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ASSESSMENT OF BACTERIAL CONTAMINATION OF COASTAL MARINE WATERS IN FISH LANDING SITES IN URBAN DISTRICT, ZANZIBAR, TANZANIA

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ABSTRACT

Pollution of Zanzibar beaches and coastal marine waters through dumping of domestic wastes, fish processing and disposal of untreated wastewater from local toilets and municipal drainage systems highlights a great health safety concern that may also harm the marine water ecosystem. This study assessed levels of bacterial contamination by evaluating the log cfu/100ml of *E. coli*, *Salmonella* and *Vibrio spp* in coastal marine waters of the four major fish landing sites in the Zanzibar Urban district; Maruhubi, Malindi, Kizingo and Mazizini. The study also investigated the seasonal variability of contamination of the three types of pathogens. Contamination levels of *E. coli* was highest at Malindi and Kizingo (58%). Malindi also showed highest contamination of *Salmonella spp*. (46%) whereas Mazizini had highest contamination of *Vibrio spp.* (44%). The log cfu/100ml levels varied between 1.8 at Mazizini and 3.2 at Malindi for total coliforms and between 0.28 and 0.45 for fecal coliforms. There was a significant zonal variability in contamination levels for coliforms at Malindi relative to other sites, where the levels were higher in inshore zone than offshore. Inter-site comparison for pooled data showed significantly higher level of contamination on average at Malindi inshore for both total coliform ($p = 0.0002$) and fecal coliform ($p = 0.003$) than all other sites. There was a significant seasonal variability in coliforms contamination levels in all sites ($p = 0.014$ to $p << 0.0001$); higher contamination levels were observed in rainy seasons and low contamination during dry seasons. Isolations of public health related bacterial pathogens in this study might indicate presence of other types of pathogens in Zanzibar coastal waters. There is therefore need for establishment of coastal water monitoring measures and define local standards for beach water quality. Further, the results of the study raise the possibility of contamination of fish catches, hence the need of introduction of fish catch hygiene monitoring schemes.

KEYWORDS: Log cfu/100ml, fish landing sites, inshore zone, Zanzibar.

1. INTRODUCTION

Zanzibar beaches play an important economic role as source of entertainment of locals and tourists as well as fish landing sites. Fisheries is one of the most important economic sectors in Zanzibar that substantially supports traditional livelihood both economically and nutritionally.[1] In the last ten years (2006-2016) the sector contributed between 2.2 – 10.4% of the GDP.[2,3,4]

Fish processing at fish landing sites form an important activity in fisheries sector in Zanzibar. It involves scales removal, gut content removal, fish slicing and cleaning before second hand sale and/or consumption. From the landing site, fish catches are either transported to a market for sell or sent home for consumption. In many local fish landing sites, wastes from fish catch processing and its accompanied waste water are dumped on the beaches or disposed directly into the sea without any prior treatment.[5] These wastes undergo bacterial decomposition and produce unpleasant scent along areas surrounding fish landing sites. Fish stakeholders including auctioneers, fishers, fish processors and customers who have limited hygiene awareness on disposal of fish wastes at fish landing site, play a significant role in increasing bacterial contamination that decrease fish quality.

Zanzibar town has fish landing sites that are adjacent to beaches. Misuse of Zanzibar beaches as recipients for wastewater drainage systems, dumping sites for the municipal wastes and defecation make them prone to contamination by gastrointestinal and other bacterial pathogens. This practice, without prior water treatment might jeopardize health of beach users and fish consumers.[6] The most harmful pathogens recognized on beaches that are associated with human and birds' fecal wastes–include *Vibrio spp, E. coli, Salmonella, Shigella and Pseudomonas aeruginosa* that may be responsible for serious health consequences including dysentery, gastroenteritis, cholera, diarrhea and even deaths.[1,7,8,9] It is thus evidently clear that fish catch processing on fish landing sites in Zanzibar require high safety measures including basic sanitary facilities and knowledge in order to maintain standard hygiene and to produce quality fish as recommended by National Seafood HACCP Alliance (2000).[5]

Understanding the contamination status and seasonal variability of human related pathogens in fish landing sites is therefore vital for beach management purposes.

Knowledge gained from this study will be useful in formulating appropriate guidelines to improve hygiene of Zanzibar fish landing sites, beaches and fisheries products.

2. METHODOLOGY

2.1 Study sites description

This investigation was a cross-sectional study conducted in Unguja Island, Zanzibar (04^0 50" and 06^0 30" S, 39^0 10" and 39^0 50" E) (Fig.1). Unguja Island has three (3) administrative regions; North Unguja, South Unguja and Urban West. The Urban West region is the most populated with estimated population size of 593,678 that constitutes 46% of total population of Zanzibar.[10] Four major fish landing sites were in this study; Maruhubi, Malindi, Kizingo and Mazizini- (Fig. 1). Sea water tides in the area are semi diurnal and the spring tidal range is about 3 – 4 meters. The four sites are the main fish landing centers for fishers of the urban district and other districts who wish to sell their fish in town. Maruhubi and Malindi are located on low energy areas, with sandy to muddy bottom, north of Shangani peninsula towards the Maruhubi gulf as they are protected from strong waves during southeast monsoon winds, though slight strong waves during northeast monsoon winds hit the sites. In contrast, Kizingo and Mazizini sites are characterized by sandy bottom, south of Shangani peninsula and become exposed to strong waves during southeast monsoon winds. Fishermen camping is not practiced at Maruhubi and Malindi sites; instead fishers visit only during day time for fish auctioning. Kizingo and Mazizini both serve as fishing camping sites (locally named 'dago') during northeast monsoon winds, in which fishermen from different areas of Zanzibar camp for fishing activities. Maruhubi, Malindi and Kizingo are in close proximity to wastes discharging points from toilets. The drainages were installed during colonial period in the years of early 1900's. In addition, Malindi fish landing site receives untreated sewages from public toilets found around fish market. Maruhubi site is close to mangrove forests that is used as dumping ground for untreated sewage from septic tanks and pit latrines collected from Zanzibar Stone Town and peri-urban areas. Malindi and Maruhubi are active fish landing sites throughout the year while Kizingo and Mazizini are mostly visited during northeast monsoon wind periods.

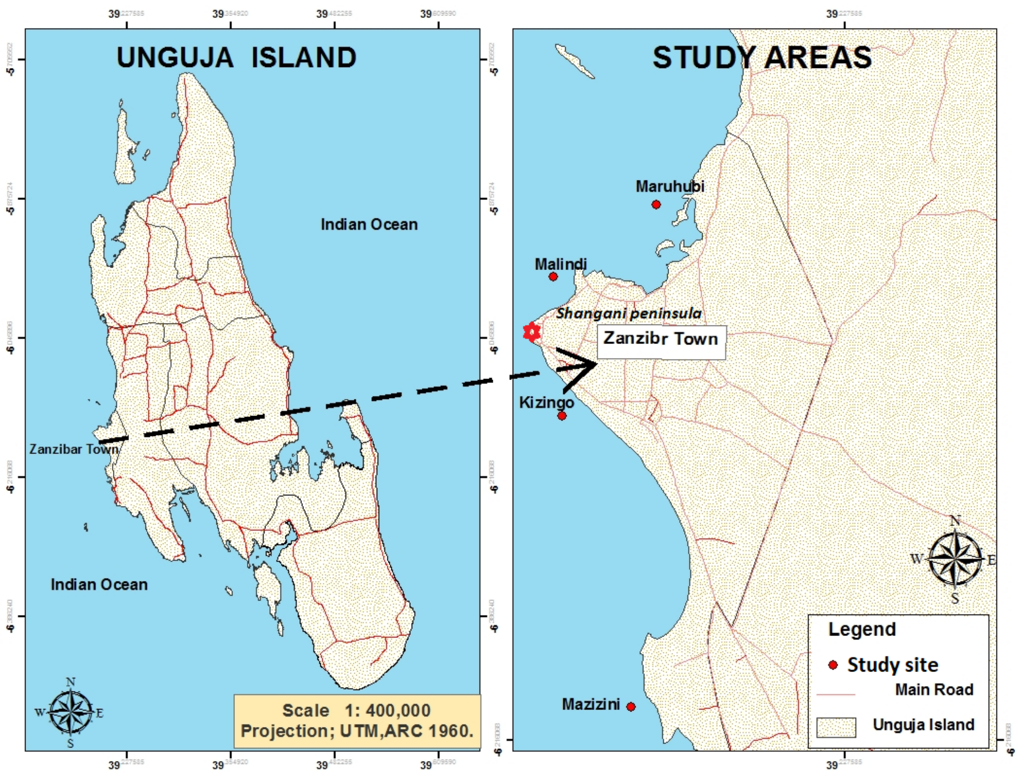

Figure 1: Map of Unguja island showing the studied Fish Landing sites; Maruhubi, Malindi, Kizingo and Mazizini.

2.2 Field sampling

Two sampling plots at each site, approximately 130m apart, were identified in each of the four sites and were permanently marked by using GPS MAP- 76. Dimensions of each plot was approximately 50m x 200m. The first plot was marked at the highest point of the high spring tide (the inshore plot) whereas the second plot was marked at the lowest point of the low spring tide (the offshore plot). Two-100m imaginary transects of 20 meters apart, parallel to the shore, were established in each plot. Each imaginary transects constituted three hot spots, 50 meters apart, from which samples were collected; six hot spots in each plot, making a total of twelve hot spots per site.

Water samples were collected in an aseptic condition by using sterile pre-labelled universal bottles (20ml each) from the identified hot spots during spring high tide. The bottles were then stored at 4^0C container and transferred to the laboratory within six hours of sample collection. First sampling was conducted in the long rain season in May 2015, followed by cool season sampling in August 2015, short rain season sampling in November 2015 and the final dry/hot season sampling in February 2016.

2.3 Laboratory analysis

Serial dilutions of seawater were done for each of the collected sea water sample (20ml) from the field using five sterile labelled test tubes each contained 9ml of sterile normal saline were placed in a test tube rack. 1ml of seawater sample was then inoculated using 1ml sterile pipette and it was put in a first labelled sterile test tube containing 9ml of sterile normal saline (0.85% Sodium Chloride). Five serial dilutions were made. For each dilution, duplicate culture was performed by using 10 micro liter pipette of that dilution.

2.3.1 Culture for coliforms

The culture of coliforms was done by using a technique described by USEPA.[11] Two replicates of 10 micro litter of each dilution was inoculated to MacConkey agar plate for total and fecal coli forms culture. A bent sterilized glass rod was used to spread out the diluted sample (bacterial suspension) in labelled sterile MacConkey agar plate. The sample plates were then incubated at 37 ^{0}C for 18-24hrs and 44 ^{0}C for 48hrs for total coli form and fecal coli forms respectively. Non-diluted samples were also cultured for total and fecal coliforms.

2.3.2 Determination of coliforms counts (colony forming unit/100ml) in a sample

Plate counter was used to count colonies after 24hrs and recorded in a work sheet. The pink colonies grown in media were described as lactose fermenting bacteria (e.g. *E. coli)* and the white colonies were described as non-lactose ferments. The levels of bacteria in terms of colony forming units (CFU per 100 ml) of original sample for both total and fecal coliforms was determined as:

CFU/100ml = Average number of colony x correction factor x dilution factor.

Where:

Average number of colonies was at an average count of two replicates-

Correction factor (10,000 that were at required volume to be reported) for CFU/100ml.

Dilution factor (a factor from respective dilution).

2.3.3 Identification of coliform bacteria (*E. coli*).

Pink lactose fermenting colonies were sub-cultured on MacConkey agar plates and incubated at 37 ^{0}C for 24hrs and at 44.5^0C for 18 -24hrs to obtain pure culture. Biochemical tests were performed for identification of common lactose ferments (*E. coli*).[12] are as follow:

Biochemical test	Results
Triple sugar test (TSI)	A/Ag
Citrate	-
Indole	+
Methyl red	+
Voges-Proskauer (VP)	-

2.3.4 Identification of Salmonella

One ml of each sample measured using sterile Pasteur pipette was introduced in a labelled sterile test tube containing 10ml of sterile enriched selenite broth. The mixture was incubated at 37 ^{0}C for 24hrs. Subculture was done in Xylose lysine deoxycholate (XLD) (Oxoid, England) agar plates. The plate was then incubated at 37 ^{0}C for 24hrs. Direct culture of each sample onto ~~a~~ XLD was also performed. Macroscopic and microscopic examinations were done and the results recorded in work sheet. Biochemical tests were performed to identify *Salmonella spp.*[12] as in Table below:

Biochemical test	Results
Triple sugar test (TSI)	Alk/A
Hydrogen sulphide (H_2S)	+
Citrate	+
Indole	+
Methyl red	+
Voges-Proskauer (VP)	-

2.3.5 Vibrio species identification

A 1ml of each sample pipetted using sterile Pasteur pipette was introduced in a sterile labelled test tube containing 10ml of sterile enriched alkaline peptone water (pH8.6). The broth was used to enhance their growth before doing subculture in agar plate. The mixture was incubated at 37 ^{0}C for 24hrs. Then, a sterile loop containing loop full of the mixture was streaked onto sterile labelled Thiosulfate citrate bile sucrose (TCBS) agar plate and incubated at 37 ^{0}C for 18- 24hrs. Macroscopic and microscopic characteristics were recorded. After sub-culture, one colony was picked out for biochemical tests for *Vibrio spp.* Identification.[12,13] Direct culture of each sample onto sterile labelled TCBS agar was also done.

Biochemical test	**Results**
Sucrose fermentation and growth on TCBS	+
Triple sugar test (TSI)	K/A
Oxidase	+
Methyl red	+
Voges-Proskauer (VP)	+
Indole	+

2.4 Data analysis

A generalized Linear Model – analysis of variance (GLM-ANOVA) was used for data analysis. The data were log-transformed to meet the parametric requirements. Two-way ANOVA test used to test for the seasonal variability and spatial variability within and among sites.

3. RESULTS

3.1 Distribution of bacterial contamination test

Total of 192 water samples from four sites, 48 samples per site, were collected during four different seasons (long rain season, cool season, short rain season and dry season) were tested for the presence of contamination of public health related pathogens; *E. coli*, *Salmonella* and *Vibrio.* Contamination of *E. coli* among sites varied from 33% in Mazizini to 58% in Malindi and Kizingo whereas contamination of Salmonella ranged between 29% at Maruhubi and 46% at Malindi (Table 1). Contamination of Vibrio ranged between 23% at Maruhubi and 44% at Mazizini.

Seasonal variability pattern for all detected human related pathogens, with exception to Maruhubi, was more or less similar among sites. The highest detection was during long rain and cool seasons relative to short rain and dry seasons (Table 1). The detection levels of *E. coli* among sites was highest at Mazizini (83%) and Malindi (100%) during long rain season and cool season respectively (Table 1) whereas during dry season the detection levels went to as low as 0% exemplified by Malindi site. On the contrary, Maruhubi revealed higher detection levels of *E. coli* during cool (75%) and short rain (50%) seasons (Table 1). Detection levels of *Salmonella* was relatively higher at Malindi (83%), Mazizini (75%) and Maruhubi (75%) during cool season than at Kizingo. Detection levels of *Vibrio spp.* was highest at Kizingo and Mazizini (83% each) during long rain season and was nil (0%) during short rain and dry season at Kizingo. By considering individual pathogen, there was no clear zonal pattern of detection levels within sites, with exception to Malindi, where detection level was relatively higher in inshore than offshore waters (Table 1).

Table 1: Seasonal and site variability of bacterial counts-shown in percentage (%) *

Species	**Site**	**Seasons**											
		Long rain			**Cool season**			**Short rain**			**Dry season**		
		inshore	**offshore**	**mean**	**inshore**	**offshore**	**mean**	**inshore**	**offshore**	**mean**	**inshore**	**offshore**	**mean**
E. coli	MR	0.0	16.7	8.3	66.7	83.3	75.0	16.7	83.3	50.0	16.7	0.0	8.3
	ML	83.3	50.0	66.7	100	100	**100**	100	33.3	66.7	0.0	0.0	**0.0**
	KZ	66.7	83.3	75.0	83.3	66.7	75.0	100.0	50.0	75.0	16.7	0.0	8.3
	MZ	83.3	83.3	**83.3**	50.0	16.7	33.3	0.0	0.0	0.0	33.3	0.0	16.7
Salmonella	MR	16.7	16.7	16.7	66.7	83.3	**75.0**	0.0	33.3	16.7	16.7	0.0	8.3
	ML	66.7	50.0	58.3	83.3	83.3	**83.3**	50.0	0.0	25.0	33.3	0.0	16.7
	KZ	66.7	66.7	66.7	66.7	66.7	66.7	16.7	0.0	8.3	16.7	0.0	8.3
	MZ	33.3	50.0	41.7	50.0	100	75.0	16.7	0.0	8.3	0.0	0.0	0.0
Vibrio	MR	16.7	16.7	16.7	16.7	66.7	41.7	33.3	0.0	16.7	0.0	33.3	16.7
	ML	66.7	50.0	58.3	66.7	66.7	66.7	0.0	0.0	0.0	16.7	0.0	8.3
	KZ	83.3	83.3	**83.3**	83.3	33.3	58.3	0.0	0.0	0.0	0.0	0.0	**0.0**
	MZ	83.3	83.3		16.7	83.3	50.0	0.0	50.0	25.0	33.3	0.0	16.7

Key: MR-Maruhubi ML-Malindi KZ-Kizingo MZ-Mazizini

* *The values are % of samples that showed presence of respective human pathogen*

3.2 Levels and spatial variability of coli form bacteria identified

When respect to log cfu/100ml, the contamination levels varied between 1.8 at Mazizini and 3.2 at Malindi for total coliform and between 0.28 and 0.45 for fecal coliform. The results revealed significant zonal variations in contamination level at only Malindi for both total coliform ($p = 0.0002$; Fig.3) and fecal coliforms ($p = 0.003$; Fig.4), where the levels were higher in inshore zone than offshore. When Malindi inshore is considered as independent site, inter-site comparison for pooled data showed significantly higher level of contamination on average for both total coliform ($p = 0.0001$; Fig. 3) and fecal coliform ($p = 0.003$; Fig.4) than all other sites. Both coliform levels were almost twice as much to other sites (Figs. 3 and 4).

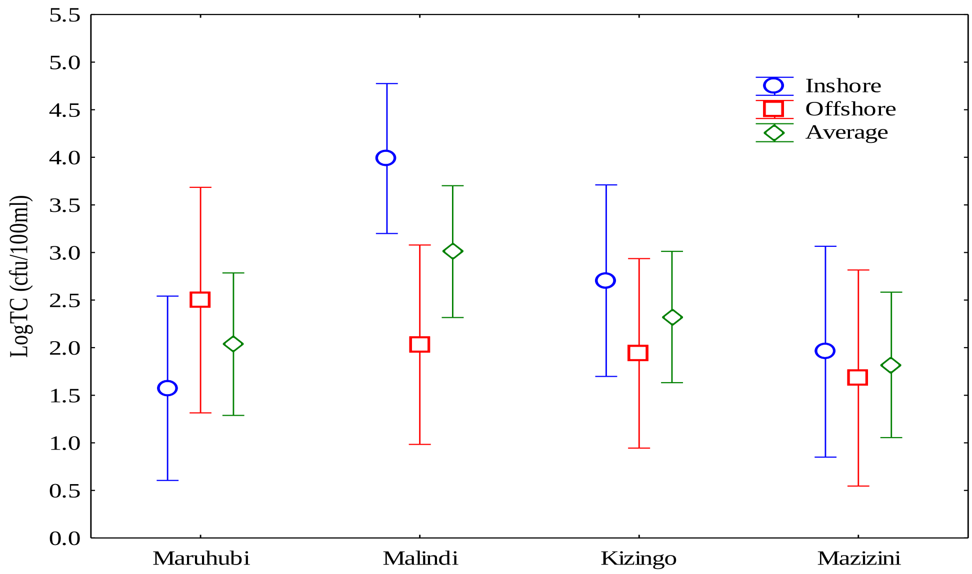

Figure 3: Inter-zonal variability and site averages in total coliform. Vertical bars denote 0.95 confidence interval.

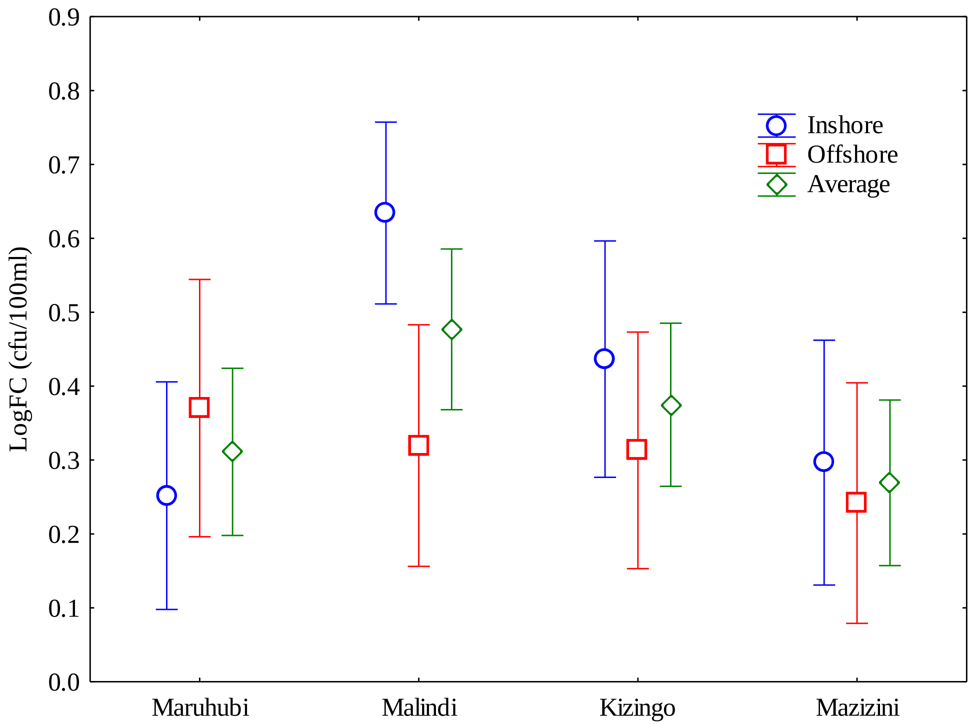

Figure 4: Inter-zonal variability and site averages in fecal coliform. Vertical bars denote 0.95 confidence interval.

3.3 Temporal variation of identified coli form bacteria

The study clearly indicated seasonal variability in coliforms within sites (Fig 5). Total coliform contamination level in log cfu/100ml revealed a wide range of variations among seasons within sites, ranging from 0 to 5.24cfu/100ml in dry season to long rain seasons respectively. There was a significant seasonal variability in total coliform contamination level in all sites; Maruhubi ($p = 0.0001$), Malindi inshore ($p = 0.002$), Malindi offshore ($p = 0.014$), Kizingo ($p << 0.0001$) and Mazizini ($p << 0.0001$). Generally, higher contamination levels were observed during cool to short rain seasons at Maruhubi and Malindi. On the contrary, for Kizingo and Mazizini, higher contamination levels were observed during long rain seasons.

In contrast to total coliform, the contamination level for fecal coliform (logcfu/100ml) showed a very narrow variation among seasons, ranged mainly from 0 in dry season to 0.78 log cfu/100ml in short rain season (Fig. 5). Despite the narrow range in variations among seasons, significant seasonal variabilities in contamination levels for fecal coliform was revealed in all sites; Maruhubi (p = 0.0002), Malindi inshore (p = 0.014), Malindi offshore (p = 0.018), Kizingo (p << 0.0001) and Mazizini (p << 0.0001). Mostly, there were higher contamination levels in rain and cool seasons and low contamination in dry seasons.

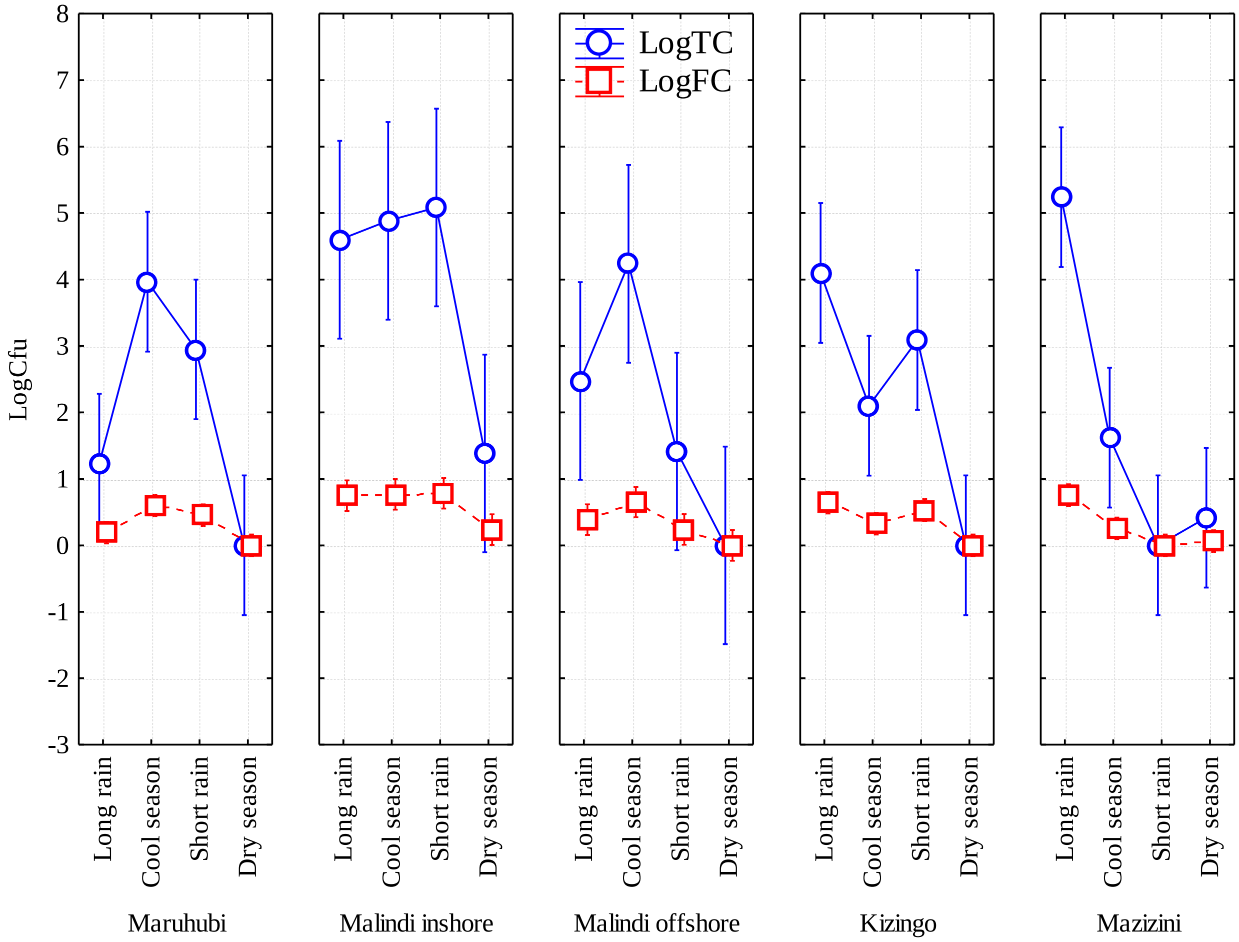

Figure 5: Seasonal variability of total and fecal coliform bacteria contamination level (log cfu/100ml) in five monitored sites.

4. DISCUSSION

Different methods have been used to assess the hygiene and quality of coastal waters. Amongst them is fecal pathogenic bacteria, of which the presence and estimate of fecal coliform bacteria implies polluted state.[14]

The detected bacterial contamination ratio within collected samples in this study was generally above 20%. Even though Zanzibar is yet to establish standard beach water quality, the observed ratio of contaminated samples is higher than the recommended international standards.[15]

The observed contamination level for intertidal areas around landing sites in stone town Zanzibar cautions the possibility of having much more varieties of disease-causing pathogens. The results further suggest that there is a great need for introducing monitoring procedures and establish local standards for beach water quality. Higher contamination of *E. coli* suggests the possible source of pollution was recent fecal contamination originating from sewage effluents from household uses. It could as well be deduced that the possibility of contaminating landed fish catches could also be high and that fish hygiene monitoring scheme needs should be introduced.

In this study higher ratios of contamination within samples was generally observed during long rain and cool seasons. Among the possible reasons that could contribute to these trends include the fact that the pathogens originated from human feces and water dependent for their proliferation, survival and spreading. During long rainy seasons (March to May) most of sewage storing chambers in stone town become over flooded as observed by Moynihan *et al.*, (2012).[16] It is thus during the rain seasons, where high rates of effluents inflow from upland in stone town into coastal waters take place, and that high rate of effluents supports proliferation and spreading of pathogens. Likewise, precipitation may cause diffuse land-based runoff that concentrates bacterial pathogens from urban effluents and accumulate into coastal waters. Moreover, the availability of prolonged effluents inflow from June to early September continues to provide suitable conditions for proliferation, survival and spreading of pathogens. On the contrary, dry and short rain seasons could play a lesser role on bacterial contamination of coastal marine waters. The results emphasize the need to consider effect of rain seasons in introducing mitigation measures to manage beach pollution.

Zonal variation in coliform bacteria was clearly observed at Malindi relative to other sites, where inshore had higher levels of coliform bacteria than offshore areas. The plausible explanation for this trend is the presence of public toilets at the edge of the beach at Malindi landing site. These toilets were found to possess a number of leaking points along their main sewage pipes that pour their sewage at far distance from the coast. The leaking effluents which produce foul-smelling at Malindi fish auction place, could thus constantly add the coliform pathogenic bacteria at inshore Malindi coastal water. This explanation could also justify for significantly higher level of coliform bacteria at Malindi inshore relative to all other sites.

This study observed distinct pattern for maximum levels of human pathogen across sites. Maruhubi and Malindi revealed higher levels of contamination during cool seasons. Maximum levels were observed to be associated with calm seasons. It can thus be suggested that the observed trend is crucial for implementation of any management intervention that seek to minimize proliferation of pathogens and reduce contamination level.

Detection of human pathogenic bacteria in collected water samples around important fish landing sites in Zanzibar town indicates vulnerability of fish catches in the investigated landing sites. The observed ranges of identified public health related pathogens; *E. coli*, *Salmonella* and *Vibrio* confirm the presence of such pathogens in the beach environment. The results imply that there is a great need of behavior change related to fish handling prior and post market. Fishers and fish mongers use to carelessly drag bunches of fish catch on the intertidal sea floor before market and after market for gut contents removal (personal observation). In addition, fish auctions are normally done in the intertidal sea floor. These practices contaminate fish catches and ultimately reduce fish quality that results in deterioration and reduction of shelf-time.[17,18] No wonder that consumers therefore become prone to various gastrointestinal diseases. Cholera outbreak occurred at Mazizini fish camp around Mazizini landing site in late 1990s' and early 2000s' could possibly be linked to such risky practices.

One of the contributing major sources of human pathogens detected in the fish landing sites is aged infrastructure that drain their effluents in the coastal waters of the stone town. Likewise, waste waters from hotels located adjacent to the beaches of stone town are also suspected to contribute to such high levels of human pathogens. There is therefore an urgent need to install new wastewater drainage systems to replace the old ones and establish regulations for wastewater handling and treatment in Zanzibar.

The observed temporal variability of detected human pathogens, being relatively higher during long rain season for Maruhubi and Malindi and during cool seasons for Kizingo and Mazizini implies the differences in suitability for implementation of management option for the control of human related pathogens. Any management intervention for control of contamination of human related pathogens should be highly considered during cool seasons for Maruhubi and Malindi landing sites and during long rain season for Kizingo and Mazizini.

5. CONCLUSION

This study revealed the presence of contamination of public health related pathogens; *E. coli*, *Salmonella* and *Vibrio* in coastal marine waters surrounding fish landing sites in Zanzibar urban district. The findings highlight the need to start considering regular monitoring and set local standards for beach water quality and that special attention of management interventions be considered during long rain and cool seasons. Likewise, the studies to isolate these pathogens from landed catches can further give more insight into the possible level of infection.

6. ACKNOWLEDGMENTS

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