Potential threat to wildlife posed by enteric pathogens from Nakuru sewage treatment plant

Josephine M. Ngari [1]*(MSc), Kiplagat Kotut [2] (PhD) and Paul Okemo [2] (PhD)

1 *Ministry of Water and Irrigation, Water Quality and Pollution Control Division, P.O. Box 30521, Industrial Area, Nairobi, Kenya.*
2 *Department of Plant and Microbial Sciences, Kenyatta University,P.O. Box 43844, Nairobi, Kenya. Mwaura Murigi [mwauramurigi@yahoo.com]*

** To whom correspondence may be addressed: Ministry of Water and Irrigation, Water Quality and Pollution Control Division, P.O.Box 30521, Industrial Area, Nairobi, Kenya. Tel. No. 0722982244.*

SUMMARY

A study was carried out to investigate the levels of faecal coliforms (FC), Salmonella spp, Shigella spp and Vibrio cholerae at different stages of wastewater treatment at Nakuru Town Sewage Treatment Plant (TSTP). The plant has two treatment lines namely trickling filter line (a combination of conventional/mechanical treatment units) and anaerobic pond line (wastewater stabilization ponds only). Standard methods were used for the enumeration of FC, Salmonella spp, Shigella spp and Vibrio cholerae. There was a decrease in microbial densities along both treatment lines up to third maturation pond followed by a progressive increase in the tertiary treatment units. The mean microbial levels achieved at different treatment stages of anaerobic pond line (APL) were significantly lower ($P < 0.05$) than that achieved along the trickling filter line (TFL). A mean of 1.08×103 FC 100 ml-1 achieved in the APL final effluent was significantly lower ($P = 0.003$, $n = 14$) than 1.49×104 FC 100 ml-1 obtained in the TFL final effluent. None of the treatment lines achieved 1000 FC 100 ml-1 recommended for discharge to the lake. The likely impact of these pathogens on wildlife is discussed.

[Afr J Health Sci. 2011; 18:85-95]

Introduction

Pathogens enter the environment through inadequately treated domestic sewage effluents [29]. The aim of wastewater treatment is to reduce, as far as possible, sanitary and ecological risks associated with raw or inadequately treated sewage disposed into aquatic systems [6]. This is achieved by reducing the Biological Oxygen Demand (BOD), levels of pathogenic microorganisms and concentration of any toxic elements present [7]. The natural habitat of most pathogens found in wastewater, such as Salmonella typhi, is the intestinal tract of man and other warm-blooded animals from where they are shed in large numbers in faeces [20]. They can also be directly isolated from the tissue of the infected animals [20]. Inadequately treated sewage effluents contain pathogens that are currently causing diseases to both man and wildlife [22]. In areas subject to human pollution there has been an increase in the emergence of new diseases in wildlife [22].

Pathogens that can be transmitted between different host species are of fundamental interest and importance to public health, conservation and economic perspectives, yet systematic quantification of these pathogens is lacking [34]. A study on pathogen characteristics, host range and the risk factors determining disease emergence has indicated that many human pathogens have a multihost characteristic [11]. This is a clear indication that there is need to understand the dynamics of bacteria that cause diseases in several host species. Identification of host species is necessary in order to mitigate disease threat to public health, livestock and wildlife [11]. When human pathogens are exposed to wildlife, there is an increased risk that the pathogens will mutate and new diseases emerge [36]. Since 1980, more than thirty five new diseases have emerged in humans at the rate of one in every eight months [24]. In August-November 1993 and August-September 1995, over 40,000 lesser flamingos died in Lake Nakuru Various reasons have been advanced for the deaths. One of the reasons advanced is infection by

Mycobacterium [26, 33] commonly found in sewage effluents [29]. The mysterious deaths of flamingos in Lake Nakuru and Lake Bogoria have alarmed conservationists and triggered investigations but the cause of the mysterious die-offs remain unknown [33]. Researchers studying chimpanzees in tropical rain forests of Ivory Coast (Tai National Park) revealed that chimpanzees have been dying in the last few years as a result of *Bacillus anthracis* infections [17]. It isnot clear how the chimpanzees got infected but it is suspected that the *Bacillus anthracis* infection may have originated from contaminated water. Bacillus anthracis is a bacterial pathogen commonly isolated from domestic sewage effluents [29].

Materials and methods

Study area

Nakuru TSTP has two sewage treatment lines, a trickling filter and anaerobic pond line. The trickling filter combines conventional/mechanical treatment units and wastewater stabilization ponds while the anaerobic pond line comprises wastewater stabilization ponds only. Both treatment lines have a tertiary treatment stage comprising of rock filters and grass plots in series.

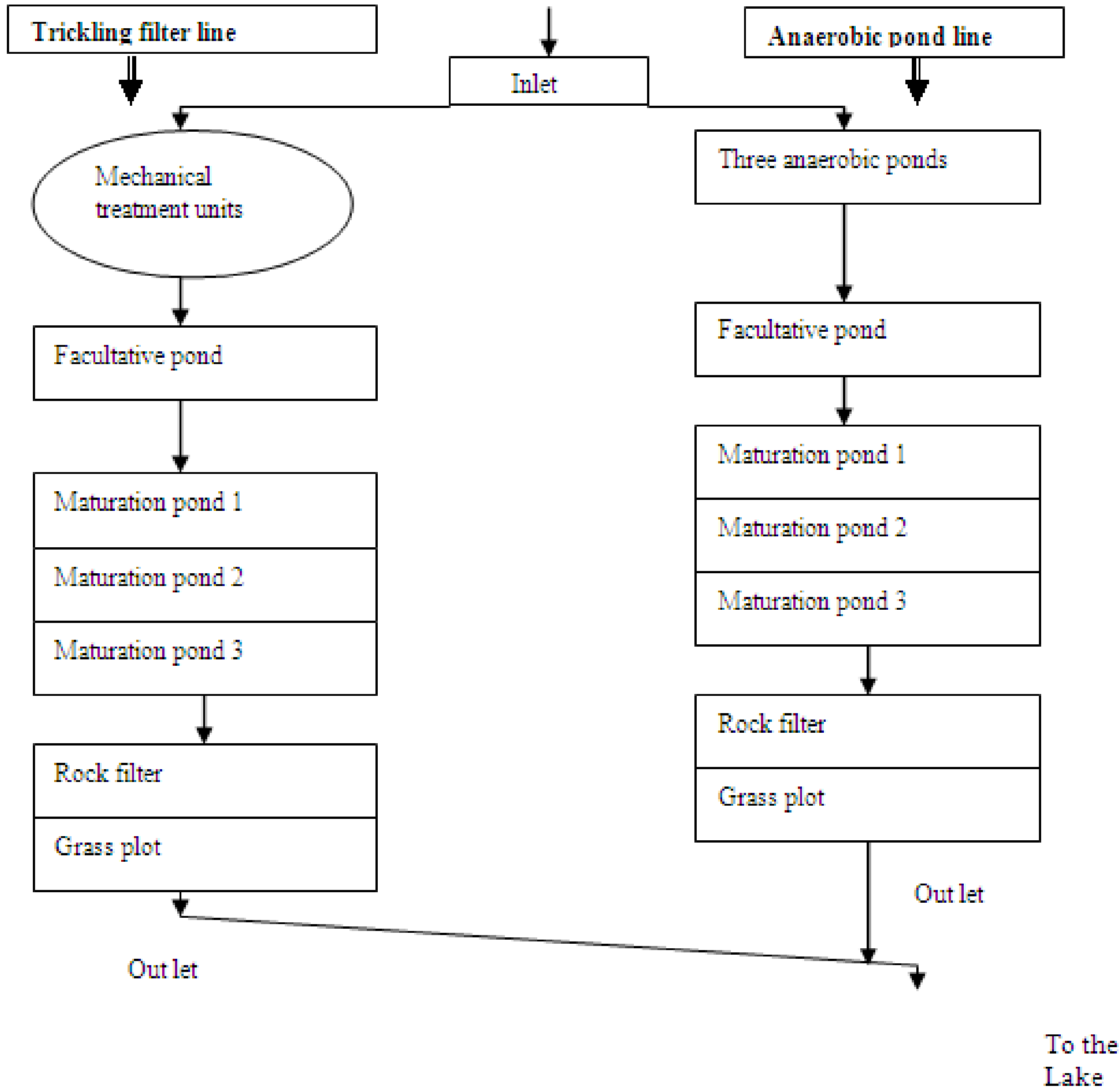

Fig. 1 A flow diagram of Nakuru TSTP

Methods

Grab samples were collected at the inlet and the outlet of each treatment unit along the two treatment systems in sterile sampling bottles and stored in a cool box with ice, then transported to the laboratory for analysis. Samples for the enumeration of general coliforms were aseptically inoculated in sterile MacConkey broth in McCartney bottles and incubated at 37 oC for 18 to 24 h. After the incubation period the MPN of general coliforms was determined from McGlady's statistical table. The positive bottles (with presence of both gas and acid) were aseptically sub-cultured in EC broth and incubated at 44.5 oC for 18 to 24 h. After the incubation period the MPN of faecal coliforms was also determined from McGlady's statistical table [4]. For the isolation of *salmonella* spp *Shigella* spp and Vibrio cholera serial dilution were of the samples were prepared and plated aseptically on highly selective *Salmonella-Shigella* agar and Thiosulphate-Citrate-Bile-salt Sucrose (TCBS) agar plates respectively. The inoculated agar plates were incubated aerobically at 35 oC for 18 to 24 h. Suspected colonies were identified morphologically and biochemically using standard procedures [4, 10].

Results

Rainfall (mm) and Temperature (oC)

Total monthly rainfall varied widely during the study period. The months of December and February were the driest months while August was the wettest of all the months (Fig. 2). March 2004 was the warmest with a mean temperature of 28.4 oC while the lowest mean temperature of 10.3 oC was observed in December 2003(Fig. 3).

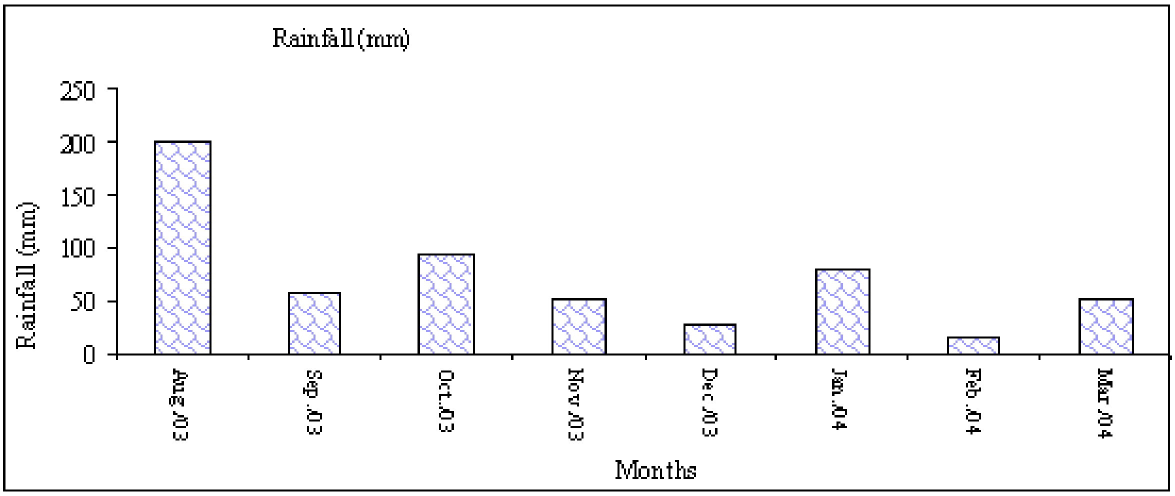

Fig. 2 Monthly total rainfall (mm) recorded at Nakuru meteorological station 9036261 in the year 2003-2004 (Source: Meteorological Department, Ministry of Transport, Nairobi).

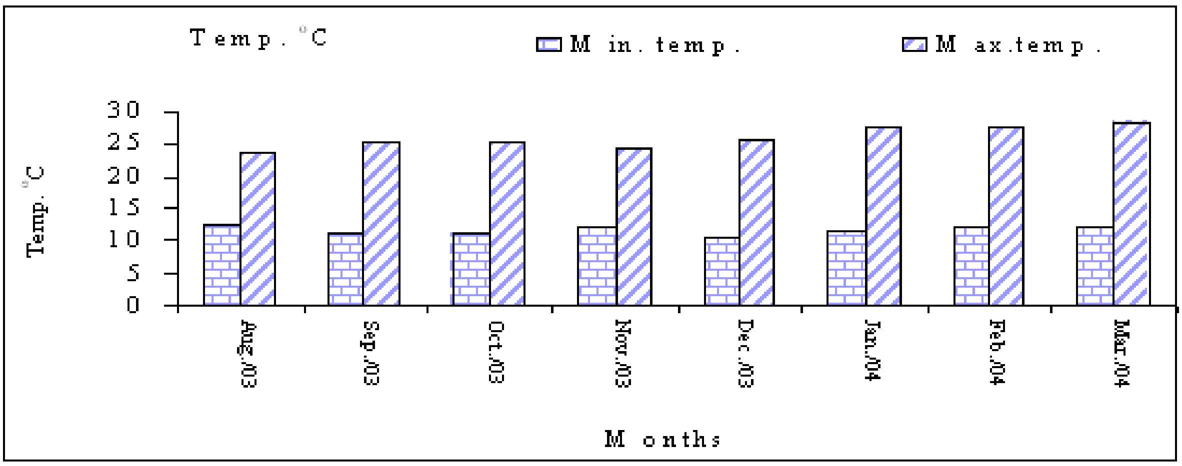

Fig. 3 Monthly temperature ranges (oC) recorded at Nakuru meteorological station 9036261 in the year 2003-2004 (Source: Meteorological Department, Ministry of Transport, Nairobi).

Feacal coliforms density per 100 ml

There was a general decrease in the density of levels of faecal coliforms from a value of 1.32x10^8 at the inlet to 2.61x10^2 at third maturation pond, followed by an increase in the effluent from the rock filter and the grass plot along anaerobic pond line. Along the trickling filter line, the decline in the levels of faecal coliforms continued up to the rock filter from where an increase was noted in the grass plot effluent (Table 1). Along the trickling filter line, the decline in the levels of faecal coliforms continued up to the rock filter (3.0×10^3) from where an increase was noted in the grass plot (1.5×10^4). The highest removal of faecal coliforms was observed in the facultative pond (99.99 %) and final maturation pond (99.98 %) of anaerobic pond and trickling filter lines respectively (Table 1). The trickling filter treatment unit achieved a low faecal coliforms removal (12.92 %) as compared to 67.09 % achieved by the anaerobic pond treatment unit. Overall faecal coliforms reduction achieved by anaerobic pond and trickling filter lines were 99.999% and 99.719 % respectively. Mean counts of faecal coliforms observed in the final maturation pond effluent of the anaerobic pond line (2.61×10^2) was significantly lower (P = 0.007, n = 22) than that observed at the trickling filter line final maturation pond (6.50×10^3). An increase in mean levels of faecal coliforms was noted in the rock filter (4.54×10^2) and the grass plot ($1.08\ \times10^3$) effluents along anaerobic pond line and the grass plot (1.49×10^4) of the trickling filter line. The mean level of faecal coliforms ($1.08\ \times10^3$) in the final effluent of anaerobic pond line was significantly lower (P = 0.003, n = 14) than 1.49×10^4 obtained in the final effluent of the trickling filter line. Occasionally, the anaerobic pond line's final effluent could stagnate at the outlet but at other times there used to be no effluent at all.

Table 1 Means, ranges and % reduction in faecal coliforms at each sampling point along anaerobic and trickling filter treatment lines.

Sampling points		MPN of faecal coliforms 100 ml-1			
		Mean (×104)	Range (×104)	PRPTU	CPR
	IN	13218	9200 - 16000	-	-
APL	AP	4350	1800 - 9200	67.09	67.09
	FP	0.1909	0.009 - 0.79	99.99	99.998
	MP	0.0261	0.004 - 0.09	86.32	99.9997
	RF	0.0454	0.008 - 0.23	-73.94	99.9996
	GP	0.1077	0.01 - 0.26	-137.22	99.99916
TFL	TF	11510	5400 - 16000	12.92	12.92
	FP	3602	220 - 9200	68.71	81.01
	MP	0.65	0.02 - 2.299.98	99.9951	
	RF	0.2994	0.05 - 0.853.93	99.9968	
	GP	1.4871	0.06 - 4.6-396.7	99.7195	

Key: AP- anaerobic pond, APL - anaerobic pond line, FP - facultative pond, GP - grass plot, , MP - maturation ponds, MPN – most probable number, RF - rock filter, TFL – Trickling filter treatment line, TL - treatment lines, PRPTU. - percent reduction per treatment unit, CPR - cumulative percent reduction.

Salmonella spp counts ml-1

The highest mean counts / ml of Salmonella spp (22.1×10^3) was recorded in the influent while the lowest observed in the effluents of third maturation pond, anaerobic pond and rock filter on a number of occasions (Table 2). There was a general decline in the counts of *Salmonella* spp up to the maturation ponds followed by a progressive increase in the effluent from rock filter and grass plot along both treatment lines. The anaerobic pond and trickling filter lines achieved an overall percent reduction of 99.98 and 98.55 % respectively at the third maturation pond. The maturation ponds of the two treatment systems achieved the highest percent reduction of *Salmonella* spp. An increase in Salmonella spp in the effluent from the rock filter and the grass plot reduced the overall counts of *Salmonella* spp in the anaerobic pond and trickling filter lines to 98.89 and 92.37 % respectively. Mean counts of *Salmonella* spp at the final maturation ponds of the two lines were significantly different (P = 0.0003, n = 15) with a higher count being realized along trickling filter line. Mean *Salmonella* spp counts (16.8×10^2) recorded in the final effluent from trickling filter line was significantly higher (P = 0.006, n = 11) than the mean count (2.44×10^2 Salmonella spp) recorded in the anaerobic pond line (Table 2).

Table 2 Means, ranges and % reduction of Salmonella spp at each sampling point along anaerobic and trickling filter treatment lines.

Sampling points		Salmonella spp counts ml-1			
		Mean	Range	PRPTU	CPR
	IN	22066	11000 -30000	-	-
APL	AP	2733	800 - 8000	87.61	87.61
	FP	291	90 - 600	89.35	98.68
	MP	6	30 - 10	97.94	99.98
	RF	40	10 - 100	-56.67	99.82
	GP	244	55 - 450	-510	98.89
TFL	TF	17333	9000 - 25000	21.45	21.45
	FP	3187	1000 - 6000	81.61	85.56
	MP	319	50 - 700	89.99	98.55
	RF	457	150 - 900	43.26	97.93
	GP	1683	300 - 5200	-268.27	92.37

Key: AP- anaerobic pond, APL - anaerobic pond line, FP - facultative pond, GP - grass plot, MP - maturation ponds, MPN – most probable number, RF - rock filter, TFL – Trickling filter treatment line, TL - treatment lines, PRPTU. - percent reduction per treatment unit, CPR - cumulative percent reduction.

Shigella spp counts ml-1

The highest mean counts for *Shigella* spp (43.53 $\times 10^2$) was recorded in the influent (Table 3). On a number of occasions, no *Shigella* spp was detected at third maturation pond of anaerobic pond line. There was a general decline in *Shigella* spp counts up to the maturation ponds followed by a progressive increase in the effluent from rock filters and grass plots of both treatment lines. An increase in *Shigella* spp counts was noted during drier month of November and December of 2003 and February 2004 (Fig. 2). The facultative pond of anaerobic pond line and the final maturation pond trickling filter line achieved the highest percent removal of 89.59 % and 95.89 % respectively, compared to other treatment units. The anaerobic pond and trickling filter lines achieved an overall percent reduction of 99.87 and 98.81 respectively, at the third maturation ponds. An increase in *Shigella* spp counts in the effluent from the rock filter and the grass plot reduced the percentage reduction of the anaerobic pond and trickling filter lines to 98.35 and 90.81 % respectively. The mean counts of *Shigella* spp (4.0 × 102) recorded in the final effluent from trickling filter line were significantly higher ($P < 0.001$, $n = 11$) than that recorded in the final effluent of anaerobic pond line.

Vibrio cholerae counts ml-1

The highest mean counts of Vibrio cholerae (4.6× 103) was recorded in the inlet while the lowest (2.2) was recorded a number of times in the effluent from third maturation and the rock filter along the anaerobic pond line (Table 4). The facultative pond of the anaerobic pond line and the final maturation pond of the trickling filter line achieved the highest percent removal

Table 3 Means, ranges and % reduction of Shigella spp at each sampling point along anaerobic and trickling filter treatment lines.

Sampling point		Shigella spp counts ml-1			
		Mean	Range	PRPTU	CPR
	IN	4353.33	1100 – 9000	-	-
APL	AP	460	100 – 900	89.43	89.43
	FP	47.86	10 – 90	89.59	98.9
	MP	5.8	0 – 11	87.86	99.87
	RF	13.18	5 – 30	-127.24	99.7
	GP	63.58	15 – 170 -	382.4	98.35
TFL	TF	3593.33	1500 – 8000	17.45	20.22
	FP	1253.33	200 – 3000	65.12	71.21
	MP	51.8	15 – 90	95.89	98.81
	RF	142	60 – 300	-174.15	95.22
	GP	400.13	12 – 800	-181.78	90.81

Key: AP- anaerobic pond, APL - anaerobic pond line, FP - facultative pond, GP - grass plot, , MP - maturation ponds, MPN – most probable number, RF - rock filter, TFL – Trickling filter treatment line, TL - treatment lines, PRPTU. - percent reduction per treatment unit, CPR - cumulative percent reduction.

Table 4 Means, ranges and % reduction of Vibrio cholerae at each sampling point along anaerobic and trickling filter treatment lines.

Sampling point		Vibrio cholerae counts ml-1			
		Mean	Range	PRPTU	CPR
	IN	4613	-		-
APL	AP	420	100 – 800	90.9	90.9
	FP	7.27	3 – 12	98.26	99.84
	MP	2.2	0 – 4	69.74	99.95
	RF	4.5	0 – 8	-104.55	99.9
	GP	14.55	2 – 30	-223.33	99.69
TFL	TF	2513.3	900 – 5000	45.52	45.52
	FP	373.33	100 – 700	84.35	91.91
	MP	22.6	9 – 80	94.03	99.51
	RF	28.33	6 – 70	-25.35	99.39
	GP	65.4	2 - 200	-130.85	98.58

Key: AP- anaerobic pond, APL - anaerobic pond line, FP - facultative pond, GP - grass plot, , MP - maturation ponds, MPN – most probable number, RF - rock filter, TFL – Trickling filter treatment line, TL - treatment lines, PRPTU. - percent reduction per treatment unit, CPR - cumulative percent reduction.

(98.26 % and 94.03 % respectively). A mean Vibrio cholerae value of 2.20 counts ml-1 realized at final maturation pond of anaerobic ponds line was significantly lower ($P = 0.003$, $n = 15$) than 2.26 × 101 recorded at the final maturation pond of the trickling filter line The greatest reduction (90.9 %) in *Vibrio cholerae* counts was noted in the anaerobic ponds effluent. There was an increase in *Vibrio cholera*e counts in the rock filter and grass plot effluents of the two treatment lines. The mean level of *Vibrio cholerae* (6.5× 101 counts ml-1) in the final effluent from the trickling filter line was significantly higher ($P = 0.023$, $n = 10$) than 1.5× 101 counts ml-1 recorded in the anaerobic pond line.

Discussion

Faecal coliforms

The quality of water is typically determined by establishing microbial presence, especially faecal coliforms (3, 18]. The mean level of faecal coliforms recorded in the influent (1.32×108 ml-1) was higher than 9.04×10^6 ml-1 recorded in the raw sewage at Akuse wastewater treatment plant in Ghana [21]. The different levels at the two treatment plants can be attributed to different influent strengths and environmental conditions at the two treatment plants. Akuse wastewater treatment plant in Ghana had a weaker influent [21] as compared to Nakuru town wastewater treatment plant. Low numbers of faecal coliforms in the anaerobic pond line final maturation pond effluent can partly be attributed to low flow (evident from incidents of little or no final effluent at all during the study period) that characterized the anaerobic pond line as compared to trickling filter line. Low flow is known to increase the exposure of faecal coliforms and other microorganisms to ultraviolet light increasing their mortality rate [12].

The high levels of faecal coliforms in the effluent from third maturation along trickling filter line is an indication of poor performance and is likely to have been due to poor performance of the trickling filter unit, overflow at the inlet caused by blockage of the screening grid at night (plant is not normally manned at night for security reseasons and any overflow at the inlet goes to this line) in addition to a non-functioning secondary clarifier. Wastewater stabilization ponds produce effluent with low counts of pathogenic microorganisms [32]. This was evident along the anaerobic pond line where 99.999 % was achieved at final maturation pond. The increase in the levels of faecal coliforms in the effluent form the rock filter and the grass plot can be attributed to an increase in organic matter in the rock filter from wildlife wastes, decomposing algae and grass in the grassplot providing nutrients needed for the survival of the microorganisms [31].

The 99.72 % reduction of faecal coliforms achieved by the trickling-filter line compares well with a report by the London Metropolitan Water Board [42] that tertiary pond treatment of effluent from a conventional secondary treatment plant can reduce E. coli by 99.5 %. Faecal coliforms reduction of 99.99 % achieved by anaerobic pond line is indicative of a better treatment efficiency for it was much higher than 90.0 %

coliforms reduction reported by New Zealand, Auckland Metropolitan Drainage Board [16] and 99.0 % faecal coliforms removal rates reported in two ponds connected in series and having a total retention time of 26 days in Israeli [40].

In tropical developing countries, effluent standards do not exist but there is a general guide followed which gives a range of $< 5.0 \times 10^3$ cells of faecal coliforms per 100 ml as acceptable for release to the environment [29]. Faecal coliform levels in the trickling filter line final effluent was well above the set guideline. This limit may have been safe in 1970s but with the current trend of emergence of new pathogen and resistant strains of known enteric pathogens, etc., there is need for more strict standards which should be adhered to. A guide of 1.0× 103 faecal coliforms 100 ml^{-1} that was based on WHO minimum requirement for unrestricted irrigation [43] was proposed by the designers of the sewage treatment plant. This proposal does not appear to have put into considerations the fact that the treated effluent from Nakuru TSTP was to be being discharged into a sensitive area, Nakuru National Park [29]. With the current trend of emergence of new wildlife diseases in wildlife in areas subject to human pollution [36, 37] stricter guidelines are mandatory for an effluent entering a wildlife habitat.

Salmonella spp

The range of Salmonella spp in the influent (1.10×10^4 - 3.0×10^4 ml^{-1}) was far much higher than a range of 5 to 80 counts ml^{-1} typical for raw domestic sewage [44]. A study on the prevalence of waterborne diseases within health facilities in Nakuru district indicated that typhoid fever caused by *Salmonella typhi* is the leading waterborne disease in the area [19]. The high values observed at Nakuru town wastewater treatment plant can be attributed to high prevalence of *Salmonella* infections in the communities served by the wastewater treatment plant. Hence it is possible that high counts of Salmonella spp in the raw sewage can be attributed to the high number of typhoid fever cases (49 %) of all waterborne diseases [19] within Nakuru district. A high *Salmonella* spp removal observed in the maturation ponds of both anaerobic pond and trickling filter lines can be largely attributed to high mortality rate due to high pH of 8.4 [35] observed in this ponds and increased exposure to UV light [16]. Increase in *Salmonella* spp counts noted in the effluent from the rock filter and the grass plot along both treatment lines (Table 2) could have resulted from the multiplication of the microorganism in the rock filter due to presence of organic matter from decomposing algae (source of nutrients) in the rock filter, decomposing grass in the grassplot and introduction of microorganisms by wild animals which graze on the grassplot and the area within the treatment plant. A 99.98 % reduction achieved by the anaerobic pond line was slightly higher than 99.5 % reported by Coetzee [12] and the documented 99.6 % [29]. Presence of Salmonella spp in the final effluent after a retention period of over four weeks confirms a study by McGarry and Bouthiller [30] in Central and South Africa sewage oxidation ponds which indicates that *Salmonella* spp are better survivors than *E. coli* in the environment and their survival is dependent on presence of nutrients [31]. The difference in the overall percent reductions of microbial numbers achieved by different treatment plants is most likely due to different environmental conditions, designs and their maintenance status. In a study by Windle [42], *Salmonella* spp and Enteroviruses were regularly isolated from effluent that had passed through wastewater stabilization ponds. Windles findings, the presence of Salmonella spp in the final effluent indicates that there is a high likelihood of enteric viruses being present in the final effluent. This poses a danger to our wildlife in that enteric viruses have a low-dose infectivity, long term survival in the environment, ability to adapt to new hosts, and limited removal and inactivation in wastewater treatment [39]. The ability of viruses to adapt to new hosts means there is a likely hood of them causing new diseases in the new hosts.

Although the trickling filter line is a combination of both mechanical system and wastewater stabilization ponds, it only achieved a 98.5 % reduction up to the third maturation pond that decreased to 92.5 % at the outlet which was lower than 98.89 % achieved by the anaerobic pond line. This is an indication of low efficiency of the trickling filter line because wastewater stabilization ponds in series with a conventional treatment system are usually referred to, as polishing ponds and their main function is to reduce the pathogenic microorganisms in the effluent by exposing them to UV light from the sun. This was not evident in this study because the ponds produced a poor quality effluent as compared to anaerobic pond line.

Shigella spp

The range of *Shigell*a spp in the influent (1.1×10^3 0 -9.0×10^3 counts ml^{-1}) was higher than the documented range of 0.01 to 10 counts ml^{-1} [44]. A low number of *Shigella* in the influent (Table 3) as compared to other pathogens *(Salmonella* spp and *Vibrio* cholerae) isolated in this study can be attributed to its low infection prevalence in Nakuru area [19] and its low infective dose (10 to 100 microorganisms) as compared to 106 to 109 for Salmonella and about 104 for *Vibrio cholerae* [14]. Increase in the density of *Shigella* spp in the effluent from the rock filter and the grassplot is an indication that the rock filter and the grass plot contribute negatively in the aspect of microbial reduction. Presence of *Shigella* spp in the environment poses a health hazard to the wildlife as it is highly virulent. In tropical countries Shigellosis is endemic and it has been estimated that some 5 M people die every year [14].

Vibrio cholerae

Although no cholera outbreak was reported in Nakuru area during this study, high counts of Vibrio cholerae was noted in the influent. This suggests that a number of people in Nakuru town are carriers *Vibrio cholerae.* Due to the fact that Vibrio cholerae grows best aerobically [10]. A high reduction of Vibrio cholerae achieved by the anaerobic pond (90.90 %) as compared to the trickling filter treatment unit (45.52 %) can be attributed to the anaerobic condition in this pond. The ability of *Vibrio cholerae* to survive for long periods in the environment was evident in this study where *Vibrio cholerae* was present in the final effluents after a retention period of more than four weeks (Table 4). Previous studies suggested that *Vibrio cholerae* can only survive in the environment for a short period [9]. However, recent surveys indicate that Vibrio cholerae is commonly found as a natural resident of aquatic environments in areas free of cholera epidemics and that its presence is not necessarily associated with recent faecal contamination [27]. The ability of *Vibrio cholerae* to survive in the environment for long periods poses a risk of cholera outbreaks in case inadequately treated sewage effluent is discharged into water bodies. This is because sewage effluent would boost the number of *Vibrio cholerae* in the water to levels that can easily cause an outbreak of cholera, hence the need to ensure that there is a high pathogen reduction in the final sewage effluent. Most cholera outbreaks are linked to faecal contamination of drinking water [29].

Likely implications of human pathogens presence in the final effluent

The presence of high levels of faecal coliforms and the enteric pathogens (*Salmonella* spp, *Vibrio cholerae*, and *Shigella* spp) isolated in the final effluent of Nakuru Town Wastewater Treatment plant (Table 2) indicates that other potentially pathogenic microorganisms (e.g. enteric viruses and nematodes) commonly isolated from sewage could be present [37, 23, 29]. It has been shown that there are risks associated with exposure to enteric viruses in biologically treated effluents and the probability of getting an infection by coming into contact with such waters are high [39]. Recent surveys have revealed that, there is a great danger associated with exposure of wildlife to human pathogens and there is an increase in emergence of new diseases in wildlife especially in areas subject to human pollution [36]. For example, marine turtles are experiencing increased rates of microbial diseases and tumors [2]. Field observations indicate a high prevalence of tumors in turtles associated with heavily polluted areas and regions of high human population. Coral reefs are facing large-scale destruction as a result of coral bleaching and infectious diseases [22]. 30 % of the world's reefs have been lost since 1980 and some of the microorganisms affecting the coral reefs are human pathogens [22].

Nearly 1,000 sea otters have been found dead a long the California coastline in the past five years [22]. The sea otters mortality is associated with peak river flows and storm events. Researchers believe that parasites are entering water bodies through untreated or partially treated sewage and terrestrial waste carried in storm-water runoff [22]

Pathogen pollution threatens all types of wildlife [37]. When human pathogens are exposed to wildlife, there is an increased risk that pathogens will mutate and new diseases emerge. Habitat destruction and chemical pollution were thought to be the main threat to biodiversity but pathogen pollution seems to be taking over as the main threat to biodiversity [36]. In Nakuru TSTP, wild animals graze on grassplots (meant for tertiary treatment of sewage effluent) with fresh partially biologically treated effluents contrary to WHO guideline which forbids animal grazing on an area irrigated with biologically treated effluent before two days are over after irrigation [43]. This exposes the wild animals to any pathogens present in the effluent. Exposure of wildlife in Nakuru National Park to enteric human pathogens should be prevented.

Conclusion

The results obtained in this study indicate that the final effluent from the trickling filter line does not meet the standards set for the discharge to the lake. It has high levels of potentially pathogenic microorganisms. This is a clear indication that wild animals grazing on the grass-plots made for tertiary treatment of sewage and drinking final effluent as it flows to Lake Nakuru are exposed to potentially pathogenic microorganisms. There is a high likelihood of the wild animals and people feeding on game roast meat developing new diseases with time as a result of the possible mutation of microorganisms in the process of adapting to the new host. Emergence of new diseases in the wildlife within Nakuru national park as a result of exposure to human pathogens would adversely affect the economy of our country since this park is one of the Kenya's major tourist attractions. This stresses the importance of protecting our wildlife from exposure to human pathogens. This can be achieved by improving the efficiency of Nakuru TSTP leading to production of high quality final effluent that is free of human pathogens. Construction of a wastewater facility within a wildlife habitat should be discouraged.

Acknowledgement

We would like to thank the Permanent Secretary, Ministry of Water and Irrigation for granting me study leave and financial support. We also appreciate the assistance offered by Nakuru Municipality and Nakuru Provincial Water Office for facilitating access to the wastewater treatment plant and laboratory for the analytical work.

References

1. Alonso A. Sea turtles are vulnerable to environmental stress. Environmental and Energy Study Institute. May 2001. Available at website: http://www.wpti.org or www.eesi.org
2. Amdur MO, Doull J and Klaassen CD. Toxicology: the basic science of poisons (4th edn). 1991; McGraw-Hill, New York.
3. Anonymous. South African Water Quality Guidelines (2nd edn) Department of Water Affairs and Forestry. Pretoria, 1996,
4. APHA. Standard Methods for the Examination of Water and Wastewater (19th edn). American Public Health Association, American Water Works Association Water Environment Federation 1998; Washington D.C.
5. Arana I, Santorum P, Muela A, and Barcina I. Chlorination and ozonation of wastewater: comparative analysis of the efficacy through the effect upon Escherichia coli membranes. *Journal of Applied Microbiology.* 1999; **86:** 883 - 888
6. Asano T. Water from (waste)water: the dependable resource. Proceedings of Stockholm Water Symposium, August 12th – 18th 2001, Stolkholm, Sweden.
7. Atlas RM. Microbiology Fundamentals. Macmillan Publishing Co., New York.
8. Bennum L and Nasirwa O. Trends in water bird numbers in the rift valley of Kenya. *Ostrich*. 2000; **71:** 220 – 226.
9. Bryan FL. Epidemiology of food-borne diseases transmitted by fish, shellfish and marine crustaceans in the United States, 1970 -1978. *Journal of Food Protection.* 1990; **43:** 859 - 876.
10. Cheesbrough M. Medical Microbiology Laboratory Manual in Tropical Countries, vol. two. Cambridge shire: England, 1984.
11. Cleaveland S, Laurenson M and Taylor, L. Diseases of humans and their domestic mammals: Pathogen characteristics, host range and the risk of emergence. *Biological sciences.* July 2001; **351:** 991- 999.
12. Coetzee T and Fourie C. Efficiency of conventional sewage purification works, stabilization ponds, and maturation ponds in respect to pathogen survival of bacteria and indicator organisms. *J. pro. Inst. Sew. Purif.* 1965; **3:** 210 - 215.
13. Craig D L, Fallowfield HJ. and Cromar NJ. Enumeration of faecal coliforms from recreational coastal sites: evaluation of techniques for separation of bacteria from sediments. *Journal of Applied Microbiology.* 2002; **93:** 557-565.
14. David G, Richard C.and John F. Medical Microbiology (15th Edn). U.K: Churchhill Livingstone, 1997.
15. Dubusk T, Reddy K, Hayes D. and Schwegler B. Performance of pilot scale water hyacinth. *Journal of Water Pollution Control Federation.* 1998; **61:** 1217 - 1224.
16. E rnest FG. Waste Stabilization Ponds. World Health Organization. Geneva, 1971.
17. George, P. Anthrax now jumps to wild chimpanzees. *Daily Nation News Paper,* July 29th 2004: 32.
18. Gray NF. Drinking Water Quality: Problems and Solutions. N.Y: John Wiley & Sons, 1994.

19. Hlupheka P and Marium M Prevalence of waterborne diseases within the health facilities in Nakuru District. A dissertation submitted for the requirement of Certificate in Applied Epidemiology. Department of Community Health, University of Nairobi. 2001.
20. Huss HH. Assurance of Seafood Quality. Food and Agriculture Organization Technical paper 334. Rome, 1994.
31. Isaac OA. Treatment of domestic sewage at Akuse,Ghana. Water Research Institute. Ghana: Accra, 2000. Available at website: http://www.wrc.org.za
22. Jessup D. Marine mammals face increased mortality rate. The Environmental and Energy Study Institute, May 2001, Washington D.C. Available at website: http://www.wpti.org or www.eesi.org
23. Jimenez L, Munir J, Toranzos G. and Hazen T. Survival and activity of Salmonella typhimurium and Escherichia coli in tropical fresh water. *Journal of Applied Bacteriology*. 1989; **67:** 61 - 69.
24. Karesh WB, Cook RA, Bennet EL and Newcomb J. Wildlife trade and global disease emergence. Wildlife Conservation Society. Available at website: http://www.cdc.gov/EID/vol11no07/05-0194.htm
25. Klutse A and Baleux B. Nematode eggs and protozoan cysts removal oin microphytic wastewater stabilizaton ponds in the Sudan-Sahel area. *Rev. Sci. Eau,* 1995; **8:** 563 – 577.
26. Kock ND, Kock RA, Wambua J, Kamau GJ and Mohad K. Mycobacterium avium related epizootic in free-ranging Lesser Flamingos in Kenya. *Journal of Wildlife Diseases.* 1999; **35:** 297 – 300.
27. Lima dos Santos CA, Josupeit H & Dos Santos DC. Impact of council directive 493/91/ EEC on seafood trade: the case of developing countries. International Conference on New markets for seafood, 28th September–1st October 1993, International fisheries Institute, University Hull, U.K.
28. Mangat I.B. Patel & partners Nakuru Water and Sanitation Study. Wastewater treatment considerations for Nakuru, 1996 Technical Report No. 5.
29. Mara D. Sewage Treatment in Hot Climates. John Wiley: New York, 1976.
30. McGarry and Bouthiller Survival of Salmonella typhi in sewage oxidation ponds` in Central and Southern Africa. American Society of Civil Engineers: New York, 1966. 31. M e r v i n OP. Water quality modeling: a guide to effective practice. 2001 World Bank report, Washington, D.C.
32. Miguel RP. Waste Stabilization Ponds for Wastewater Treatment. FAQ sheet on stabilization ponds. Universidad del valle, Instituto Cinara, Cali. Colombia, 2003.
33. Motelin, G. K., Thampy, R. J., and Ndetei, R. The mysterious lesser flamingo deaths in Lake Nakuru: a cross-sectional ecotoxicological study of the potential roles of algal toxins, heavy metals and pesticides. Report by World Wildlife Fund, Nakuru: Kenya, 1995.
34. National Biological Information Infrastructure. Wildlife Disease Information Node, 2002.
35. Ngari J. The efficiency of Nakuru Town Sewage Treatment Plant. Kenyatta University: Msc Thesis, 2006.
36. Peter D, Andrew AC and Alex DH. Emerging infectious diseases of wildlife-Threat to biodiversity and human health. *Science.* 2000; **287:** 443 – 445. Available at website: http://www.conservationmedicine.org/papers/daszak -et - al 2000pdf
37. Raymond LH. Carol reefs are dying. In: wildlife and Human diseases congress Environmental and Energy Study Institute, May 2001, Washington DC. Available at website: http:// www.eesi.org
38. Reilly PJ and Twiddy DR. Salmonella and Vibrio Cholerae in brackish water cultured tropical shrimps. *International Journal of Food Microbiology.* 1992; **16:** 293 – 301.
38. Tanaka H, Asano T, Schroeder ED and Tchobanoglous G. Estimating the safety of wastewater reclamation and reuse using enteric viruses monitoring data. *Water Environment Research.* 1998; **70:** 39-51.
40. Wachs AM. Study on wastewater stabilization ponds in Israel. Part 1: Oxidation ponds at Herzliya. Haifa Institute of Technology. Israel, 1961.
41. Wanjiru J. Kenya pink flamingo weighted down by heavy metals. Enviroment News Service. Available at website: wysiwyg://4/http://www.ens-news.com/ens/jul/2001
42. Windle TE. Improvement of quality of sewage works effluent during passage through a series of lagoons. In: Metropolitan Water Board Report for the year 1963-1964. London, 1965.

43. World Health Organization. Health guidelines for the use of wastewater in agriculture and aquaculture: Reports of a WHO scientific group (Technical Report Series No. 778). Geneva, 1998.
44. Yate MV and Gerba CP Microbial considerations in wastewater reclamation and reuse. In: Asano, T. (Ed) Wastewater reclamation and reuse. *Water Quality Management Library.* 1998; **10:** 437 - 488.