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Full Length Research Paper

Diurnal and seasonal variations of pathogenic bacteria in Dandora Sewage Treatment Plant wastewater, Nairobi, Kenya

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Diurnal and seasonal variation of pathogenic bacteria diversity and loads at Dandora Sewage Treatment Plant (DSTP), and compliance of effluent with local and international statutory requirements was assessed. Standard bacteriological techniques were used to describe bacteria content from wastewater samples collected from influent and effluent sources. Diurnal variation of bacterial loads occurred only in the effluent ($F = 22.788$, $p = 0.000$) with lower counts in the afternoon. Seasonal variation was observed in both influent ($F = 14.795$, $p = 0.001$) and the effluent ($F = 23.574$, $p = 0.000$), with more pollution during the dry season. The effluent microbiological quality, irrespective of diurnal and seasonal changes, did not adhere to local and international statutory requirements for discharge into natural environment. The effluents were polluted with pathogens including; *Escherichia coli*, *Enterococcus faecalis, Staphylococcus typhi, Pseudomonas aeruginosa,* and *Klebsiella aerogenes*. The health risk posed to downstream users of DSTP effluent occurs notwithstanding the time of the day or season. The findings in this study suggest need for appropriate measures to monitor and control the microbiological quality of DSTP effluent and other similar facilities in sub-Saharan Africa, to ensure public health safety in line with the millennium Development Goals.

Keywords: Wastewater, bacterial pathogens, seasonal variation, diurnal variation, water scarce region

INTRODUCTION

Wastewater represents a major source of microbial pollution in water bodies receiving raw or even partially treated sewage (Okoh *et al.,* 2007). The microbes in wastewater include bacteria, viruses, protozoa, helminths and fungi (Kim *et al.,* 2007). These microbes are mainly excreted in the faeces of humans, birds, and animals (Bitton, 2005). Waterborne pathogens pose health risk when wastewater is reused either as raw drinking water or for agricultural purposes (WHO, 1989).

In regions with water scarcity such as sub-Saharan Africa, water bodies that receive wastewater pollution also serve as major sources of domestic water to vast number of population (Sabae and Rabeh, 2007). Studies have associated wastewater microbes with increased incidences of waterborne diseases (Shuval, 2003; Hamner *et al.,* 2006). The diseases are acquired through direct contact with wastewater (Habari *et al.,* 2000), inhaling aerosols generated in sprinkler irrigation (Shuval *et al* 1989), drinking contaminated water (CDC, 2004), and eating sea foods harvested from wastewater polluted sources (Shuval, 2003).

The diversity and density of pathogens in wastewater vary depending on the diversity and prevalence of infections in the population producing the wastewater (Petterson and Ashbolt, 2003), seasonal changes (Wemedo *et al.,* 2012), and the time of estimation (Kim *et al.,* 2009). This suggests that wastewater must not be discharged into natural environment without proper treatment, and that wastewater treatment must be reliable and subject to frequent monitoring in order to

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ensure public health safety within the Millennium Development Goals (MDGs) adopted by the United Nations General Assembly in the year 2000.

In order to safeguard public health and protect environment from wastewater discharge, both international and local guidelines have been put in place. The international guideline developed by World Health Organization (WHO) is based on intended use of effluent. Microbiological quality of effluent used in irrigation of crops that are eaten uncooked, sports fields, and public parks in unrestricted regions should not exceed 10^3 faecal coliforms (FC) per 100 mL (WHO, 1989). In the US, the Environmental Protection Agency (EPA) has set 0 FC / 100 mL standard for effluent use in irrigation of any food crops not commercially processed including crops eaten raw (EPA, 1992). In Kenya, the National Environmental Management Authority (NEMA) standard dictates that effluents being discharged into natural water bodies should not contain *Escherichia coli* per 100 mL, and that Total Coliforms (TC) should not exceed 30 organisms per 100 mL (Kenya Gazette, 2006).

Dandora sewage treatment plant (DSTP) at Ruai in Nairobi City processes wastewater which is generated from the city. This plant is the largest in Kenya and was established in 1980 for a projected population of one million inhabitants in the Nairobi area. Since then, the population has increased three-fold to 3.5 million residents (CBS, 2010). Wastewater is processed through physical and biological processes in waste stabilization ponds, and finally discharged into Nairobi River without further processing.

The DSTP effluent together with Nairobi River water is extensively used for crop irrigation downstream along river Athi. It is therefore important to assess the human pathogens associated with the processed wastewater from DSTP in order to safeguard public health of communities downstream of Nairobi River. This study therefore aimed at assessing the potential pathogenic bacteria diversity, density, and seasonal and diurnal variations in the sewage treatment plant as well as effluent compliance with national and international standards.

MATERIALS AND METHODS

Study area

Dandora sewage treatment plant (DSTP), the largest wastewater stabilization system in East Africa, is located in Ruai location, of Nairobi County, Kenya. It is situated 26 kilometers east of the City. It receives domestic sewage, and industrial wastewater, as well as run-off water from the city. It has a dry weather capacity of 80,000 m^3/d but it is designed to handle peak capacity of 240,000 m^3/d full treatments and storm water treatment capacity of 300,000 m^3/d (Pearson *et al.,* 1996).

Wastewater pre-treatment processes include physical screening at the plant intake followed by biological processing in waste stabilization ponds. The plant has a design retention value of 52 days, and faecal coliform die-off value of 0.65 per day (Pearson *et al.,* 1996). The reclaimed wastewater from DSTP is discharged into river Nairobi, a tributary of river Athi, without further processing. The combined waters of Nairobi and Athi rivers constitute a major source of domestic water and agricultural irrigation for downstream communities.

Study design

The study design was purposive. Sampling points were deliberately chosen to account for microbial loads in the DSTP influent before physical treatment and effluent wastewater, discharged into Nairobi River, after treatment in waste stabilization ponds. The sampled wastewater volumes and depth of sampling were as recommended by standard methods for water and wastewater examination (APHA, 1998).

In order to include both dry and wet seasons in the sampling frame, 12 water samples were taken weekly during the dry spell (January to March, 2010) and a similar number taken during the wet season (late March to June, 2010). To evaluate diurnal microbial variation, water samples were collected in the morning (0900 hours) and again in the afternoon (1700 hours) for the entire study period (January to June, 2010).

Wastewater sampling procedures

Four duplicate samples were taken weekly from each sampling point from January to June, 2010. A total of 96 bacteriological samples were collected in clean sterile screw capped 250 millilitres (ml) polypropylene bottles. The sampled wastewater volumes and depth of sampling were done in accordance with standard methods for water and wastewater examination (APHA, 1998). The samples were transported to the DSTP laboratory in ice packed cooler boxes and analyzed within 2 hours.

Isolation and characterization of bacterial isolates

Bacterial diversity and loads were determined by serial dilution and plating of water samples on differential culture media. The isolates were then identified and biochemically characterized following the methods described in Bergey's Manual of Systematic Bacteriology (Kreig and Holt, 1984).

Data analysis

Statistical Package for Social Sciences (SPSS) version

Table 1. Diurnal bacterial variation in influent wastewater

Bacteria Type	Mean Count (CFU / 100 mL)		Student *t*-test
	Morning Session	Afternoon Session)	
Escherichia coli	$2.1 \times 10^7 \pm 4.2 \times 10^7$	$2.8 \times 10^7 \pm 3.4 \times 10^7$	0.644
Klebsiella aerogenes	$1.2 \times 10^6 \pm 3.5 \times 10^6$	$5.9 \times 10^6 \pm 1.7 \times 10^7$	0.353
Enterococcus faecalis	$3.2 \times 10^6 \pm 3.6 \times 10^6$	$3.6 \times 10^6 \pm 2.7 \times 10^6$	0.767
Pseudomonas aeruginosa	$1.6 \times 10^6 \pm 3.5 \times 10^6$	$4.9 \times 10^6 \pm 4.4 \times 10^6$	0.058
Salmomella typhi	$3.1 \times 10^5 \pm 6.8 \times 10^5$	$1.1 \times 10^6 \pm 1.3 \times 10^6$	0.091
Salmomella paratyphi	$9.3 \times 10^2 \pm 1.0 \times 10^3$	$1.1 \times 10^3 \pm 4.7 \times 10^2$	0.758
Vibrio cholerae	$7.5 \times 10^4 \pm 1.3 \times 10^5$	$8.5 \times 10^4 \pm 1.5 \times 10^5$	0.094
Proteus mirabilis	$2.8 \times 10^3 \pm 3.6 \times 10^3$	$6.4 \times 10^3 \pm 1.3 \times 10^4$	0.390
Shigella flexneri	$5.4 \times 10^2 \pm 1.2 \times 10^3$	$1.2 \times 10^3 \pm 2.3 \times 10^3$	0.384
Vibrio parahaemolyticus	$6.2 \times 10^2 \pm 1.6 \times 10^2$	$4.5 \times 10^2 \pm 4.8 \times 10^2$	0.598

Table 2. Diurnal **b**acterial variation in the effluent

Bacteria Type	Mean Count (CFU / 100 mL)		Ten-fold Variation	Student *t*-test
	Morning Session	Afternoon Session		
Enterococcus faecalis	$5.1 \times 10^4 \pm 2.6 \times 10^3$	$1.6 \times 10^3 \pm 5.5 \times 10^2$	1	0.004
Escherichia coli	$2.4 \times 10^4 \pm 7.2 \times 10^3$	$1.1 \times 10^3 \pm 1.5 \times 10^2$	1	0.003
Pseudomonas aeruginosa	$4.0 \times 10^2 \pm 4.5 \times 10^2$	$1.0 \times 10^2 \pm 1.2 \times 10^2$	0	0.036
Salmonella typhi	$7.8 \times 10^1 \pm 7.8 \times 10^1$	$9.0 \times 10^0 \pm 1.4 \times 10^1$	1	0.006
Klebsiella aerogenes	$7.5 \times 10^1 \pm 9.1 \times 10^1$	$6.0 \times 10^0 \pm 7.0 \times 10^0$	1	0.016

16 for Windows was used to calculate means and Standard Deviations. Student *t* - test was used to test the significance of diurnal and seasonal microbial loads variation.

RESULTS

Diurnal variation of bacteria in influent wastewater

The bacterial types at DSTP influent were similar irrespective of whether the measurement was carried out in the morning or in the afternoon (Table 1). *Escherichia coli* was the most dominant bacteria regardless of the time of day and its levels were between 2.1×10^7 and 2.8×10^7. The least dominant bacteria were *V. parahaemolyticus*. Pollution levels associated with the other bacteria namely, *K. aerogenes*, *E. faecalis, P.aeruginosa, S. typhi, S. paratyphi,V. cholerae, P. mirabilis,* and *S. flexneri* are shown in Table 1. Bacterial loads were higher in the afternoon session. However, the difference between the morning and the evening loads was not statistically significant ($F = 0.138$, $p = 0.710$).

Diurnal variation of bacteria in effluent wastewater

The bacterial types found in effluent wastewater were similar in the morning and in the afternoon session (Table 2). Nevertheless, bacterial loads varied; with morning loads being ten-fold higher than observed in the afternoon with respect to *Escherichia coli, E. faecalis*, *S. typhi* and *K. aerogenes* (Table 2).

E. faecalis was the most dominant bacteria regardless of the time of day and its levels were between 1.6×10^3 and 5.1×10^4. Generally, microbial levels in the effluent varied between the morning and the afternoon sessions, with lowest counts being recorded in the afternoon ($F = 22.788$, $p = 0.000$). The highest variation was observed with *E. faecalis* and *E. coli* ($p = 0.000$).

Seasonal variation of bacteria in influent wastewater

Bacterial types in the influent wastewater remained the same as found earlier with respect to diurnal variation (Tables 2 and 3). There was bacterial loads variation between the dry and rainy seasons, with lowest counts being recorded during the rainy season. The variation was in range of 1 to 3 ten-fold (Table 3).

Seasonal bacterial variation in the effluent

Bacterial types in the effluent during the dry season were

Table 3. Seasonal bacterial variation in influent wastewater

Bacteria Type	Mean Count (CFU / 100 ml)		Tenfold Variation	Student *t*- test
	Dry Season	Rainy Season		
Escherichia coli	$4.9 \times 10^{7} \pm 3.7 \times 10^{7}$	$2.8 \times 10^{5} \pm 3.4 \times 10^{5}$	2 fold	0.011
Klebsiella aerogenes	$7.1 \times 10^{6} \pm 1.6 \times 10^{7}$	$1.5 \times 10^{4} \pm 3.4 \times 10^{4}$	2 fold	0.015
Pseudomonas aeruginosa	$6.1 \times 10^{6} \pm 3.7 \times 10^{6}$	$4.2 \times 10^{4} \pm 3.2 \times 10^{4}$	2 fold	0.012
Enterococcus faecalis	$5.8 \times 10^{6} \pm 3.9 \times 10^{4}$	$7.7 \times 10^{3} \pm 4.4 \times 10^{2}$	3 fold	0.001
Salmonella typhi	$1.4 \times 10^{6} \pm 1.3 \times 10^{6}$	$9.4 \times 10^{3} \pm 1.0 \times 10^{4}$	3 fold	0.002
Vibrio cholerae	$1.6 \times 10^{5} \pm 1.7 \times 10^{5}$	$8.5 \times 10^{2} \pm 1.5 \times 10^{3}$	3 fold	0.003
Proteus mirabilis	$9.1 \times 10^{3} \pm 1.8 \times 10^{4}$	$1.6 \times 10^{2} \pm 2.2 \times 10^{2}$	1 fold	0.034
Salmonella paratyphi	$2.0 \times 10^{3} \pm 1.9 \times 10^{3}$	$1.1 \times 10^{1} \pm 5.0 \times 10^{0}$	2 fold	0.002
Shigella flexneri	$1.8 \times 10^{3} \pm 2.9 \times 10^{3}$	$1.8 \times 10^{1} \pm 2.5 \times 10^{1}$	2 fold	0.014
Vibrio parahaemolyticus	$1.0 \times 10^{3} \pm 1.4 \times 10^{3}$	$3.2 \times 10^{1} \pm 2.7 \times 10^{1}$	2 fold	0.018

E. faecalis, S. typhi and *V. cholera* had the highest variation (3 ten-fold) and the least variation was associated with *P. mirabilis* (1 ten-fold) (Table 3). In general, the levels for the ten bacteria in the influent wastewater were lower during the rainy season (F= 14.795, p = 0.001).

Table 4. Seasonal variation of bacteria in the effluent

Bacteria Type	Mean Count (CFU / 100 mL)		Ten-ford Variation	Student *t*-test
	Dry Season	Rainy Season		
Enterococcus faecalis	$5.1 \times 10^{4} \pm 1.2 \times 10^{2}$	$1.2 \times 10^{3} \pm 3.0 \times 10^{2}$	2	0.000
Escherichia coli	$2.5 \times 10^{4} \pm 7.9 \times 10^{3}$	$9.4 \times 10^{2} \pm 3.6 \times 10^{2}$	2	0.000
Pseudomonas aeruginosa	$4.5 \times 10^{2} \pm 4.1 \times 10^{2}$	$5.6 \times 10^{1} \pm 7.7 \times 10^{1}$	1	0.004
Salmonella typhi	$8.8 \times 10^{1} \pm 7.5 \times 10^{1}$	0	1	0.000
Klebsiella aerogenes	$8.0 \times 10^{1} \pm 1.0 \times 10^{1}$	0	1	0.012

Enterococus faecalis and *Escherichia coli* had the highest variation (2 ten-fold) and the least variation was associated with *Pseudomonas aeruginosa, Klebsiella aerogenes* and *Salmonella typhi* (1 ten-fold) (Table 4). In general, bacterial levels were reduced during the rainy season for all the five bacteria found in the effluent (Table 4). Cumulatively, the difference in bacterial levels between the rainy and the dry season were statistically significant (F = 23.574, p = 0.000).

similar to those found in rainy season. However, bacterial level varied, with more counts being found during dry season than rainy season. The bacterial counts during the rainy season were 1 to 2 ten-fold lower than in dry season (Table 4).

DISCUSSIONS

The aim of this study was to assess the diurnal and seasonal patterns in the occurrence of pathogenic bacteria in Dandora Sewage Treatment Plant (DSTP) wastewater. This was achieved by sampling influent wastewater before physical screens, and effluent before discharge into the Nairobi River.

The types and concentrations of bacteria isolated from DSTP influent were similar in the morning (0900 h) and the afternoon samples. The diversity and density of wastewater microbes depends on the health status (Petterson and Ashbolt, 2003), the time of estimation (Kim *et al.,* 2009), as well as the defecation patterns of the sewered population (Horan, 2005). The finding in DSTP suggests similarity in diversity of microbes, prevalence of infections, and defecation patterns among Nairobi city dwellers.

E. coli was the most dominant bacteria and the least dominant was *V. parahaemolyticus* regardless of the time of day. The level of a particular pathogen, secreted in faeces or urine of infected person into wastewater depends on the prevalence of infections in the community producing the wastewater (Mara, 2004). This suggests that infections associated with *E. coli* are higher than any other among Nairobi city residence. *E. coli* causes a wide range of infections, including urinary tract infections (UTI) and diarrhoea diseases in all age groups (Chesbrough, 2006). It is the dominant pathogen in UTI causing approximately 80% of the infections in human population (Al-Haddad, 2005).

Similar to the finding at the DSTP intake wastewater, there was no bacterial variation between the morning and the afternoon sessions at the effluent. Variation in bacterial concentrations was observed with higher pollution in the morning than in the afternoon. This finding differs from that of Machibya and Mwanuzi (2006) at Kilombero Sugar Wastewater Stabilization Ponds in Tanzania. Machibya and Mwanuzi (2006) observed one log increase of *Escherichia coli* levels during the afternoon hours; bacterial die-off is expected to be higher during the day due to the influence of light-mediated factors (Mara, 2004; Kim *et al.,* 2009). Machibya and Mwanuzi (2006) attributed their finding to poor design of waste stabilization ponds.

Seasonal changes brought about variation in levels of bacterial pathogens in both influent and effluent wastewater at DSTP, with lower microbial load being recorded during rainy season (late March to June, 2010). The current study finding corroborates that of Wemendo *et al.* (2012) who reported higher bacterial densities in dry season than wet season. Seasonal changes in the prevalence of bacterial diseases are common and the concentration of bacteria in wastewater may be related to the number of people with a disease in any given day (Feachem *et al.,* 1983; Horan, 2005). Additionally, in combined sewer system, like the case of DSTP, wastewater quality is subject to dilution by rain water (Ulrich *et al.,* 2004; Rhee *et al.,* 2009).

Effluent bacterial densities showed seasonal variation with higher counts being observed during dry season than rainy season. The low bacterial levels in wastewater during the rainy season can be attributed to dilution of wastewater microbial quality in stabilization ponds (Ulrich *et al.,* 2004; Rhee *et al.,* 2009). The current study finding corroborated with those of Hodgson (2007) who observed low bacterial counts in the effluent of Akosombo Waste Stabilization Ponds, Ghana due to rain water dilution.

DSTP failed to meet local and international requirements for discharge of effluents irrespective of day or seasonal changes. The international guidelines have been set by World Health Organization (WHO) dictate that, effluent used for irrigation of crops likely to be eaten raw should not exceed 10^3 faecal coliform per 100 mL of wastewater (WHO, 1989). When the WHO guideline is met, no pathogen should be detectable in the wastewater effluents, but this was not the case for DSTP effluent, containing pathogens such as *E. coli, E. faecalis*, *S. typhi, P. aeruginosa,* and *K. aerogenes*. The local standard for discharge of effluents into natural environment has been published by National Environmental Management Authority (NEMA). NEMA standard states that no E. coli should be detectable per 100 mL of wastewater discharged into environment (Kenya Gazette, 2006).

The reclaimed effluent from this plant is discharged into Nairobi River without further processing that pours its contents into Athi River. The combined water of the two rivers form the main source of domestic and irrigation water for communities downstream. This suggests that poor microbiological quality of DSTP effluent poses serious public health risk to the downstream users along Athi River.

Discharge of untreated or partially treated wastewater is attributed to the on-going global pollution challenges of natural water bodies (Doughari *et al.,* 2007). Consequently, waterborne diseases have increased considerably among populations relying on natural water bodies as a primary source of domestic water (Hamner *et al.,* 2006). Wastewater must not be discharged into natural environment without proper treatment, and that the treatment must be reliable and subject to frequent monitoring in order to ensure public health safety within the Millennium Development Goals (MDGs) adopted by the United Nations General Assembly in the year 2000.

CONCLUSION

Diurnal variation of bacteria occurred only at the effluent wastewater of the Dandora Sewage Treatment Plant (DSTP) with higher loads in the morning (0900 h) than afternoon (1700 h). Seasonal changes affected bacterial load in both influent and effluent of the DSTP. Pollution was lowest during the rainy season (late March to June, 2010) due to rain water dilution of wastewater.

The microbiological quality of DSTP effluent, irrespective of diurnal and seasonal changes, did not meet both international and local statutory requirements for discharge into natural environment. Therefore, health risk posed to downstream users of the effluent occurs notwithstanding the time of the day or season. In resource scarce region of the world, these findings underline the challenges a number of developing countries are facing currently and in long-term into the future. Lessons learnt in this study suggest appropriate measures to monitor and control the microbiological quality of similar wastewater treatment plants in sub-Saharan regions in particular and developing countries in general to ensure public health safety in line with the MDGs

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MA and MJ participated in designing the experiments, executing them, performing data analysis and writing the manuscript. MS and JM participated in performing the evaluation of tests. MA (corresponding author) organized and supervised the collection and analysis of samples. All authors read and approved the final manuscript.

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