agricultural farm land: Njoro river, Kenya

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A significant proportion of the population living along River Njoro depend on direct river use to carry out domestic activities. Antibiotic pollutants in wastes of treated farm animals that have not undergone any disinfection and sewage treatment processes pose a significant environmental health risk. The current study investigated the presence of total antibiotic resistant bacteria to a range of antibiotics used in the treatment of infectious diseases that may find their way into water and sediments in the river. This was done by culturing samples on nutrient agar media amended with various types of antibiotics. The study showed significant ($P< 0.05$) spatial variations in total bacteria resistant to chroramphenical, tetracycline, ampicillin and streptomycin antibiotics. Faecal pollution in river Njoro can transmit various diarrhoea pathogens as well as being a reservoir for antibiotic resistant genes that can be transmitted to consumers through water.

Introduction

There is wide use of antibiotics in human and veterinary medicine to control infectious disease. Antibiotics are also used as feed additives and growth promoters in livestock. This widespread use is an important factor for the emergence, selection, and dissemination of antibiotic resistant bacteria (Babic *et al*., 2006). Antibiotic resistant bacteria and drug resistance genes are important environmental contamination. The antibiotic resistance genes can be transferred between bacteria in the environment through plasmids, integrons and transposons (Van Elsas and Bailey, 2003).

Materials and methods

Study site

The River Njoro spans a distance of about 60 km from its origin in the native forests of the Eastern Mau Escarpment (elevation of 2700-3000 meters (m)) to its terminus at Lake Nakuru in the Rift Valley floor. Treated sewage effluents are also discharged in the river. Non point sources of pollution are rampant on the stream There is direct river water use by nearby communities. In-stream activities such as bathing, water fetching, laundry cleaning and cattle watering occur (SUMAWA, 2005).

Sampling sites on Njoro River were chosen from upstream to downstream as Sigotik which is assumed as unpolluted upstream site, Turkana cattle watering point. To capture discharges from Njokerio area, Njoro canning - to capture effluents from the canning factory and effluents from Egerton University, Njoro Bridge to capture effluents from Kenya Orchards canning factory, Kiptanui, Daneside and KARI farms, Kerma Watering point. Ngata to capture discharges from Njoro and Kenyatta areas, Mogoon to Capture discharges from Rift Valley Technology Institute and nearby farms.

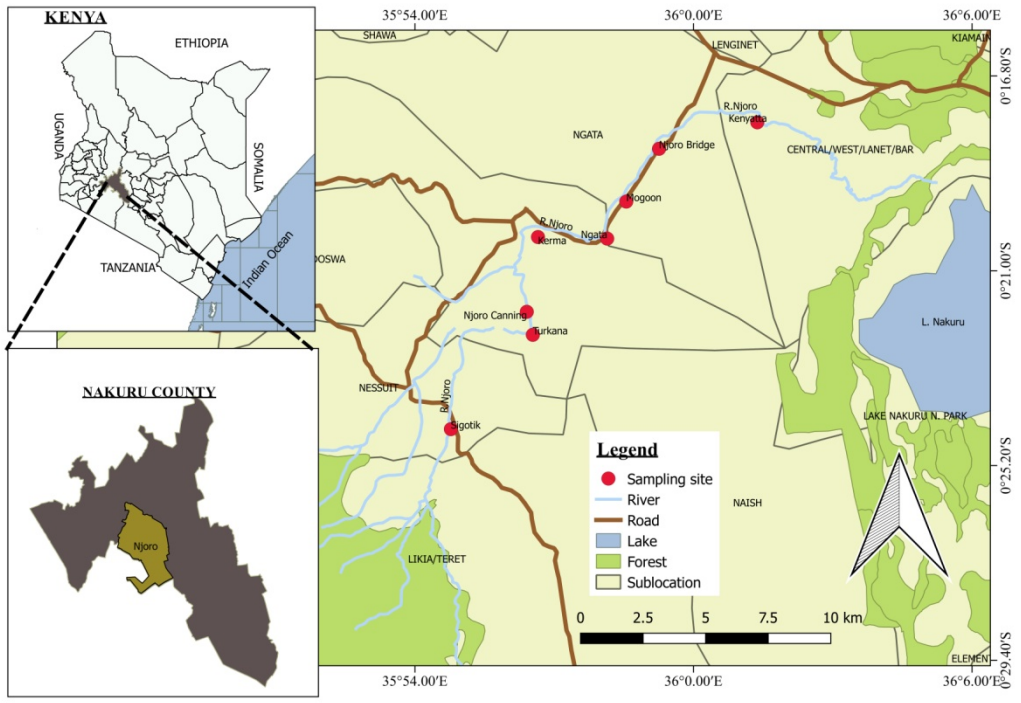


Figure 1. WEDC – Map of river Njoro

Source: Koech, 2014

Sample collection and processing

Three replicates of water and sediment samples were collected at the sampling sites during both the dry and wet seasons. About 10cm sediment core was sampled using a 5cm diameter PVC core at the sampling site.

Microbiological water quality indicators

Microbiological quality assessment of water samples was carried out as described in APHA (2005). Thus, membranes for total coliforms and *E. coli* were grown on Chromacult agar (Merck) at 37°Cfor 24 hours. *E. coli* CFUs appeared blue in this medium while other coliforms appeared pink. The numbers of colonies of each type were counted and total number multiplied by volume filtered and dilution factor to give the number per 100ml.

Isolation and identification of antibiotic resistant bacteria

To test for total bacteria resistant to antibiotics proportion in water or sediment resistant to specific antibiotics the procedure described by McArthur and Tuckfield (2000) was used. Ten serial dilutions of sediment or water were made by suspending I gm of the first 2 cm sediment layer in 9ml of 1% peptone water, vortexed gently and 100 µl spread plated on nutrient agar containing 100µg ml-1cycloheximide and 100µg ml-1of antibiotics including: tetracycline, streptomycin, chloramphenicol and ampicillin.

Morphological, cultural identification and biochemical characterisation

The pure cultures were streaked on nutrient agar plates and single colonies examined for colonial characteristics (size appearance, colour, margins, elevation, texture etc.). A loopful of 24 hr old culture were gram stained and observed for cell shapes and gram reaction under oil immersion objective of a bright field microscope. Results for each isolate were tabulated. Standard biochemical tests were done on each isolate as per Bergys Manual of Systematic Bacteriology (Holt *et al.,* 1994) and results recorded.

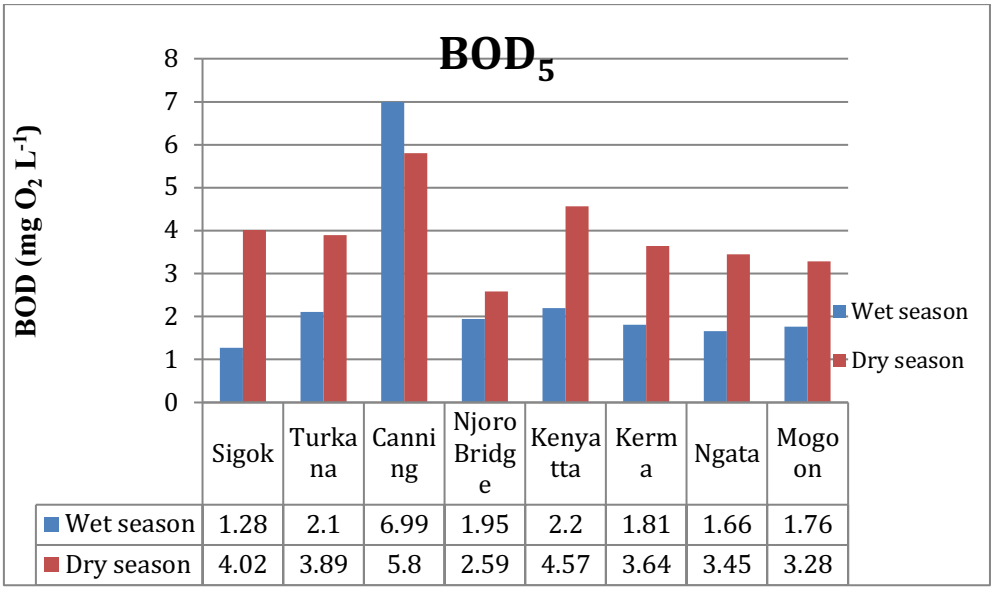
Statistical analysis

Data obtained was represented as Tables or graphs in Ms. Excel^{TM}. Statistical analysis was carried out on appropriate programs in SPSS^{R} software version 19. Significant level was set at $\alpha = 0.05$. Turkey test was performed to separate the means. The bacterial species for different sites was compared by descriptive statistics.

Results

Generally, there was higher BOD values recorded in the dry seasonas compared to the wet season. Canning Factory site demonstrated highest BOD_5 values whereas sigotik had lower BOD values.

Faecal pollution was observed in all sites of river Njoro even Sigotik the furthest site at 2400m upstream. Concentration of *E.coli* indicators increased downstream in wet season. Significantly higher ($p< 0.05$) concentrations were detected in the wet season compared to dry season. In dry season similar levels of pollution occurred in all sites.



	Sigok	Turkana	Canning	Njoro Bridge	Kenyatta	Kerma	Ngata	Mogoon
Wet season	1.28	2.1	6.99	1.95	2.2	1.81	1.66	1.76
Dry season	4.02	3.89	5.8	2.59	4.57	3.64	3.45	3.28

Figure 2. BOD_5 for both wet and dry months for the five sampling sites

Isolation and Identification of antibiotic resistant bacteria

There was resistance towards all the test antibiotics evidenced by growth of bacteria in media amended with antibiotics. The results for the first season and the second season i.e. the rainy season and the dry season indicated in the table below.

Biochemical tests were performed to identify the antibiotic resistant isolates. The coliform bacteria identified based on their IMViC reactions were mainly *E. coli* and *Enterobacter* species. The enteric pathogens identified up to the genus level were classified as *Salmonella* and *Shigella* species based on their TSI reaction and motility, while cytochrome oxidase positive organisms were identified as *Vibrio* species. *E coli* was identified and further tests were performed to determine its pathotype. Most of the *E. coli* isolated were non pathogenic while a few strains based on PCR pathotyping were entero -aggregative *E. coli (*EAEC), entero - pathogenic *E. coli* (EPEC) and entero- toxigenic *E. coli* (ETEC). *Klebsiella*species were also isolated and these were *K. Oxytoca* and *K. pneumonia*. The *Enterobac*ter species isolated were *E. aerogenes, E. cloacae* and *E. amnigenus*. Two *pseudomonas* species were also isolated and these are *P. aeruginosa*and*P. Putida*.*Aeromonas* species isolated were *A. hydrophila*and*A. sobria*. *Yersinia enterocolitica* and *Citrobacterfrendii* were also isolated.

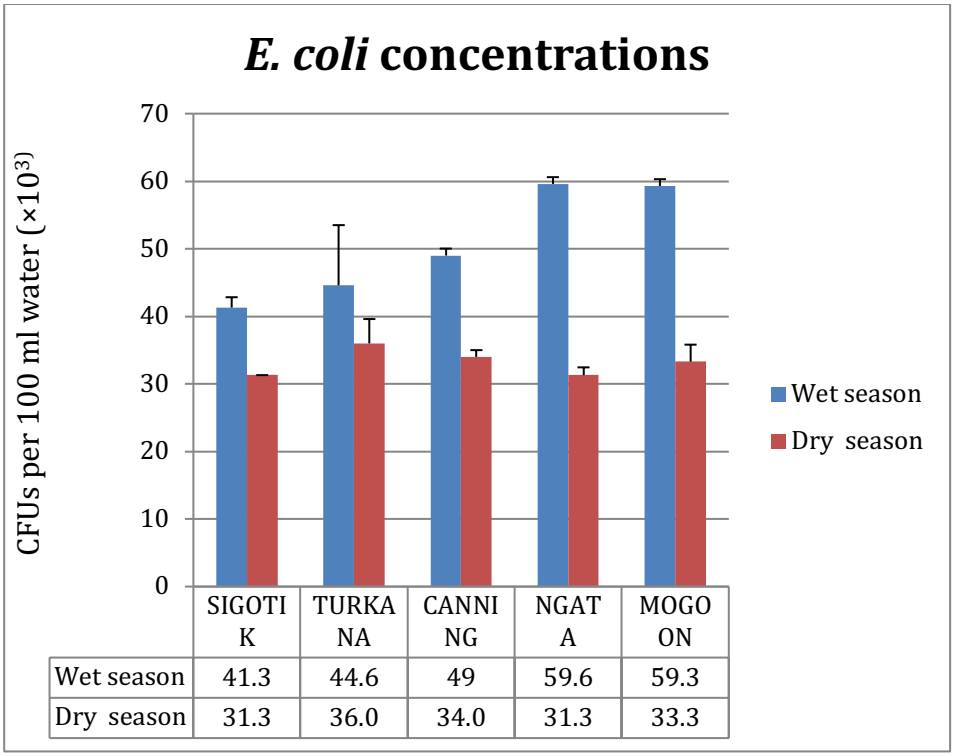


Figure 3. Concentration of faecal coliforms for the dry and wet season

Discussion

Microbiological water quality

According to water quality guidelines for drinking water, the results indicated that the various water sources were of poor microbiological quality. The lowest level of faecal coliforms recorded in both months was 3.13 x 10^4 cfu·ml^{-1}. However, according to (DWAF, 1998) the maximum limit for no risk of faecal coliforms is 0 cfu·100 ml^{-1}. The lowest total coliform recorded throughout the sampling times was 6.20 x 10^4 cfu·ml^{-1}. The counts exceeded the 5 cfu·100 ml^{-1}, which is the maximum recommended limit for no risk (DWAF, 1996: WRC, 1998).

Isolation of antibiotic resistant bacteria

There were high numbers of antibiotic resistant organisms in all the study sites along River Njoro even in the furthest point upstream (Sigotik). Generally there were more resistant strains in the dry season as opposed to the rainy season this could be probably be attributed to dilution effects during the rainy season as opposed to more stagnant waters with higher microbial activities in the dry season, which facilitates selective pressure and horizontal gene transfer. The significantly higher numbers of antibiotic resistant bacteria found in Turkana and Njoro Canning factory sites compared to other sites could be due to the high rate of pollution in these sites as evidenced by physiochemical parameters and microbiological quality indicators.

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