THE BACTERIOLOGICAL QUALITY OF DANDORA SEWAGE TREATMENT PLANT AND THE RECEIVING WATERS OF NAIROBI AND ATHI RIVERS

MUSYOKI ABEDNEGO MOKI, B.Sc. (MLS)

156 / PT / 10318 / 2008

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (INFECTIOUS DISEASES) IN THE SCHOOL OF HEALTH SCIENCES OF KENYATTA UNIVERSITY
DECLARATION

This thesis is my original work and has not been presented for a degree in any other
university or any other award

MUSYOKI ABEDNEGO MOKI

I56 / PT / 10318 / 2008

SIGNATURE ___________________ DATE 02/06/2011

Supervisors:

We confirm that the work reported in this thesis was carried out by the candidate under
our supervision:

1. DR. JOHN J. N. MBITHI

   DEPARTMENT OF MEDICAL LABORATORY SCIENCE,
   SCHOOL OF HEALTH SCIENCES, KENYATTA UNIVERSITY

   SIGNATURE ___________________ DATE 02/06/2011

2. DR. SULEIMAN A. MBARUK

   INSTITUTE OF PRIMATE RESEARCH, NATIONAL MUSEUMS OF
   KENYA, KAREN, NAIROBI

   SIGNATURE ___________________ DATE 2011
DEDICATION

I wish to dedicate this work to my mother, Susan Ngayau and my brothers and sisters: Wilfred Mboya, Lucas Kyai, Esther Nthambi and Sheila Nzisa
Acknowledgement and gratitude are expressed to all those who contributed in suggestions and preparation of this thesis. Special thanks are to my supervisors: Dr. John J. N. Mbithi of Department of Medical Laboratory Science, School of Health Sciences, Kenyatta University and Dr. Suleiman A. Mbaruk of Institute of Primate Research for their tireless mentorship and sage advice to complete this work.

I should also express my gratitude to Nairobi City Water and Sewerage Company for granting research permission and access to Dandora sewage treatment plant. Finally, I thank the staff members at Dandora sewage treatment plant for their cooperation and assistance during study period.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>APHA</td>
<td>American Public Health Association</td>
</tr>
<tr>
<td>APHRC</td>
<td>African Population and Health Research Center</td>
</tr>
<tr>
<td>BOD</td>
<td>Biological Oxygen Demand</td>
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<td>CBS</td>
<td>Central Bureau of Statistics</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
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<tr>
<td>CFU</td>
<td>Colony Forming Units</td>
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<tr>
<td>DSTP</td>
<td>Dandora Sewage Treatment Plant</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
</tr>
<tr>
<td>FC</td>
<td>Faecal Coliform</td>
</tr>
<tr>
<td>IAWQ</td>
<td>International Association on Water Quality</td>
</tr>
<tr>
<td>KEBBS</td>
<td>Kenya Bureau of Standards</td>
</tr>
<tr>
<td>KNBS</td>
<td>Kenya National Bureau of Statistics</td>
</tr>
<tr>
<td>NEMA</td>
<td>National Environmental Management Authority</td>
</tr>
<tr>
<td>NRDC</td>
<td>Natural Resource Defense Council</td>
</tr>
<tr>
<td>RBC</td>
<td>Rotating Biological Contractors</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>TC</td>
<td>Total Coliform</td>
</tr>
<tr>
<td>TF</td>
<td>Trickling Filters</td>
</tr>
<tr>
<td>UASB</td>
<td>Upflow Anaerobic Sludge Blanket</td>
</tr>
<tr>
<td>US</td>
<td>United States of America</td>
</tr>
<tr>
<td>WASREB</td>
<td>Water Service Regulatory Board</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>WSP</td>
<td>Waste Stabilization Ponds</td>
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ABSTRACT

Wastewater is known to contain microbes that are deleterious to human health. Epidemiological reports show that wastewater associated disease outbreaks are common around the world. This suggests that wastewater must be processed carefully before release into natural waters and the environment. The purpose of this study was to determine the total bacterial load and microbial types in Dandora Sewage Treatment Plant (DSTP) and its handling capacity in terms of pathogen removal. In addition, the seasonal and temporal relationship of bacterial load variation was estimated. Antecedent bacterial load of Nairobi and Athi rivers was also established to reveal the microbial load emptied into Athi River by the DSTP. Standard methods for collection and examination of wastewater were used to elucidate bacterial counts in the samples. The mean bacterial load at the influent was $7.1 \times 10^7$ CFU / 100 mL. *Escherichia coli* was the dominant bacterial type and the least common bacterial type was *Vibrio parahaemolyticus*. Other bacterial types found in the influent were *Klebsiella aerogenes*, *Enterococcus faecalis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Vibrio cholerae*, *Proteus mirabilis* and *Shigella flexneri*. At the end of DSTP processing, the bacterial characteristic of the effluent was as follows: *Enterococcus faecalis* with mean total load of $2.7 \times 10^4 \pm 1.7 \times 10^3$ CFU / 100 mL ($k_i = 0.0806$), *Escherichia coli* estimated at $1.3 \times 10^4 \pm 1.1 \times 10^3$ CFU / 100 mL ($k_i = 0.1260$), *Pseudomonas aeruginosa* measured as $2.5 \times 10^2 \pm 1.0 \times 10^1$ CFU / 100 mL ($k_i = 0.1581$), *Salmonella typhi* with load of $4.4 \times 10^1 \pm 5 \times 10^0$ CFU / 100 mL ($k_i = 0.1615$) and *Klebsiella aerogenes* measured as $4.1 \times 10^1 5 \times 10^0$ CFU / 100 mL ($k_i = 0.1897$). In this regard, *Enterococcus faecalis* ($k_i = 0.0806$) was the most resilient human pathogen. Seasonal variation in terms of quantity of bacteria was significant in both influent ($F = 14.795, p = 0.001$) and the effluent ($F = 23.574, p = 0.000$) with more bacteria found during the dry season. Bacterial load in the DSTP effluent showed diurnal variation with higher counts being found in the morning session ($F = 22.788, p = 0.000$). The bacteria types in Nairobi River, DSTP and Athi River were similar. The levels were higher in Nairobi River than in the DSTP effluent ($F = 55.12, p = 0.001$) and Athi River upstream ($F = 13.638, p = 0.009$). The performance of DSTP in terms of efficiency to remove bacteria from wastewater is below the set World Health Organization (WHO), Environmental Protection Agency (EPA) and National Environmental Management Authority (NEMA) guidelines for processed wastewater. The failure was replicated irrespective of the seasonal and diurnal variation. In conclusion, the DSTP and the Nairobi River portends a health risk to downstream communities and remedial intervention is urgently required.
CHAPTER ONE

1 INTRODUCTION

1.1 Background of the Study

Wastewater is known to contain microbes that are deleterious to human health (Toze, 1997; WHO, 2000; NRDC, 2004; Okoh et al., 2007). Worldwide epidemiological surveys suggest numerous disease incidences have been reported in which wastewater was implicated as the source (NRDC, 2004; CDC, 2006). These diseases have been associated with direct contact with wastewater (Bouhoum and Schwartzbrod, 1998; Habbari et al., 2000), aerosols produced by sprinkler irrigation (Linneman et al., 1984; Fattal et al., 1986; Shuval et al., 1989), contaminated drinking water (CDC, 1996, 2004), and sea foods harvested in wastewater polluted sources (Shuval et al., 2003; NRDC, 2004).

Further, global water shortfalls have forced the use of wastewater in agriculture out of which farm produce is contaminated with human pathogens (WHO, 2000). In addition, several epidemiological studies have reported increased disease incidences among consumers of uncooked vegetables irrigated with untreated wastewater (Shuval et al., 1984; Hopkins et al., 1993; Cifuentes, 1998; Peasey, 2000; Blumenthal et al., 2001; Ait Melloul and Hassani, 1999). Specifically, wastewater disease incidences are associated with parasites (Shuval et al., 1984; Cifuentes, 1998; Peasey, 2000), viruses (Blumenthal et al., 2001), and bacteria (Hopkins et al., 1993; Cifuentes, 1998; Ait Melloul and Hassani, 1999; Blumenthal et al., 2001).
In the meantime, the level and diversity of human pathogens in wastewater depends on the season (Okoh, 2007), the time of estimation (Hodgson, 2007), population disease incidence including carrier states and per capita water use of population contributing the wastewater (Petterson and Ashbolt, 2003). In unprocessed wastewater, pathogen load is highest when disease incidence is high in the population and carrier states are a result of constant presence of the pathogens in the wastewater (Petterson and Ashbolt, 2003). During the rainy season pathogen number may increase while the total load in pre-processed wastewater may be low due to dilution of rain water (WHO, 2000; Hodgson, 2007).

Taken together, this would suggest wastewater must be processed properly and to microbiological standards before discharge into natural water systems (Toze, 1997; WHO, 2000). In this regard, global standard for processed wastewater requires that coliform counts should not exceed $10^3$ CFU / 100 mL (WHO, 2000). Yet, processed wastewater for reuse in agricultural crops eaten raw should not contain any coliform per 100 mL of the wastewater (US EPA, 1992).

Dandora sewage treatment plant (DSTP) at Ruai is the biggest wastewater treatment facility in Kenya. This facility was established in 1980 for a projected population of one million inhabitants in the Nairobi area. Since then, Nairobi population has increased three-fold to 3.5 million residents (CBS, 2010). In terms of DSTP wastewater loading, this is about three times the intended capacity for this system.
In the meantime, there are no systematic microbiological surveys that show the performance of DSTP in terms of microbial removal. Similarly, human pathogen load before the DSTP effluent load into Nairobi and Athi rivers is also unknown. In addition, DSTP effluent together with Nairobi and Athi River water is extensively used for crop irrigation downstream river Athi.

It is estimated that the population dependent on river Athi is about 2 million and use the river for both domestic including drinking and agricultural irrigation. The crops irrigated with Athi River water are used locally and also sold to City of Nairobi and the nearby towns. Most of the farm produce grown with this water may be eaten raw and include tomatoes, onions and carrots. Since the human pathogen content of this water is unknown, the crop contamination with these pathogens is also unknown. In the interest of public health of downstream communities, it is important to assess the human pathogens associated with the processed wastewater from DSTP.

1.2 Statement of the Problem

Since the establishment of Dandora Sewage Treatment Plant in 1980 there has been no systematic surveys conducted to ascertain the efficiency of DSTP to remove human pathogens even though similar systems elsewhere have been associated with waterborne illnesses (Katzenelson et al., 1976; Fattal et al., 1986; Shuval et al., 1989; Fattal, 1987; Margalith, 1990).
DSTP is upstream a farming community meaning its effluent in addition to that of Nairobi and Athi rivers form a major source of drinking and irrigation water. This means that the residual chemicals and microbial hazards in these waters are a health risk to the communities that depend on Athi River for domestic and recreational uses. Yet, data on the efficiency of DSTP to remove microbial hazards and the persistence of these pathogens to occur in Nairobi and Athi rivers is unavailable. In the interest of public health of the communities downstream DSTP, this study was designed to establish the microbial hazards present in pre - and post - processed DSTP wastewater and their persistence in Nairobi and Athi rivers.

1.3 Justification

Wastewater has been severally associated with different microbial diseases. Global estimates show that 60 million diarrhoeal cases resulting in about 2 million deaths annually are associated with wastewater reuse in agriculture (WHO, 2000; NRDC, 2004). About 99 percent of the wastewater associated deaths occur in developing countries (WHO, 2000; Prüss-Ustün and Corvalan, 2006; Bos et al., 2009). This suggests that wastewater must be processed properly and to global standards to safeguard human health especially in situations where such water is used for domestic, recreational and agricultural purposes.

DSTP water is a major component of both Nairobi and Athi rivers. Both rivers are in turn used by communities downstream as a major domestic and agricultural water source yet, there are no systematic surveys to ascertain the microbiological status of these waters.
In the meantime, disease outbreaks associated with improperly processed wastewater abound in the literature (Shuval et al., 1986; Hopkins et al., 1993; Cifuentes, 1998; Ait Melloul and Hassani, 1999; Peasey, 2000; Blumenthal et al., 2001). This suggests that microbiological monitoring should be regular and systematic to assure health of the communities downstream. Therefore, this study was designed to ascertain the microbiological quality of DSTP effluent subjected to both Nairobi and Athi rivers and the capacity of the processing system to remove microbial hazards as directed by both local and international standards.

1.4 Research Questions

1. What are the human pathogenic bacteria in pre- and post-processed wastewater at Dandora Sewage Treatment Plant?

2. Does the microbial pollution in the DSTP show a temporal and seasonal variation characteristic?

3. What are residual bacterial types in effluent receiving waters of Nairobi and Athi rivers?

1.5 Null Hypotheses

1. There are no human pathogenic bacteria in pre-processed and post-processed wastewater at Dandora Sewage Treatment Plant

2. There are no temporal and seasonal variation of the bacteria present in both influent and effluent wastewater at Dandora Sewage Treatment Plant
3. Bacteria of human origin are undetectable in the effluent receiving waters of Nairobi and Athi rivers

1.6 Study Objectives

1.6.1 General Objective
To assess the bacteriological quality of wastewater at the Dandora Sewage Treatment Plant

1.6.2 Specific Objectives

1. To determine bacterial types in both pre- and post-processed wastewater at Dandora Sewage Treatment Plant
2. To establish the diurnal and seasonal variation of the bacteria present in both influent and effluent wastewater at Dandora Sewage Treatment Plant
3. To estimate the residual bacteria in effluent receiving waters of both Nairobi and Athi rivers
CHAPTER TWO

2 LITERATURE REVIEW

2.1 Microbiology of Wastewater

Wastewater contains microbes such as bacteria, protozoan, helminthes, archaea, fungi, and rotifers. However, bacteria constitute the major component and the main groups are Zooglea, Pseudomonas, Flavobacterium, Alcaligenes, Achromobacter, Corynebacterium, Comomonas, Brevibacterium, Acinetobacter, Bacillus, Sphaerotilus, Beggiatoa, Vitreoscilla, and Sphingomonas (Ziglio et al., 2002; Bitton, 2005). Other groups include Proteobacteria, Cytophaga-flavobacterium and Arcobacter (Snaidr et al., 1997), Caulobacter, Hyphomicrobium (Holm et al., 1997), Nitrosomonas, Nitrobacter and Rhodospirillaceae (Bitton, 2005).

In addition, protozoans such as ciliates and flagellates are also found in wastewater. Ciliates include Chilodonella, Colpidium, Blepharisma, Euplotes, Paramecium, Lionotus, Trachelophyllum, Spirostomum. Aspidisca, Vorticella (for example Vorticella convallaria, Vorticella microstoma), Carchesium, Opercularia, and Epistylis. On the other hand, wastewater flagellates are Bodo ssp., Pleuromonas ssp., Monosiga ssp., Hexamitus ssp., Poteriodendron ssp., Rhizopoda (amebae) (Bitton, 2005).
Moreover, wastewater contains rotifers, metazoans, which are multicellular organisms. This includes *Philodina* spp., *Habrotrocha* spp., *Lecane* spp. and *Notommata* spp. (Stott *et al*., 2003). Wastewater also contains fungi such as *Geotrichum*, *Penicillium*, *Cephalosporium*, *Cladosporium* (Guest and Smith, 2003) and algae including *Ulothrix*, *Phormidium*, *Anacystis*, *Euglena*, and *Chlorella* (Bitton, 2005). Moreover, other microbes such as archaea, which include Methanogens, are also found in wastewater (Ritchie *et al*., 1997).

### 2.2 Human Microorganisms in Wastewater

Wastewater may contain human microbial pathogens such as viruses, bacteria, protozoa and helminthes (Leclerc *et al*., 2002). Most of pathogens are enteric and therefore, they are present in water environment following human faecal contamination (NRDC, 2004). However, some of the pathogens are non-enteric being excreted in urine or sputum of infected individuals and may gain entry into wastewater (NRDC, 2004).

Occurrence of human pathogens in wastewater depends on the prevalence of diseases, carrier state, per capita water use and disease seasonality (WHO, 2002; Petterson and Ashbolt, 2003). In light of all these, wastewater portends public health risk and it is for this reason that wastewater is processed before release into natural environment (Okoh *et al*., 2007).
2.3 Bacterial Pathogens in Wastewater

Among the human pathogens in wastewater, bacteria are the most common (Toze, 1997; Okoh et al., 2007). They include *Salmonella* species, *Shigella* species, *Escherichia coli*, *Vibrio* species, *Yersinia enterocolitica*, *Campylobacter* species, *Leptospira*, *Legionella pneumophila* and *Bacteriodes fragilis*. Moreover, bacteria, which cause opportunistic infections in human, can also be found in wastewater. These include pathogens in genus *Pseudomonas*, *Aeromonas*, *Klebsiella*, *Flavobacterium*, *Enterobacter*, *Citrobacter*, *Serratia*, *Acinetobacter*, *Proteus*, *Providencia* and *Mycobacteria* (Feachem et al., 1983; Bitton, 2005; Samie et al., 2009).

2.3.1 *Salmonella* species

*Salmonella* species such *Salmonella typhi*, *Salmonella paratyphi*, *Salmonella enteritidis*, *Salmonella typhimurium* among others are enterobacteriaceae widely distributed in the environment and include more than 2000 serotypes. In the wastewater, these bacteria range from $2 \times 10^1$ to $8 \times 10^4$ CFU / 100 mL (Yates and Gerba, 1998). Salmonellae are the most predominant of all wastewater bacteria and they cause typhoid and paratyphoid fever as well as gastroenteritis. In the United States, *Salmonella* species numbers in wastewater range from $10^2$ to $10^4$ CFU / 100 mL but concentrations up to $10^9$ / 100 mL have been reported in the developing countries (Jimenez and Chavez, 2000).

It is estimated that 0.1% of the population excretes Salmonellae at any given time (Bitton, 2005). Moreover, *Salmonella* species survive for less than sixty (< 60) days in wastewater, less than seventy (< 70) days in soil and less than thirty (< 30) days on crops.
when temperature range between 20°C to 30°C (Feachem et al., 1983). In the mean time, the minimal infective dose for *Salmonella* spp. to cause infections in humans is $10^4$ to $10^7$ (Bitton, 2005).

### 2.3.2 *Shigella* species

*Shigella* species are the causative agents of bacillary dysentery or shigellosis, an infection of the large bowel that leads to cramps, diarrhea, and fever. This disease produces bloody stools as a result of inflammation and ulceration of the intestinal mucosa. There are four pathogenic species of *Shigella*: *Shigella dysenteriae*, *Shigella flexneri*, *S. boydii* and *Shigella sonnei* (Bitton, 2005).

Importantly, an infected individual may excrete up to $10^9$ *Shigella* per gram of feces and a level of $7 \times 10^2$ *Shigella* organisms / 100 mL has been reported in wastewater (Feachem, 1983). Further, Yates and Gerba (1998) reported a level of 0 to $10^3$ *Shigella* organisms / 100 mL of domestic wastewater. In addition, Samie *et al.* (2009) reported levels less than $10^8$ CFU / 100 mL in Mpumalanga, South Africa and noted that *Shigella* species were the least dominant bacterial type in wastewater.
Shigella species persists less in the environment than fecal coliforms and they survive for less than thirty (< 30) days in wastewater and less than ten (< 10) days on crops (Feachem, 1983). However, the pathogen has a very low infectious dose and can be as low as 10 organisms (Bitton, 2005).

2.3.3 *Vibrio* species

*Vibrio* species are also present in wastewater. The most encountered species are *Vibrio cholerae* and *Vibrio parahaemolyticus* (Samie et al., 2009). *Vibrio cholerae* is the causative agent of cholera by releasing enterotoxin that causes mild to profuse diarrhea, vomiting leading to a very rapid loss of fluids, which may result in death in a relatively short period of time (Bitton, 2005).

*Vibrio* species levels in wastewater have been reported at $10^{-4}$ to $10^{4}$ CFU / 100 mL (Kott and Betzer, 1972) and $10^{2}$ to $10^{5}$ CFU / 100 mL (Yates and Gerba, 1998). Survival rate for the bacteria is less than thirty (< 30) days in both wastewater and soil and, less than five (< 5) days on crops (Feachem et al., 1983). However, the infectious dose for these bacteria ranges from $10^{4}$ to $10^{6}$ organisms (Bitton, 2005).
2.3.4 *Escherichia coli*

Several strains of *Escherichia coli*, many of which are harmless, are found in wastewater. However, there are several categories of *Escherichia coli* strains, that bear virulence factors and cause diarrhea. They include Enterotoxigenic *Escherichia coli* (ETEC), Enteropathogenic *Escherichia coli* (EPEC), Enterohemorrhagic *Escherichia coli* (EHEC), Enteroinvasive *Escherichia coli* (EIEC), and Enteroaggregative *Escherichia coli* (EAggEC) (Bitton, 2005). Moreover, Enterotoxigenic *Escherichia coli* cause gastroenteritis with profuse watery diarrhea accompanied with nausea, abdominal cramps, and vomiting. Approximately 2 to 8% of the *Escherichia coli* present in wastewater is Enteropathogenic *Escherichia coli*, which causes Traveler’s diarrhea (Bitton, 2005).

*Escherichia coli* level has been documented in Brazil at $1.8 \times 10^8$ CFU / 100 mL and $3.9 \times 10^5$ CFU / 100 mL in raw and processed wastewater of Waste Stabilization Ponds (WSP) respectively (Pivelli *et al.*, 2008). However, the infective dose for these bacteria is relatively high ranging between $10^6$ and $10^8$ organisms (Bitton, 2005). Nevertheless, enterohemorrhagic *E. coli* O157:H7, has a relatively low infectious dose of $<10^2$ organisms (Herwaldt *et al.*, 1992).
2.3.5 Campylobacter species

Campylobacter species which are often present in wastewater are Campylobacter fetus and Campylobacter jejuni. These pathogens are known to infect humans as well as wild and domestic animals. They are ubiquitous in domestic wastewater and in effluents from abattoirs and poultry processing plants (Bitton, 2005).

Seasonal trend similar to that of the incidence of infections in humans has been reported in the occurrence of Campylobacter species in wastewater (Jones, 2001). Further, the levels of these pathogens in wastewater have been reported at 3.7x10³ CFU / 100 mL. Campylobacter species have relatively low infectious dose of approximately 500 organisms. Owing to their sensitivity to oxygen and inability to grow at temperatures below 30°C, C. jejuni survives but does not grow in the environment (Bitton, 2005).

2.3.6 Opportunistic Bacterial Pathogens

Opportunistic bacterial pathogens cause diseases to immunocompromised people, elderly and young children. This group includes heterotrophic gram-negative bacteria belonging to genera Pseudomonas, Aeromonas, Klebsiella, Flavobacterium, Enterobacter, Citrobacter, Serratia, Acinetobacter, Proteus, Providencia, and nontubercular mycobacteria. Pseudomonas aeruginosa is ubiquitous in the environment and is frequently found in water, wastewater, soils and plants. Further, Pseudomonas aeruginosa level in wastewater ranging from 10³ to 10⁶ organisms / 100 mL has been reported (Bitton, 2005)
2.3.7 *Leptospira interrogans*

*Leptospira interrogans* is a small spirochete that can gain access to the host through abrasions of the skin or through mucous membranes. It causes leptospirosis, a disease characterized by the dissemination of the pathogen in the patient’s blood and the subsequent infection of the kidneys and the central nervous system. The disease can be transmitted from animals (rodents, domestic pets, and wildlife) to humans coming into contact (for example, bathing) with waters polluted with animal wastes. This zoonotic disease may strike sewage workers. This pathogen is not of major concern because it does not survive well in wastewater (Rose, 1986).

2.3.8 *Legionella pneumophila*

This bacterial pathogen causes Legionnaires’ disease. This is a type of acute pneumonia with a relatively high fatality rate; it may also involve the gastrointestinal and urinary tracts as well as the nervous system. This organism is transmitted mainly by aerosolization of contaminated water as occurs in sprinkler irrigation (Bitton, 2005). The level of this pathogen ranges from 1 to $10^3$ CFU / 100 mL (Leoni *et al.*, 2001).

2.4 Wastewater Viruses

Human viruses are also potentially present in wastewater and may include Rotaviruses, Norwalk viruses, Echoviruses, Adenoviruses, Caliciviruses, Astroviruses, Reoviruses, enteroviruses and other small round structured viruses (Toze, 1997; Okoh *et al.*, 2007). But the most commonly isolated viruses are enteroviruses such as *Poliovirus* type 1 and 2, many strains of Echovirus, Enterovirus (including Hepatitis A virus) and
Coxsackieviruses (Toze, 1997; Tanji et al., 2002).

Enteroviruses survive for about four months (< 120 days) in wastewater, three months (< 100 days) in soil, and about two months (< 60 days) on crops (Feachem et al., 1983). The levels of viruses in wastewater range from $10^2$ to $10^3$ viral particles / 100 mL (Toze, 1997; Tree et al., 2003). However, the minimal infectious dose for viruses such as HAV ranges from 1 to 10 PFU (Bitton, 2005).

2.5 Wastewater Protozoa

Protozoan such as Cyclospora, Cryptosporidium spp., Giardia spp., Microsporidium, Entamoeba histolytica, Toxoplasma gondii, and Naegleria are often present in wastewater. These pathogens are present in form of cyst or oocyst and their prevalence in wastewater is higher than any other environment (Toze, 1997; Okoh et al., 2007). The survival of protozoan is less than thirty days (< 30) days in wastewater, less than twenty (< 20) days in soil, and less than ten (< 10) days on crops (Feachem et al., 1983).

Entamoeba histolytica, Giardia lamblia and Cryptosporidium parvum are the most commonly isolated protozoan pathogens in wastewater (Caccio et al., 2003; Toze, 1997). Further, the level of Giardia cysts may be as high as $10^4$ / 100mL in raw wastewater (Caccio et al., 2003). Meanwhile, a level of 48 cysts / 40 L has been reported in processed wastewater (Rose et al., 1989). The minimal infectious dose for this parasite is however $10^1$ to $10^2$ cysts.
Cryptosporidium occurs in raw wastewater at levels varying between 85 and 1370 oocysts / 100 mL but the range of this pathogen in processed wastewater varies between 0 and 396 oocysts / 100 mL. The minimal infectious dose for Cryptosporidium is $10^1$ oocysts (Bitton, 2005). The level of Entamoeba histolytica cysts in raw wastewater may be as high as 500 cysts / 100 mL (Bitton, 2005).

2.6 Wastewater Helminths

Helminthes which are common in wastewater are the round worm (Ascaris lumbricoides), the hook worm (Ascaris duodenale or Nector americanus), the whip worm (Trichuris trichiura) and Strongloides stercolaris (Feenstra et al., 2000). These helminths survive for many days in wastewater, soil and on crops (Feachem et al., 1984). However, seasonal fluctuations in the number of helminth ova in wastewater are expected. For example, in Marrakech, Morocco, the level of ova in raw wastewater varied between 0 and 12 ova / 100 mL with an annual mean of 3 ova / 100 mL. Further, the minimum infectious dose of helminthes such as Ascaris lumbricoides is relatively low and has been reported to be 1 to 10 eggs (Bitton, 2005).

2.7 Wastewater Fungi

Blue algae (cyanobacteria) such as Anabaena flos aquae, Microcystis aeruginosa, and Schizothrix calcicola are often present in wastewater. These algae produce exotoxins (peptides and alkaloids) as well as endotoxins (lipopolysaccarides) which are toxic to humans and may be responsible for syndromes such as gastroenteritis (Toze, 1997).
2.8 Wastewater Chemistry

Wastewater potentially contains both organic and inorganic chemicals. Inorganic chemicals include dissolved constituents, nutrients, nonmetal constituents, metals and gases while organic chemicals may include chlorinated organic, phenolic compounds, surfactants and pesticides. Organic compounds such as aliphatic and aromatic hydrocarbons are typically benign (Bitton, 2005).

The principle inorganic cations naturally present in wastewater are calcium ions ($\text{Ca}^{2+}$), magnesium ions ($\text{Mg}^{2+}$), potassium ions ($\text{K}^+$), and chloride ions ($\text{Cl}^-$). However, some residual inorganics in wastewater are carcinogenic and they include arsenic, lead and cadmium compounds. Several inorganic compounds such as aluminium, chromium, copper, manganese, molybdenum, nickel, selenium, zinc and sodium which are essential in human at low doses cause adverse health effects at high doses (NRDC, 2004).

The endocrine disrupters (ED) such as natural estrogens (for example, 17b-estradiol), synthetic steroids (for example, 17a-ethynylestradiol entering in the composition of contraceptive pills), phytoestrogens, pesticides, and alkylphenols may also be present in wastewater (Folmar et al., 1996).

2.9 Wastewater Treatment Strategies

Strategies employed in wastewater treatment include activated sludge systems, attached microbial growth based systems, and anaerobic treatment based systems, Waste Stabilization Ponds, and disinfection (Okoh et al., 2007). The activated sludge systems
are the most widely applied wastewater treatment strategy in the world (Mara, 2004). These systems are relatively efficient in removing pathogenic microorganisms and parasites compared to other biological treatment processes such as trickling filters. However, the removal of indicator and pathogenic bacteria may vary from 80 percent to more than 99 percent (Bitton, 2005).

Studies in India have shown that the activated sludge systems remove 90 to 99 percent of enteric viruses. However, lower removal of 48 percent and 69 percent been been documented at Haut de Seine and Nancy wastewater treatment plants in France respectively (Bitton, 2005). On the other hand, removal of protozoan parasites is estimated to be 0 to 90 percent for Cryptosporidium and 60 to 90 percent for Giardia cysts (Robertson et al., 2000). Further, Giardia removal following activated sludge treatment and disinfection has been reported ranging from 87 to 98 percent in Italy (Caccio et al., 2003). Removal of Cryptosporidium oocysts ranging between 94 and 99 percent has been documented following biological treatment in the United States (Gennaccaro et al., 2003).

Another wastewater processing strategy is that based on attached microbial growth. This include systems such as trickling (percolating) Filters (TF) and rotating biological contractors (RBCS) (Al-Sa’ed, 2001). Trickling Filters are inconsistent in removal of bacteria and this varies from 20 to 90 percent. Further, removal of Salmonella species is lower than in activated sludge systems and may vary from 75 to 95 percent. However, removal of total and fecal coliforms at the Kerrville, Texas, was reported to be 92 and 95
percent respectively while enteroviruses varied from 59 to 95 percent using this system (Bitton, 2005).

Removal of cysts and oocysts by TF is also generally low and varies from 10 to 99 percent. For example, 74 to 91 percent removal of *Entamoeba histolytica*, and *Giardia* cysts have been documented in India (Panicker and Krishnamoorthi, 1978). Rotating Biological Contractors (RBC) method of wastewater processing removes a total of 1 log unit or more of fecal coliforms and between 79 and 99 percent of *Campylobacters* species (Jones, 2001).

Another strategy in wastewater processing is based on anaerobic treatment. The systems adopting this strategy include septic tanks, upflow anaerobic sludge blankets (UASB), and anaerobic rotating biological contactor (Okoh *et al.*, 2007).

Waste stabilization ponds (WSPs) which are used for secondary treatment of wastewater or as polishing ponds is yet another method of wastewater management. The ponds are classified as facultative, aerobic, anaerobic, aerated, high-rate aerated, and maturation ponds. The anaerobic process utilizes naturally occurring bacteria to break down biodegradable material in wastewater (Toze, 1997). Anaerobic ponds have a depth of 2.5 to 9 meters (Hammer, 1986) and relatively long retention time of 20 to 50 days (Metcalf and Eddy, 1991). These ponds are pre-treatment step for BOD and organic matter is
biodegraded under anaerobic condition to methane (CH₄) and carbon dioxide (CO₂) and other gases such as hydrogen sulfide (H₂S).

On the other hand, facultative ponds depth range from 1 to 2.5 meters and the detention time varies between 5 and 30 days (Hammer, 1986). Waste treatment is because of natural biological processes carried out mainly by bacteria and algae. A mixture of aerobic, anaerobic and facultative microorganisms is involved in waste treatment (Bitton, 2005).

These ponds allow accumulation of solids that are degraded anaerobically at the bottom of the pond releasing soluble degradable organic material and nutrients, which diffuse upwards in the pond. Near the top of the pond, oxygen is supplied by algal photosynthesis and to a limited extent by diffusion from the air. Dissolved oxygen is present to only a few centimeters depth at night, but diffuses deeper during daylight (Tchobanoglous and Angelaki, 1996; Al-Sa’ed, 2001). Thus there exists a fully aerobic zone at the top of the pond, and between this and the anaerobic zone at the bottom there is a middle zone where oxygen is cyclically present and bacterial respiration is "facultatively" aerobic-anaerobic (Tanik et al., 1996; von Sperling and de Lemos Chernicharo, 2005).
The other type of waste stabilization ponds are aerobic ponds. These ponds are shallow with depth of 0.3 to 0.5 meters and detention time of 3 to 5 days. The ponds contain bacteria and algae in suspension and maintain aerobic conditions throughout their depth. The ponds obtain oxygen via oxygen transfer between air and water surface, and oxygen produced by photosynthetic algae (George and Andrew, 2003). Maturation or tertiary ponds are yet other type of waste stabilization ponds. They are 1 to 2 meters deep and have a detention time of approximately 20 days. This type of ponds serve as tertiary treatment for wastewater effluent from activated sludge or trickling filters. Oxygen provided by surface re-aeration and algal photosynthesis is used for nitrification. Their role is to further reduce BOD, suspended solids, and nutrients (nitrogen and phosphorus), and to further inactivate pathogens (Okoh et al., 2007).

The removal or inactivation of pathogens is controlled by a variety of factors among which are temperature, pH, sun light, lytic action of bacteriophages, predation by microorganisms and attachment to settleable solids. Level of 90 to 99 percent removal of indicator and pathogenic bacteria was reported in WSP (Saqqar and Pescod, 1992). Bacteria die-off increases with an increase in retention time and temperature (Saqqar and Pescod, 1992). Other factors include aeration, antimicrobial extracellular algal compounds, depletion of nutrients, sunlight intensity and growth of algae (Fernandez et al., 1992).
A study in Marrakech, Morocco, reported 98 percent inactivation of fecal coliforms, and 92.2 percent for *Pseudomonas aeruginosa* in a series of two experimental ponds with a total detention time of 16 days. However, *Vibrio cholera* was not reduced and it showed a peak during the summer season (Mandi *et al.*, 1994). Moreover, 99.1 percent (25 days retention time) and 99.7 percent (40 days retention time) removal of *Giardia* cysts has been reported in Kenya and France respectively. Further, removal of parasites (*Ascaris lumbricoides*, hookworm) in aerobic pond varies between 50 and 100 percent (Grimason *et al.*, 1996).

Disinfection is yet other wastewater treatment strategy. This is the destruction of microorganisms such as viruses, bacteria and protozoan parasites which are capable of causing disease (Bitton, 2005). Disinfection is achieved either through use of ozone, chlorine, chloramines, or ultraviolet (UV) light. Ozone and UV light are more preferred because chlorine produces products that can be toxic or genotoxic to humans and animals (Bitton, 2005). However, ozone and UV light can only be used on secondary treated wastewater whereas chlorination can be used on partially or fully treated wastewater. Further, the disinfection efficiency of UV light is affected by the amount of turbidity, color, dissolved organic and inorganic in wastewater with increase of any of these factors decreasing the disinfection efficiency (Toze, 1997).
2.10 Microbiological Guidelines on Treated Wastewater

For the interest of protecting public health, international guidelines for safe disposal of wastewater effluents have been published by World Health Organization (WHO, 1989) published international guidelines for safe disposal of effluent. In turn, policy makers in different countries legislating quality of effluents have derived their standards and guidelines from the WHO framework.

The WHO microbiological guidelines are based on faecal coliform and intestinal nematode eggs aimed at reducing disease incidences associated with wastewater especially in agriculture. Consequently, the effluents quality guidelines are determined by reuse purpose and have been categorized ‘A’, ‘B’ and ‘C’) (WHO, 2000). In this way, effluents in category ‘A’ are to be reused in unrestricted areas for irrigation of crops likely to be eaten uncooked, sports and public parks. In this category, effluent are required to contain $\leq 10^3$ faecal coliforms (FC) per 100 mL (reflecting >99.99% removal) and $\leq 1$ nematode ova per litre; equivalent to a removal efficiency of up to 99.9% (3 log removal) (WHO, 2000) (Appendix V). On the other hand effluents in category ‘B’ are to be reused in restricted areas for irrigation of cereal crops, industrial crops, fodder crops, pasture and trees. In this category there is no faecal coliform guideline, however, nematode guideline of $\leq 1$ nematode ova per litre is recommended (WHO, 2000).
Further, the United States, Environmental Protection Agency (USEPA) recommends zero (0) faecal coliforms/100 mL for effluent intended for irrigation of any food crops not commercially processed including crops eaten raw. This standard is similar to Japan's standard in which the water quality is achieved through secondary treatment, filtration and disinfection. In addition, USEPA requires a guideline of \(\leq 200\) faecal coliforms/100 mL to be meet for effluent irrigating commercially processed foods crops such as orchards, and vineyards. This water quality is achieved through filtration and disinfection of biologically treated wastewater (USEPA, 1992; Kramer and Post, 2005).

In California, less than 2.2 total coliform bacteria/100 mL is set as standard for irrigation of food crops and the water quality is achieved through secondary treatment followed by filtration and disinfection. In addition, less than 23 total coliform bacteria/100 mL are recommended for effluent used in irrigation of pasture and landscaped areas. This water quality is produced through secondary treatment and disinfection (Toze, 1997; Kramer and Post, 2005).

In Saudi Arabia, 2.2 coliforms/100 mL standard is set for irrigation of crops, such as vegetables eaten raw (uncooked), parks and lawn in unrestricted areas. Further, a guideline of 250 coliforms/100 mL is set for irrigation of deciduous fruits, conserved vegetables, cooked and peeled vegetables (Toze, 1997; Kramer and Post, 2005).
In South Australia, a microbiological guideline of $<10$ thermo tolerant coliforms / 100 mL is set for residential use of wastewater, municipal irrigation and unrestricted crop irrigation. This water quality is attained through secondary treatment, filtration and disinfection. Moreover, for ornamental pond with public access, restricted crop irrigation, irrigation of pasture, and fodder crops for grazing animals, effluent quality should meet a guideline of $<100$ coliforms / 100 mL. This guideline is similar to the set guidelines for irrigation of crops eaten raw in Kuwait (Toze, 1997; WHO, 2000).

Tunisia, has adopted $\leq 1$ helminth ova per litre the guideline as set by WHO for irrigation in restricted areas. However, irrigation of vegetables likely to be eaten raw using reclaimed wastewater is prohibited (Toze, 1997; Bahri, 1998). In this country, effluent of secondary treatment plants supplemented by retention in ponds or reservoirs is mainly used to irrigate fruit trees, fodder crops, industrial crops, cereals and golf courses (WHO, 2000).

In Kenya, the Environmental Management and Co-ordination (Water Quality) Regulations, 2006 describes guideline values for effluent discharge into public water in Kenya. In these regulations, 30 total coliforms per 100mL and zero (0) *Escherichia coli* per 100 mL are set for effluent discharge into natural environment (Kenya Gazette, 2006). Further, Kenya Bureau of Standards (KEBS) requires that *Escherichia coli*, *Staphylococcus aureus*, *Shigella* species, *Salmonella* species, *Pseudomonas aeruginosus*, and coliforms should not be detectable in 250 mL of drinking water while *Streptococcus faecalis* should not be detectable at any volume of drinking water (Kenya Gazette, 2006;
2.10.1 Reported Cases of Wastewater Treatment Failure

Waste Stabilization Ponds (WSP) are expected to achieve the WHO microbiological guidelines for disposal and reuse of wastewater effluents (WHO, 2000). However, failure by these systems to meet the guidelines has been documented. For instance in Valle Del Cauca, Colombia, although 4 log units of *Escherichia coli*, 1 log unit of *Streptococcus* species was removed, *Salmonella* species were still detectable in the effluent and the *Escherichia coli* surpassed the WHO microbiological guidelines for irrigation in unrestricted areas (Madera *et al.*, 2002).

A study in Khartoum, Sudan, Ahmed (2010) reported 79 percent reduction of total coliforms (TC) which is below the expected removal efficiency (99.99 %) for these systems. Another study in Egypt reported total coliform (TC), faecal coliform (FC), *Escherichia coli*, faecal streptococci (FS), *Salmonella* species and *Listeria* reduction as 98.8, 95.6, 79.4, 96.8, 97.9 and 89.5% respectively. Therefore, the effluent quality did not meet the WHO microbiological guideline and the expected performance efficiency for WSP (Mahassen *et al.*, 2008).
In Maracaibo, Venezuela, a level of $5.4 \times 10^4$ to $1.4 \times 10^5$ for total coliforms and $5.2 \times 10^4$ to $1.3 \times 10^5$ for faecal coliforms was found in effluent wastewater despite the high (93 to 98 percent) removal efficiency of microorganisms an indication that the WSP system failed the WHO guideline (Botero et al., 1997). Further, in Brazil, an average density of total coliforms and *Escherichia coli* in WSP effluent was $1.1 \times 10^7$ CFU / 100 mL and $3.9 \times 10^5$ CFU / 100 mL respectively and *Ascaris* eggs were observed in 80.8% of the samples collected. Moreover, *Salmonella* species was detected in 36.4% of the samples and therefore the effluent quality did not meet bacteriological quality recommended by World Health Organization (Pivelli et al., 2008).

*Giardia* species cysts were detected in 28% of final maturation pond effluents samples in Matsapha sewage treatment plant, Swaziland and a maximum of 600 cysts / L in the final maturation effluent was reported again an indication that the WHO microbiological guideline (< 1 oval / L) was far much exceeded (Nkambule et al., 2010). In another case, a total *Listeria* density of $2.9 \times 10^2$ to $2 \times 10^7$ CFU / 100 mL was reported in WSP effluent in Eastern Cape Province, South Africa. Thereby, the treated effluent quality fell short of recommended WHO microbiological guideline and demonstrated a potential negative impact of the wastewater effluent on the receiving environment thus suggesting a serious risk to public health (Odjadjare et al., 2010).
In Kenya, *Ascaris* sp. ova in range of 0.7 to 88.9 ova / L was reported in Waste Stabilization Ponds in Eldoret, Dandora and Nakuru. The findings showed failure by the systems to meet the WHO guideline of < 1 nematode ova / L. (Grimason et al., 1996).

Taken together, these studies suggest that treatment failure do occur in WSP thereby failing to adhere to the WHO microbiological guidelines, yet systematic studies on the performance of the Dandora Sewage Treatment (DSTP) is unavailable. Therefore, public health risk posed by microbes in the effluent discharged into Nairobi and Athi rivers is unknown.

### 2.10.2 Consequences of Wastewater Treatment Failure

Wastewater treatment is principally is carried out to remove pathogens and other chemicals which are harmful to both human and aquatic health as well as causing environmental degradation (NRDC, 2004). Therefore, wastewater should be processed to meet the WHO guidelines as well as locally acceptable standards before discharging effluent into natural environment (Toze, 1997).

In case of treatment failure, wastewater processing plants may discharge effluents with high ammonium or nitrate concentration into receiving aquatic environment. This may result in formation of un-ionized ammonia which is toxic to fish. Further, ammonia may result in oxygen demand in the receiving waters and consequently depleting oxygen thus adversely affecting aquatic life.
Discharge of nitrogen into receiving water may stimulate algal and aquatic plant growth. This in turn exerts a high oxygen demand at night time which adversely affects fish and other aquatic life and has a negative impact on the beneficial use of water resource for drinking or recreation. Further, nitrates may be responsible for methemoglobinemia in infants and certain susceptible proportion of adult population. Methemoglobinemia (‘blue babies syndrome) is due to conversion of nitrate to nitrite by nitrate-reducing bacteria in gastrointestinal tract (Bitton, 2005).

In addition, raw or partially treated wastewater can result in acute and chronic toxicity to aquatic life from chemical contaminants, as well as bioaccumulation and biomagnification of chemicals in the food chain (Boesch et al., 2002). Moreover, the untreated or inadequately treated wastewater may put public health at risk from drinking water, bathing, fishing or irrigating crops using water contaminated with pathogenic bacteria, protozoa, viruses and several toxic substances some of which are carcinogenic (Paillard et al., 2005).

2.10.3 Epidemiology of Wastewater-Borne Human Diseases

Globally, approximately 60 million cases of diarrhoea resulting in about 2 million deaths annually are associated with wastewater microbes. Further, about 99 percent of these deaths occur in developing countries. For instance, typhoid causes approximately 16,000,000 illness cases resulting in 0.5 million deaths per year. Moreover, global
Ascaris lumbricoides infections are up to about 1.45 billion annually. Of these, 350 million people suffer adverse health effects. Further, Hookworm infections are estimated at 1.3 billion cases, of which 150 million suffer adverse health effects. In addition, Viruses such as Hepatitis A are estimated to be 1.4 million cases per year worldwide (WHO, 2006; Pruss-Ustun and Corvalan, 2006; Bos et al., 2009).

Studies among consumers of uncooked vegetables irrigated with untreated wastewater in Israel, Mexico and Chile have reported disease incidences caused by parasites, viruses and bacteria (Shuval, 1984; Hopkins, 1993; Cifuentes, 1998; Peasey, 2000; Blumenthal, 2001).

Disease incidences have been documented in farm workers and, their families, and to nearby populations exposed to untreated (Krishnamoorti, 1973; Bouhoum, 1998; Habbari, 2000; Srivastava, 1986) or treated (Katzenelson, 1976; Linneman, 1984; Fattal, 1986; Camann, 1987; Shuval, 1989; Blumenthal, 2001) wastewater in agriculture. Further, disease incidences have also been reported in populations using both treated and untreated wastewater (Sehgal, 1991; Cifuentes, 1998; Cifuentes, 2000; Peasey, 2000; Blumenthal, 2001).

An epidemiological study in Morocco, found increased incidences of giardiasis and amoebiasis in the study population (children) where wastewater is reused for agriculture in a peri-urban area of Marrakesh (Amahmid and Bouhoum, 2000). In Hanoi, Vietnam diarrheal incidence rate of 28.1 episodes per 100 person-years with wastewater,