Surveillance of Viral, Faecal Indicator Bacteria and Vibrio Pathogens in the Final Effluents of Wastewater Treatment Facilities in the Eastern Cape Province: A Vehicle for Capacity Development in Microbial Water Quality Science in the Province

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

BACKGROUND TO THE STUDY AND STATEMENT OF PROBLEM

The majority of Wastewater Treatment Plants (WWTPs) discharge their effluents into environmental water bodies. Hence guidelines were established for the qualities of such wastewater effluents destined for discharge into the environment. However, production of effluents of acceptable quality has become a major challenge for wastewater treatment facilities in South Africa, especially in the Eastern Cape Province. This is based on the acknowledgement that it is mostly non-urban, poor and without adequate infrastructure, and that a significant proportion of its communities lack pipe-borne water, and as such rely on beaches, streams, rivers, groundwater and other water bodies for recreation, drinking and domestic purposes. Many of these water bodies are often polluted by industrial and municipal wastewater effluents. These effluents harbour the potential to impact on agricultural, recreational and drinking-related water uses, and their characteristics may, depending on the health status of the community contributing wastes to a WWTP, contain enteric bacterial and viral pathogens.

Human enteric viruses are causative agents of many non-bacterial gastrointestinal and respiratory tract infections, as well as other clinical syndromes, including conjunctivitis, hepatitis and other diseases such as aseptic meningitis, encephalitis and myocarditis with high morbidity and mortality especially in immunocompromised individuals. The majority of these viruses are non-enveloped, which makes them highly resistant to decontamination processes used in both wastewater and drinking water treatment. The monitoring of sewage effluents for viral and bacterial pathogens may prove a suitable approach for the study of circulating pathogens and their persistence in treated effluents.

The efficiencies of the WWTP in the Eastern Cape Province with regards to producing final effluents of acceptable bacteriological and virological qualities remains inadequately documented, and are to a significant extent reflective of the shortage of skilled manpower in the water sector in the Province especially in the area of microbial water quality. This study was therefore designed to assess the prevalence of human viral, faecal indicator bacteria and Vibrio bacteria pathogens in the final effluents of 14 WWTP in Eastern Cape Province as a vehicle for skills development in microbial water quality science amongst previously disadvantaged demographic groups.

PROJECT AIMS

The aims of this project were as follows:

1. To carry out a survey of existing wastewater treatment facilities in the entire Eastern Cape Province, noting their dates of establishment, working capacity and current statuses.
2. To assess the incidence of human viral pathogens, faecal coliform and *Escherichia coli* in the selected WWTP effluents.

3. To assess the incidence and antibiogram characteristics of Vibrio bacteria pathogens and pathogenic *E. coli* in the selected WWTP effluents.
4. To determine the physicochemical qualities of the selected WWTP effluents.
5. Compare data obtained from typical urban, semi-urban and rural communities of the seven main districts that make up the province.
6. Submit a report of our findings to the WRC and Eastern Cape Provincial Government

METHODOLOGY

Wastewater final and discharge point (where available and accessible) effluent samples were collected from 14 WWTP in the Eastern Cape Province over a period of a year from September 2012 to August 2013 and transported in cooler boxes to the Applied and Environmental Microbiology Research Group (AEMREG) Laboratory at the University of Fort Hare, Alice for analyses within 6 h of collection.

Free chlorine, Turbidity, Electrical conductivity, total dissolved solids, temperature, pH, dissolved oxygen of the effluent samples were determined *in situ* using a multi-parameter ion-specific meter. Concentrations of orthophosphate, total nitrogen (nitrate + nitrite), chemical oxygen and biochemical oxygen demands, were determined by standard photometric methods.

Faecal coliforms and Vibrio counts were determined by membrane filtration and direct plating methods. Viruses in water samples were concentrated using the adsorption-elution method, followed by extraction of viral nucleic acids and purification done using commercially available kits. The concentrations of human enteric viruses in the river-water samples were estimated using quantitative PCR. The RNA viruses were quantified in a two-step protocol where RNA was first transcribed into cDNA in a separate reverse-transcription step.

Antibiogram characterisation was carried out for confirmed pathogens to determine the prevalence of antibiotic resistant determinants in the environment.

SUMMARY OF MAJOR FINDINGS

The major findings of this study are as summarised below:

- About 78.5% of the WWTPs chlorinated their effluents to compliant levels for ≥ 50% of the times. Cases of chlorine under-dosing were also reported, with 50% of the plants applying substandard dosages of chlorine for > 20% of the times.

- With regards to temperature and pH, effluents from all the 14 WWTPs were compliant with the set regulatory guideline values.

- With the exception of effluents from Amalinda, Fort Beaufort and Queenstown WWTPs, effluents compliance to set guidelines for both DO and BOD was higher than 50% indicating the efficiency of the WWTPs in removing organic matter from the effluents.

- With respect to phosphate concentrations, there was overall compliance indicated by 75% of the effluents from Amalinda, 92% of effluents from Keiskammahoek and Komga and 100% for the remaining effluents throughout the study period.

- The levels of nitrates, nitrites, EC and TDS were largely compliant to set guidelines and, where non-compliance was observed, the overshoot was mostly marginal.

- We conclude therefore, that with respect to physicochemical parameters, the WWTPs performed optimally and produced effluents of acceptable standards for discharge into freshwater ecosystems without upsetting their nutrient balance. These effluents can potentially be used for irrigation without increasing the salinity of the soils.

- On the average, 86% of the WWTPs had a compliance rate of ≥ 50% with respect to the faecal coliform guideline of 1000 CFU/100 ml in their effluents. This contrasts with the Green Drop 2012 average compliance value of 36% for the selected WWTPs. *E. coli* O157 was not detected at all.

- An independent samples T-Test (IBM SPSS version 20) comparison of mean faecal coliform bacteria counts from the discharge point samples with the mean faecal coliform bacteria counts from the final effluent samples (of all WWTPs) showed no significant differences ($P > 0.05$) between the bacteriological qualities of the final effluent and discharge point samples.

- Both *Vibrio* and *E. coli* pathotypes were detected in final effluent samples. Confirmed *Vibrio* pathogens included *V. parahaemolyticus* (11.6% prevalence), *V. fluvialis* (28.6% prevalence) and *V. vulnificus* (28% prevalence) while 31.8% belonged to other *Vibrio* spp. not assayed for in this study. Confirmed *E. coli* pathotypes includes Enteropathogenic *E. coli* (EPEC) (1.2%), Enteroaggregative *E. coli* (EAEC) (2.7%) and Uropathogenic *E. coli* (UPEC) (3.8%).

- Multiple antibiotic resistance patterns were also evident especially against such antibiotics as tetracyclin, polymixin B, chloramphenicol, penicillin G, sulfamethazole and erythromycin against which prevalence of resistance was greater than 60% for *Vibrio* pathotypes.

- While prevalence of antibiotic resistance of *E. coli* pathotypes to the test antimicrobials was remarkably lower than what was observed for *Vibrio* pathotypes, resistance against sulfamethazol, tetracycline and ampicillin was > 50% for all the three *E. coli* pathotypes detected.

- The dynamics of RNA viruses (hepatitis A virus, enterovirus and rotavirus) in wastewater effluents were acutely different from those of adenovirus, the only DNA virus in this study. Enterovirus and hepatitis A virus were not detected in any of the 14 WWTPs while rotavirus was detected in 4 of the 14 WWTPs. Detection frequencies of rotavirus were, by WWTP, Amalinda (33%), East Bank (17%), Komga (33%) and Whittlesea (8%). Adenovirus was detected in 93% of the WWTPs.

- Risk of infection calculations (with adenovirus) showed that effluents from the Alice WWTP presented the highest risk of infection values for irrigated crop consumption and accidental ingestion of pond water. Other WWTPs whose effluents presented substantial risk of infection when irrigated crop is consumed fresh and wet (with irrigation water) included Mdantsane, Fort Beaufort and Amalinda.
- Also, the calculated risk arising from inhalation of aerosol during irrigation using wastewater effluents was negligible even though the risk presented by ingestion of fruit or salad crop irrigated with wastewater was quite substantial for some WWTPs.
- The presence of enteric viruses in wastewater final effluents suggests that a significant portion of the human population contributing wastes to these WWTP are infected with these viruses.

SUMMARY OF CONCLUSIONS REACHED

The following conclusions were reached:

- We conclude that 24% of the WWTPs did not comply to set microbiological (faecal coliform) guidelines and the release of pathogenic enteric micro-organisms into aquatic environments can be a source of disease when water is used for drinking, recreational activities or irrigation.
- The public health risk is increased if the pathogenic enteric bacteria present in wastewater effluents (and hence in receiving water sources) are antibiotic resistant because of the reduced efficacy of antibiotic treatment against human diseases caused by such bacteria.
- WWTPs constitute important reservoirs of enteric bacteria which carry potentially transferable resistance genes which are aided by a large concentration of donor and recipient bacteria of transferable genes and availability of nutrients in the wastewater matrix.
- Presence of viruses in treated sewage will considerably contribute to the virus burden of the receiving water bodies.
- Consumption of even treated drinking water may result in infection if it coincides with failed water treatment while exposure to recreational activities and shellfish consumption may present a public health risk.

RECOMMENDATIONS FOR FUTURE INTERVENTIONS

- Municipalities may need to consider installing influent flow meters and automated chlorine dosing systems to curb cases of irregular chlorine dosing regimens. This will result in economic, public health and ecological gains.
- Municipal managers may also need to assess the qualifications of the technical staff employed to operate the WWTP and to conduct refresher courses for their technical staff to keep them up-to-date with the latest operating and maintenance procedures

for optimal WWTPs performance. This will positively contribute to the municipalities improving in their compliance with the green drop assessment.

- Detection of bacterial and viral pathogens in sewage effluents points to large pockets of infected individuals in the communities, most of which go unreported and untreated. Health awareness campaigns may need to be carried out to educate people on the benefits of hygiene and seeking early treatment in cases of illness. This may be a collaborated effort between the municipality and the local public health practitioners.

- The design of some WWTPs may have to be modified to allow for the minimum chlorine contact time before effluent discharge as stipulated by the operational guidelines of the WWTP.

- Other pathogens such as *Salmonella*, *Shigella* and *Vibrio* may need to be included in routine monitoring of wastewater final effluent quality to complement general faecal indicator bacteria.

- Municipalities may consider beneficial disposal of wastewater effluents by using them for irrigation purposes. The properly planned use of municipal wastewater for irrigation purposes will alleviate surface water pollution problems and not only conserve valuable water resources but also take advantage of the nitrogen and phosphorus content of sewage to grow crops with reduced requirements for commercial fertilizers. This may be accomplished through a collaboration between the municipalities and the local water user association (farmers' association).

RECOMMENDATIONS FOR FUTURE RESEARCH

- Enteric viral and bacterial pathogens have been detected in sewage final effluents, implying that they are in circulation in the communities concerned. These findings provide a strong link with the findings of our previous study (Assessment of the incidence of faecal indicator bacteria and human enteric viruses in some rivers and dams in the Amathole District Municipality of the Eastern Cape Province of South Africa WRC Report No. 1968/1/12) where viruses were also detected in surface water sources noted to receiving effluents from some WWTP along its course. As the previous study as well as the current study only evaluated viral nucleic acids, there is need for a large scale investigation on the ***"prevalence of infectious enteric viruses including epidemiological survey of diarrheal infections in the catchment".***
- An interesting observation was made with regards to chlorine dosing regimens and prevalence of viral pathogens at the Reeston WWTP:
    - Was it that there were no viruses in the influent sewage for the whole year?;
    - Did the high chlorine concentrations completely eradicated intact viruses from wastewater effluents?
    - How about the resultant nucleic acids that could not be detected by PCR?
- We recommend a laboratory based investigation into the effects of different chlorine dosing regimens on the survival and detectability of viral particles in water. Also, the isolation of some bacteria from effluents with high chlorine dose supports previous reports on increasing incidence of chlorine resistant bacteria. There is need for future

in-depth study on this subject pursuant to coming up with probably new guidelines for chlorine dosing.

- Huge disparities were observed between the faecal coliform based microbiological compliance of the WWTPs in this study compared to the Green Drop Report 2012 results. While the results might suggest that the WWTPs have an improved performance since 2012, the conclusion is hard to make because of the different analytical methods used. We recommend that the Colilert Method (used by the municipalities) and the Membrane Filtration Method (used in this study) be evaluated against samples containing standardised inoculum and the best performing method be adopted for the Green Drop requirements.
- Multiple antibiotic resistant bacterial pathogens (*Vibrio* and *E. coli*) were also isolated from sewage effluents in this study; the general assumption is that these pathogens acquired their resistance either by lateral gene transfer or from repeated exposures to antibiotics in human or animal bodies:
    - But, what role(s) could antibiotic residues contribute to the multiple antibiotic resistances observed?
- We recommend a future in-depth investigation of ***"the role of final effluents of WWTP as reservoirs of antibiotic resistance determinants in the watershed, to also include development of biosensors for the detection and quantification of relevant antibiotic resistance genetic elements in final effluents, and probably results in development of set guidelines for nucleic acids in final effluents***".

CAPACITY-BUILDING: The following students listed in the table below were trained directly on this project:

Student name	**Nationality**	**Project title**	**Programme**	**Status**
Miss Vuyokazi Nongogo	RSA	Evaluation of final effluents of some wastewater treatment plants as source of Vibrio pathogens in the aquatic environment of the Eastern Cape Province: Amathole and Chris Hani district municipality	MSc (Microbiology)	Submitted dissertation for external examination. Will graduate in October 2014.
Miss Sinazo Mazwi	RSA	Evaluation of some waste water treatment plant facilities in Chris Hani and Amathole District municipalities as potential sources of *Escherichia coli* in the Environment	MSc (Microbiology)	Submitted dissertation for external examination. Will graduate in October 2014.

Student name	Nationality	Project title	Programme	Status
Miss Unathi Badela	RSA	Prevalence of Vibrio species in the final effluents of three wastewater treatment facilities in the Buffalo City Local Municipality of the Eastern Cape Province, South Africa	MSc (Microbiology)	Submitted dissertation for external examination. Will graduate in October 2014.
Miss Nozuko Seti	RSA	Prevalence of pathogenic *Eschericia coli* isolated from final effluents of four wastewater treatment plants in the Buffalo city municipality	MSc (Microbiology)	Submitted dissertation for external examination. Will graduate in Oct 2014.
Mr. Thanduxolo Kulati	RSA	Evaluation of physicochemical qualities and heavy metal levels of some wastewater treatment facilities in the Eastern Cape, South Africa.	MSc (Chemistry)	Writing up dissertation.
Miss Onele Gcilitshana	RSA	Quality indices of the final effluents of two suburban based Wastewater treatment plants in Amathole District Municipality in the Eastern Cape.	MSc (Microbiology)	Submitted dissertation for external examination. Will graduate in October 2014.
Mr Siyabulela S Gusha	RSA	Studies on the Green Drop status of some wastewater treatments facilities in the Eastern Cape Province with respect to the physicochemical and microbiological qualities of their discharged effluents.	PhD (Microbiology)	Writing up thesis.
Mr Olayinka O Osuolale	Nigeria	Evaluation of the quality indices of the final effluents of three selected wastewater treatment plants in Buffalo city metropolitan municipality in the Eastern Cape Province.	PhD (Microbiology)	Writing up thesis.

Student name	Nationality	Project title	Programme	Status
Mr Adefisoye Martins	Nigeria	Assessment of the physicochemical, bacteriological and virological qualities of two wastewater treatment plants in Amathole district municipality area of the Eastern Cape Province of South Africa.	MSc (Microbiology)	Graduated in May 2014. Registered for PhD
Students not directly involved in project K5/2145, but benefit from the facilities for their projects.				
Miss Nolonwabo Nontongana	RSA	Prevalence and antibiogram profiling of *Eschericia coli* isolated from the Kat river and Fort Beaufort abstraction water.	MSc (Microbiology)	Submitted dissertation for external examination. Will graduate in October 2014.
Miss Phindiwe Ntloko	RSA	*Enterococcus* pathotypes as reservoirs of antibiotic resistance determinants in the Kat river and Fort Beaufort abstraction water.	MSc (Microbiology)	Submitted dissertation for external examination. Will graduate in October 2014.
Mr Olayinka Titilawo	Nigeria	Assessment of the quality indices and prevalence of *Escherichia coli* pathotypes in selected rivers of Osun State, South Western, Nigeria.	PhD (Microbiology)	Data collection.

KNOWLEDGE DISSEMINATION

Published paper (s)

No papers published to date

Paper (s) submitted for publication

No papers published to date

Conference presentation(s)

1. Mazwi S.N, Green E and Okoh AI. (2013). Evaluation of Some wastewater treatment Facilities in Chris Hani and Amathole district Municipality as Source of enteropathogenic *E. coli* in the Environment. 4th Municipal Water and Quality Conference, 7-11 July, Sun City, South Africa.
2. Badela AU, Green E, Ngwenya E, Mazomba N and Okoh AI. (2013). Prevalence of Vibrio species in the final effluents of three wastewater treatment facilities in the Buffalo City Local Municipality of the Eastern Cape Province, South Africa. 4th Municipal Water and Quality Conference, 7-11 July, Sun City, South Africa.
3. Osuolale O. Olayinka*[1] and Anthony I. Okoh. (2013). Incidences of Escherichia coli and physicochemical qualities of the final effluents of wastewater treatment plant in a peri-urban community in Buffalo City Local municipality. 4th Municipal Water and Quality Conference, 7-11 July, Sun City, South Africa.
4. Adefisoye MA, Aghdasi F, Green E and Okoh AI (2013). Assessment of the Prevalence of Faecal Coliforms and *Escherichia coli* O157:H7 in the Final Effluents of two Wastewater Treatment Plants in Amathole District Municipality. 4th Municipal Water and Quality Conference, 7-11 July, Sun City, South Africa.
5. Kulati T.C, Manene N, Okoh O.O and Okoh A.I (2013). Evaluation of the Physicochemical Qualities and Heavy Metals Levels of the Final Effluents of Some Wastewater Treatment Plants in the Eastern Cape. 4th Municipal Water and Quality Conference, 7-11 July, Sun City, South Africa.
6. Nongogo V, Green E, Sibanda Tim, Obi L and Okoh A.I (2013). Evaluation of final effluents of some wastewater treatment plants as source of Vibrio pathogens in the aquatic environment of the Eastern Cape Province: Amathole and Chris Hani district municipality as case study. 4th Municipal Water and Quality Conference, 7-11 July, Sun City, South Africa.
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9. S.S. Gusha, E.G. Ngwenya and AI Okoh (2013). Quality indices of the final effluents of two wastewater treatment plants in Nkonkobe local municipality of the Eastern Cape Province, South Africa. 14th WaterNet/WARFSA/GWP-SA Symposium 2013:

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12. M.A. Adefisoye; C.L. Obi and A.I. Okoh (2013). Evaluation of performance indices of two wastewater treatment facilities in Amathole District Municipality of South Africa. 14th WaterNet/WARFSA/GWP-SA Symposium 2013: Transboundary Water Cooperation: Building Partnerships; 30th October-1st November 2013, Dar es salaam, Tanzania.
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15. Gcilitshana O., Zhou L. And Okoh AI (2013). Assessment of the physicochemical and microbiological qualities of the wastewater treatment plants from the Eastern Cape, South Africa. The 18th Biennial South African Society for Microbiology (SASM 2013), 25-27 November 2013, Bela-Bela, South Africa.
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20. Nontongana N, Sibanda T and Okoh A.I (2014). Prevalence and antibiogram profiling of *Escherichia coli* pathotypes isolated from the Kat River and Fort Beaufort abstraction water. A poster presented at the Global Water Conference on Water, Gender and Development to be held between 7th and 11th July, 2014 at the International Conference Center, East London.
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Reference Group	**Affiliation**
Dr. Kevin Murray	Water Research Commission (Former Chairperson)
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Mr Andrew Lucas	Eastern Cape Department of Water Affairs

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ACRONYMS

AEMREG	Applied Environmental Microbiology Research Group
ATCC	America Type Culture Collection
BOD	Biological Oxygen Demand
CFU	Colony Form Unit
COD	Chemical Oxygen Demand
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DWAF	Department of Water Affairs
EOR	Efficiency of Recovery
EU	European Union
NTU	Nephelometric Turbity Unit
PCR	Polymerase Chain Reaction
TCBS	Thiosulfate Citrate Bile Salt Sucrose
TSS	Total Suspended Solids
WHO	World Health Organisation
WRC	Water Research Commission
WWTP	Waste Water Treatment Plant

CHAPTER ONE : BACKGROUND

1.1 INTRODUCTION

Wastewater may contain an array of pathogens that are excreted by diseased humans and animals (Wen *et al*., 2009). Whenever wastewater effluents are to be discharged into sensitive water courses, wastewater treatment and/or disinfection is required in order to protect both environmental integrity and public health. Wastewater treatment technologies, although primarily designed to remove such contaminants as biodegradable organic compounds, toxic metals, suspended solids and nutrients (nitrogen and phosphorus) from wastewater (Bitton, 2011; Godfree and Farrell, 2005; Horan, 1990), can, with optimised performance, also reduce bacterial and viral pathogens by approximately 90%, protozoan (oo)cysts by 0-1 log unit and helminth eggs by around 2 log units, depending on the concentration of suspended solids (Jiménez *et al*., 2004; Asano and Levine, 1998).

Removal/ inactivation of pathogens and parasites is important to prevent the potential outbreak of waterborne diseases, including enteric bacterial and viral diseases like cholera and gastroenteritis, as well as parasitic diseases such as cryptosporidiosis and giardiasis. Typical pathogen removals by primary sedimentation and the activated sludge processes are 30-65% and 80-90%, respectively (Fu *et al.,* 2010; Godfree and Farrell, 2005; Rao *et al*., 1977) and occur by a combination of physical, chemical and biological processes (Figure 1.1). Mara and Horan (2003) reported that the rate of adsorption of bacteria and viruses onto sludge flocs is directly related to the rate of sedimentation and thus, removal. Bacteria and viruses have been observed to adsorb onto sludge flocs either chemically by ion exchange and/or physically by electrostatic attraction, as they are relatively too small and of low density to settle (Bitton, 2011; Gray, 1999). Higher doses of coagulants (higher rates of coagulation) will, therefore, increase the removal of pathogenic microorganisms (Mara and Horan, 2003). Also, it was found that temperature is directly related to the rate of digestion of bacteria by protozoa.

Figure 1.1: Typical wastewater treatment plant, receiving watershed in the background

Sherr *et al.* (1988) reported that the digestion rate increased exponentially from 12°C to 22°C although bacterial ingestion rates varied significantly depending on protozoan cell size and total bacteria abundance. Water resource pH also plays an important role in pathogen removal. Bitton (2011) reported that most microorganisms are sensitive to pH of water because the pH affects the ionization of chemicals and therefore plays a role in the transport of nutrients and toxic chemicals into the cell.

Whereas removal of helminth eggs, bacteria and viruses is commonly achieved by ‘natural’ treatment processes like wastewater stabilization ponds, disinfection methods such as chlorination, ozonation and UV radiation are required for pathogen inactivation when more conventional (energy-intensive) processes like the activated sludge process are used (Jiménez, 2003). While disinfection methods are effective for the removal of bacteria and viruses, they are less efficient in the removal of helminth eggs as these are very resistant to disinfection methods and as such, techniques like sand filtration of final or treated effluent prior to disinfection are recommended to deal with helminth eggs and round worms. Chlorine kills microorganisms by destroying cellular materials and can be applied to wastewater as a gas, liquid or in a solid form (Okoh *et al*., 2007). However, free residual chlorine remaining in the water, even at low concentrations, is highly toxic to beneficial aquatic life (Hijnen *et al*., 2006) and, if in excess of acceptable levels (0.2 mg/L), may need to be removed by dechlorination to protect fish and aquatic life.

The public can be exposed to wastewater by several routes; the most common of which is through ingestion during recreational activities such as swimming, through bathing for health and when undertaking religious ceremonies. In the rural areas where the availability of piped water is limited and in most cases non-existent, the communities utilise stream/ river water for drinking and other domestic uses. The water used may include ground water sources that may also be contaminated with wastewater. These are direct exposure routes.

People can also be indirectly exposed to wastewater through consumption of shellfish produced in contaminated waters. Filter feeders such as molluscs have been found to concentrate pathogenic microorganisms occurring in contaminated water by filtration leading to the infection of consumers (Tamburrini and Pozio, 1999). For instance, Hernroth (2002) reported that enteric viruses were found in 50-60% of mussel samples at a mussel-farm used for bioremediation. Many of these viruses are stable in water and have long survival times with half-lives ranging from weeks to months (Banks *et al*., 2001).

In agriculture, people can be directly exposed to aerosols during irrigation using wastewater as well as through contact with the irrigated area or ingestion of irrigated crops. Since gastrointestinal pathogenic microorganisms do not occur as a natural part of the normal intestinal microbiota, their presence (and density) in wastewater is dependent on the number of infected people in the population contributing to the wastewater flow.

The production of effluents of acceptable quality by WWTP in South Africa is a challenge, especially in the Eastern Cape Province acknowledged as mostly non-urban, poor and without adequate infrastructure. Also, the documentation of final effluent compliance of the WWTP to set guidelines with respect bacteriological and virological quality remains poor in the Province.

This study was therefore designed to assess the prevalence of human viral, FIBs and Vibrio bacteria pathogens in the final effluents of 14 wastewater treatment facilities in Eastern Cape Province as a vehicle for skills development in microbial water quality science amongst previously disadvantaged demographic groups.

1.2 PROJECT AIMS

The aims of this project were as follows:

1. To carry out a survey of existing wastewater treatment facilities in the entire Eastern Cape Province, noting their dates of establishment, working capacity and current statuses.
2. To assess the incidence of human viral pathogens, faecal coliform and *Escherichia coli* in the selected WWTP effluents.
3. To assess the incidence and antibiogram characteristics of Vibrio bacteria pathogens and pathogenic *E. coli* in the selected WWTP effluents.
4. To determine the physicochemical qualities of the selected WWTP effluents.
5. Compare data obtained from typical urban, semi-urban and rural communities of the seven main districts that make up the province.
6. Submit a report of our findings to the WRC and Eastern Cape Provincial Government.

1.3 CHANGES TO ORIGINAL WORKPLAN

When the proposal was submitted to the Water Research Commission (WRC) the interaction between the Project Team and the Eastern Cape Department of Water Affairs (EC DWA) had not materialised. The original (signed contract) required selection of two wastewater treatment facilities in each of the seven District Municipalities in the Province making a total of 14 study WWTP. The EC DWA made inputs as follows which altered the project proposal:

- The need to stick to the total number of plants to the selected (14) due to budget constraint, noting that the budget as initially proposed was drastically reduced.
- The plants should fall within the EC DWA priority target plants based on current functionality and interests of the department.
- The need to ensure that plants are not located more than three hours' drive from Alice such that sampling will be a day return event, and as such spare the project logistic inconveniences of overnight accommodation and related HR expenses as initially proposed

Hence, the only change from the original plan was in the selection of the study plants to reflect the interests articulated above.

CHAPTER TWO : LITERATURE REVIEW

Clean, safe and readily available water is critical to the survival of human and other life forms. While safe water remains a critical resource world over, South Africa, being a country located in a semi-arid part of the world faces some challenges in preserving and conserving this scarce and limited resource. Compounding these challenges are the concerns over the environmental health and economic implications of water pollution (Osode and Okoh, 2010; Basson *et al.,* 1997). Water pollution arises from many sources, and occurs when pollutants or contaminants are discharged into water bodies without adequate treatment to remove harmful substances. Of major importance is the concern over pollution from untreated or inadequately treated municipal wastewater (sewage) effluents (Owili, 2003).

South Africa is one of the few countries in the world that enshrines the basic right to sufficient water in its Constitution, stating that *"Everyone has the right to have access to sufficient food and water"* (Constitution of South Africa, 1996 Chapter 2, Section 27b), and, though the South Africa government made it mandatory from the old South African Water Act (Act 54 of 1956) that effluent be treated to acceptable standards and returned to the water course where water was originally obtained, much remains to be done to fulfil that right (Mema, 2009; Morrison *et al*., 2001). Significant problems remain concerning the financial sustainability of service providers, leading to poor maintenance culture. The uncertainty about the government's ability to sustain current funding levels in the sector is also a concern (Mema, 2009).

According to a Water Supply, Sanitation and Hygiene (WASH) news Africa report in 2010, "*Many of South Africa's municipal wastewater treatment plants (WWTP) are not performing to acceptable water quality standards and there are several issues surrounding the performance of these plants. Contributing to the challenges experienced by municipalities is a lack of skills for the operation of facilities and a lack of infrastructures investment over the past decades. A lack of good-quality drinking water leads to health problems, which is serious, given the fact that many poor citizens source water directly from the rivers, where not only municipalities, but also industrial water users, discharge polluted water. Since South Africa does not have large rivers, the discharged effluents concentrate into small watercourses*" (WASH news Africa, 16 July, 2010). This situation is more pronounced in such poor provinces as the Eastern Cape Province.

The Eastern Cape Province has been well acknowledged as mostly non-urban, poor and without adequate infrastructure, with a significant proportion of its communities lacking pipe-borne water, and as such rely on beaches, streams, rivers, groundwater and other water bodies for recreation, drinking and domestic purposes. Many of these water bodies are often polluted by industrial and municipal wastewater effluents (Igbinosa *et al*., 2011; Igbinosa and Okoh 2010; Momba *et al*., 2009; Okoh *et al*., 2007; Fatoki *et al*., 2003; Obi *et al*., 2002) amongst others. The attendant negative consequences of the impact of such pollutions on the water bodies is the compromising of the primary health of people especially with death threatening gastrointestinal diseases and other infectious illness (Momba *et al*., 2006, 2009;

Hoebe *et al*., 2004; Obi *et al*., 2004; Bourne and Coetzee, 1996) caused by several viral and other pathogens (such as toxic *Escherichia coli* and Vibrio bacteria), especially amongst children as well as the immunocompromised.

There is evidence that suggest that the organisms causing many of these illnesses are not necessarily the ones that are routinely tested for in microbial water quality assessments, in order to establish the quality of water. Hence the need to monitor not only the classical pollution indicator organisms like culturable total, faecal coliforms and coliphages as are seldom done, but also viral pathogens (Wyn-Jones *et al*., 2011; Lipp *et al*., 2001) and such specific, highly infectious bacterial pathogens as *Vibrio* bacterial and toxic *E. coli*, more so in the light of the emergence of *E. coli* O157:H7 that is causing serious havocs in Europe since mid-2011 (CDC, 2012). The prevalence of this new toxic *E. coli* strain in the South African environment remains to be ascertained; neither is there adequate information on the epidemiology of pathogenic *Vibro* bacteria in the Eastern Cape aquatic environment. Also, though some studies have been carried out on the occurrence of *Vibrio* pathogens in the Eastern Cape Province by the AEMREG group (Igbinosa *et al*., 2011; Okoh and Igbinosa, 2010; Igbinosa *et al*., 2009), the studies were restricted to the final effluents of only three of the WWTP studied, and as such not a good representation of the picture in the Eastern Cape Province.

Furthermore, several studies have indicated that levels of indicator bacteria do not correlate with those of viruses, particularly when faecal indicator concentrations are low (Contreras-Coll *et al*., 2002). Also, in our previous reports on final effluents of three WWTP, some Vibrio species appeared to survive the activated sludge based WWTP as free cell and as plankton associated entities (Igbinosa *et al*., 2009; 2011). In the same vein we observed that Hepatitis A virus and Coxsackie virus also escaped the treatment processes of typical sub-urban and rural wastewater treatment facilities in the Eastern Cape (unpublished report). We hypothesize that these scenarios are common occurrences in the entire Eastern Cape Province and hope to confirm this through this proposed research.

Also, viruses are known to be more resistant to environmental degradation than bacteria (de Roda Husman *et al*., 2009; Rzezutka and Cook, 2004; Thurston-Enriquez *et al*., 2003). The enteric virus group is the most meaningful, reliable and effective virus index for environmental monitoring. These viruses, cause diseases such as paralysis, meningitis, respiratory disease, epidemic vomiting and diarrhoea, myocarditis, congenital heart anomalies, infectious hepatitis, and eye infections mostly in children or elderly among bathers at recreational beaches (van den Berg *et al*., 2005).

Literature search revealed that aquatic virology research in South Africa was pioneered by one of the seminal names in that field, Grabow and his colleagues in the 1980s, in Gauteng (Grabow, 2007; Taylor *et al*., 2001; Grabow *et al*., 1996; Grabow, 1986; Grabow *et al*., 1983). In one of their studies, they reported on the molecular epidemiology of Group A rotaviruses in water sources and selected raw vegetables in South Africa; studied water samples collected from water treatment plants, and irrigation waters and associated vegetables located in the Western Cape, Gauteng and Limpopo (van Zyl *et al*., 2006). Similar studies on aquatic virology in the Eastern Cape Province were reported by the AEMREG and include reports on the Buffalo and Tyume Rivers (Chigor *et al*., 2014; Sibanda and Okoh, 2012, 2013). Nevertheless, shortage of skills in microbial water quality remains a major problem in the water sector in South Africa. Hence the need to increase skills development especially amongst the previously disadvantaged demographic groups on microbial water quality science becomes imperative.

CHAPTER THREE : DESCRIPTION OF STUDY AREA

The Eastern Cape Province borders the provinces of the Western Cape, Northern Cape, Free State, and KwaZulu-Natal, as well as Lesotho in the north (DWA, 2009). The province is mostly rural with high percentage of people living in poverty (67.4 %) and a very low Human Development Index (HDI) of 0.52 (Eastern Cape Department of Social Development, 2008). It is the second largest province in South Africa and mainly comprised of rural settlements with little or no adequate sanitary facilities. The Eastern Cape Province is divided into seven district municipalities, namely, Alfred Nzo, Amathole, Chris Hani, Ukhahlamba, O.R. Tambo, Cacadu and the Nelson Mandela Metropolitan Municipality (Figure 3.1).

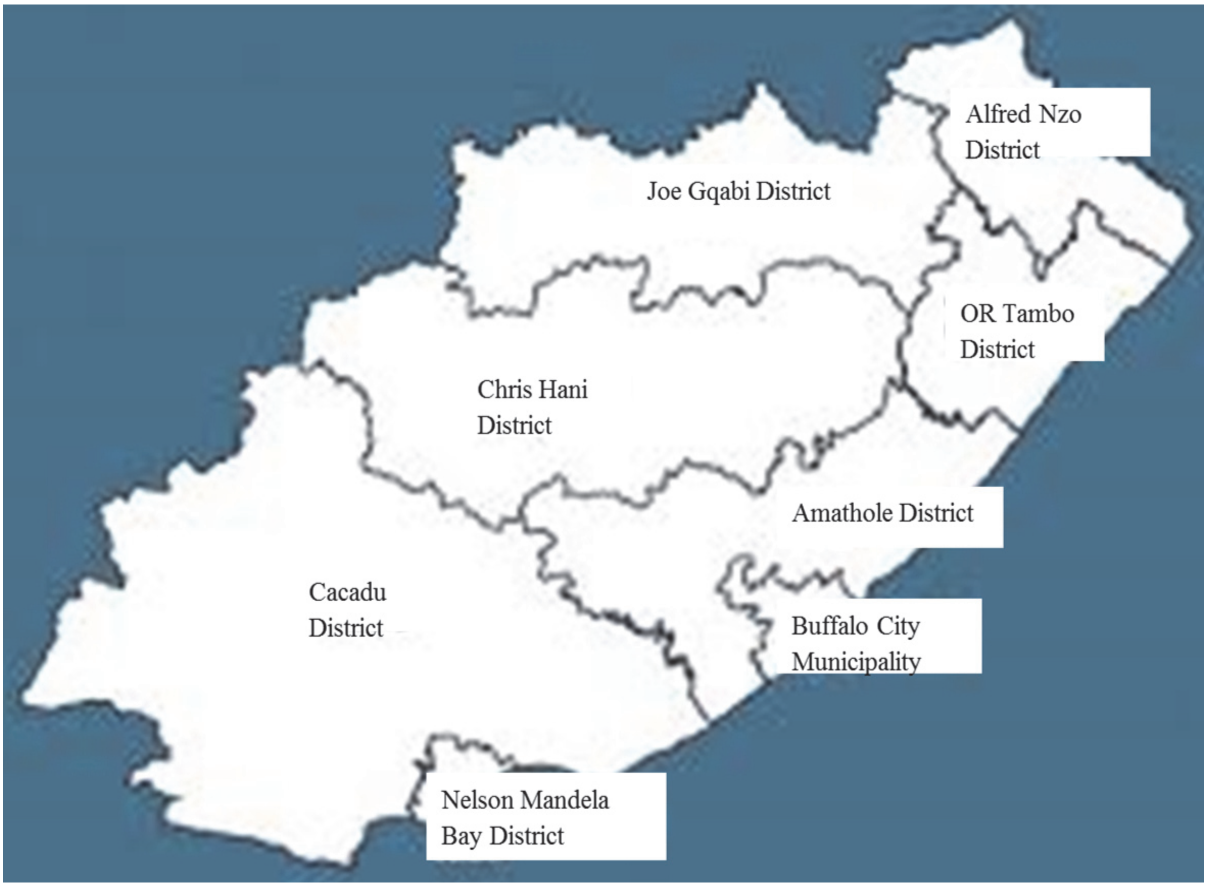

Figure 3.1: The seven District Municipalities of the Eastern Cape Province

In collaboration with the Eastern Cape Department of Water Affairs, 14 WWTPs were selected for study from two district municipalities *viz* Amathole District Municipality and Chris Hani District Municipality based on the criteria articulated earlier.

Amathole District Municipality is situated in the central part of the Eastern Cape stretching along the Sunshine Coast from the Fish River Mouth, along the Eastern Seaboard to just south of Hole in the Wall along the Wild Coast. It is comprised of eight local municipalities: Mbhashe, Mnquma, Great Kei, Amahlathi, Buffalo City, Ngqushwa, Nkonkobe and Nxuba. According to the Greendrop report (2012), Buffalo City being the largest, is also the best performing district with 15 out of 15 plants being in low and medium risk positions.

Also, according to the Greendrop report (2012), Chris Hani District Municipality represents one of the highest risk municipalities in the Eastern Cape with 93% of its WWTPs in critical and high risk positions. Tables 3.1 and 3.2 are summaries of the WWTP indicating the treatment technology, design capacity and compliance ratings according to the Green Drop Report of 2012. Also included are the motivations for the inclusion of each of the plants in this study.

Table 3.1: List of the selected wastewater treatment plants (WWTPs) in Buffalo City Local Municipality in the Eastern Cape Province

WWTP	**Schornville**	**Zwelitsha**	**Mdantsane**	**Reeston**	**Dimbaza**	**East Bank**	**Amalinda Central**
Technology	Activated sludge and BNR, biofilters, anaerobic digestion and sludge drying beds	Biofilters, anaerobic digestion and sludge drying beds	Biofilters, anaerobic digestion and sludge drying beds	Activated sludge and sludge lagoons	Activated sludge and sludge lagoons	Activated sludge and marine outfall	Petro system, Biofilters, anaerobic digestion and sludge drying beds
Design Capacity (Ml/d)	4.8	9.3	24	2.5	7	40	5
Operational % in relation to Design Capacity	133.3	84.9%	43.8%	44%	112.9%	85.5%	154%
Microbiological Compliance	0.0%	16.0%	0.0%	68.0%	8.0%	74.0%	46.0%
Chemical Compliance	64.7%	61.7%	84.5%	92.0%	92.8%	88.5%	47.0%
Physical Compliance	84.3%	76.3%	79.3%	84.3%	88.7%	89.7%	1.7%
Critical Risk Area	Poor effluent compliance, operating capacity exceeds	Poor effluent compliance	Poor effluent compliance	Poor effluent compliance	Poor effluent compliance, operating capacity exceeds	Poor effluent compliance	Poor effluent compliance, operating capacity exceeds design

WWTP	Schornville	Zwelitsha	Mdantsane	Reeston	Dimbaza	East Bank	Amalinda Central
	design capacity				design capacity		capacity
Motivations for choice of plant	This works is supported as it discharges to Buffalo River upstream of Laing Dam. This works is very close to community houses and will be closed in medium term, after new regional WWTW constructed (in planning phase.	This works is supported as it discharges to Buffalo River upstream of Laing Dam. Zwelitsha WWTW will be upgraded into a new Regional WWTW (medium term).	Currently these has been no disinfection, but it is intended that chlorination needs to be introduced probably in the time frame of this study	Probably the newest works on the list, this small WWTW is due to be upgraded as the site is planned for a regional facility that will be capable of recycling back to domestic use.	Conventional works receiving mixture of industrial & domestic effluent	The largest works in the BCM area and there is disinfection & irrigation of golf course and potential for reclamation of final effluent for industrial use.	The final effluent from this works is used for irrigation of golf course and to farmers (food crops). The balance of the effluent flows a short distance before flowing over the beach into the sea, where recreational use standards need to apply.

Table 3.2: List of the selected WWTPs in Amathole and Chris Hani District municipalities in the Eastern Cape Province

Location	**Amathole District Municipality**					**Chris Hani District Municipality**	
WWTP	**Fort Beaufort**	**Keiskammahoek**	**Komga**	**Stutterheim**	**Alice-Fort Hare**	**Whittlesea**	**Queenstown**
Technology	Activated sludge and sludge drying beds	Activated sludge and sludge lagoons	Activated sludge and sludge drying beds	Activated sludge and sludge drying beds	Not applicable*	Biofilters, sludge composting	NI
Design Capacity (Ml/d)	3.0	0.67	0.63	4.0	Not applicable*	4.99	NI
Operational % in relation to Design Capacity	233.3%	NI	NI	62.5%	Not applicable*	50.1%	NI
Microbiological Compliance	20.0%	70.0%	70.0%	5%	Not applicable*	84.0%	NI
Chemical Compliance	65.0%	70.0%	70.0%	5%	Not applicable*	75.0%	NI
Physical Compliance	80.0%	56.7%	56.7%	5%	Not applicable*	55.7%	NI
Critical Risk Area	Poor effluent compliance, operating capacity exceeds design	Poor effluent compliance, no influent monitoring	Poor effluent compliance, no influent monitoring	Poor effluent compliance	Not applicable	Poor effluent compliance	No information

Location	**Amathole District Municipality**					**Chris Hani District Municipality**	
	capacity						
Motivations for choice of plant	The WWTW is situated in an agricultural area where potential health impacts could have a negative impact on market / export market agreements.	This is a small works in a rural community. Such works often have less operation & maintenance support, and may have bigger health & environmental impacts that larger works.	Small works in Rural area.	The Stutterheim WWTW is upstream of the Wriggleswade Dam which is battling with Water Hyasinth growths. Wriggleswade dam is a major impoundment in the Amathole Water System.	The Alice works is a privately owned WWTW serving municipal & private needs and would be a good comparison to Municipal owned works. As the works is in a University town, it is anticipated that it be visited by researchers & students and be the subject of their reports.	Final effluent is discharged to Klipplaat River where extensive use is made by Farming Activities.	Final Effluent from this WWTW is used by farmers as compensation water, by long standing agreement, when Bonkolo Dam was built. Health issues could compromise farming activities.

Fort Beaufort WWTP is under the management and authority of Amatola Water Board (AWB)[1] and currently operates without a license. This WWTP discharges its final effluents into the Kat River. The Alice sewage treatment works[2] discharges into the Tyume River. Some communities living downstream of the river use the water for irrigation purposes. The Komga WWTP discharges its effluents into Kei River while Stutterheim WWTP discharges its final effluents into Cumakala River (DWA, 2009; DWA, 2012). Keiskammahoek WWTP discharges its effluents into the Keiskamma River (DWA, 2009; DWA, 2012) while the Dimbaza WWTP discharges into Mdizeni River, which links to the main Keiskamma River. The Amalinda and East Bank WWTPs discharge their effluents into the Indian Ocean[3] while the Mdantsane WWTP discharges its effluents into the Buffalo River as does the Reeston WWTP, and while the Whittlesea WWTP discharges its effluents into the Klipplaat River.

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[1] The works are owned by Amathole District Municipality who is the Water Services Authority in the area. Amathole District Municipality then appointed on contract a Water Service Provider, Amatola Water Board (AWB) to operate these works. However, Amathole District Municipality are still responsible for compliance of these works.

[2] Alice WWTP is owned by the University of Fort Hare, and operated under a contract between the University and a service provider namely: Pollution Control Technologies. However, in terms of compliance, the University is held responsible

[3] This discharge is at Bat's Cave and effluent shall comply with quality requirements of General Standards except for bacterial quality *E. coli*, which shall not exceed 1000 per 100 ml

CHAPTER FOUR : PHYSICOCHEMICAL ANALYSIS

4.1 Introduction

Whereas rivers and other open aquatic ecosystems have a self-purification capacity that enables the quality of the water thereof to maintain some equilibrium, continuous discharge of sewage effluents into these systems gradually decreases their self-purification capacity and increases the accumulation of pollutants within the river systems as well as in the sediments Plants and animals living in these systems, as well as humans and land animals which drink from these water bodies may be poisoned or otherwise harmed if discharged effluents contain excessive amounts of salts, nutrients, detergents, toxic metals or organic matter which may harbor or encourage microbial growth. The physicochemical qualities of wastewater effluents must be routinely monitored to ensure that the discharged effluents are of acceptable quality so as to preserve the integrity of the receiving water bodies. Even in cases where effluents are used for irrigation purposes, there are maximum allowable standards in terms of salinity, pH, heavy metals and microbial concentrations which will not either directly harm the crops or the people who either ingest the crop or the irrigation aerosol.

In this chapter, we report on the sampling methodologies and results of the physicochemical analysis of effluent samples from the 14 WWTP assessed in this study.

4.2 Sampling and Analytical Procedures

Samples were collected once monthly from each of the 14 WWTP for twelve months in the period commencing September 2012 to August 2013. Effluent samples were collected from the final effluents (as it leaves the works) and the discharge point (end-of-pipe)[4]. There were onsite analysis of some parameters (Figure 4.1, see also Table 4.1) while other parameters assessed in the laboratory. The collected samples were transported in cooler boxes to the AEMREG laboratory at the University of Fort Hare, Alice for analysis following standard methods. Storage of samples in the laboratory was done within 6 hours of collection.

[4] There is a dearth of information regarding the scientific significance of increased contact time between chlorine and microbes in chlorinated final effluents in the pipeline between the final effluent tank and the discharge point. This study therefore sought to investigate if there could be a significant improvement in the microbiological qualities of the effluent at the discharge point as compared to the final effluent tank. This was done by collecting samples from both the final effluent tank and the discharge point, analysing them concurrently and comparing the results. It is presumed that the longer the distance between the two the more the contact time of the chlorine and more efficient in eliminating the bacteria.

Figure 4.1: Students carrying out *on site* physicochemical analyses

The physicochemical parameters assessed in this study, the methodologies employed and the regulatory guidelines by different statutory bodies are as presented in Table 4.1.

Table 4.1: Physicochemical parameters, methodology and regulatory guideline values for wastewater effluents[5]

Parameters, units	**Methodology**	**Regulatory Guidelines (General limit)**
pH*	Multiparameter ion specific meter (Hanna_BDH laboratory supplies)	5.5-9.5 (DWAF, 2004)
Total Dissolved Solids* (mg/l)		450 mg/L (DWAF, 1996b)
Electrical Conductivity* (µS/cm)	Conductivity meter (CRISON CM35, Crison instrument)	70 mS/m above intake to a maximum of 150 mS/m (DWAF, 2004)
Dissolved Oxygen (mg/l)*	Merck DO meter, Model Ox 330 (Merck Pty Ltd)	≥ 5mg/L (WHO,2006)
Biochemical Oxygen Demand (BOD)**	Oxitop WTW BOD meter (Merck Pty Ltd)	3-6 mg/L (EU standard)
Temperature (°C)*	Thermometer	Maximum of 35°C (DWAF,

[5] All WWTPs discharging final effluent less than 2000 m^3/day need to comply with General Limit Values

Parameters, units	Methodology	Regulatory Guidelines (General limit)
		2004)
Turbidity (NTU)*	Microprocessor turbidity meter (HACH company, model 2100P)	< 5 NTU (WHO, 2008)
Free chlorine (mg/l)*	Multiparameter ion specific meter (Hanna_BDH laboratory supplies)	0.25 mg/l (DWAF, 2004)
Nitrite (NO_2) (mg/l)**	Standard photometric method (DWAF, 1992) using the Spectroquant Pharo 100 photometer (Merck Pty Ltd)	15 mg/l (DWAF, 2004)
Nitrate (NO_3) (mg/l)**		15 mg/l (DWAF, 2004)
Phosphate (P) (mg/l)**		10 mg/l (DWAF, 2004)
Chemical Oxygen Demand (COD) (mg/l)**		75 mg/l after removal of algae (DWAF, 2004)

*analysed onsite

**analysed in the laboratory

4.3 Data Analyses

Detailed analysis of the data is focussed on the few of the parameters as detailed below.

4.3.1 pH Analysis

pH is defined as the negative logarithm to base 10 of the hydrogen ion concentration (UNESCO, WHO & UNEP, 1996), any whole number change in the pH level of the receiving water bodies as a result of discharging highly acidic or basic effluents will result in the water conditions being either 10 times more acidic or alkaline. This is especially so in rivers with low flow volumes which receive high volumes of effluent, resulting in minimal dilution effect. This may create unconducive environments for aquatic organisms, from microbes to higher organisms like fish, most of which can only live in a narrow pH range. Also, pH affects the solubility of both nutritive metals and heavy (toxic) metals and whole number changes in the pH of lotic systems can affect primary productivity as well as increase toxicity, which may result in fish kills and have long reaching socio-economic consequences.

4.3.2 Dissolved Oxygen (DO) and Biochemical Oxygen Demand (BOD)

The DO and BOD levels in wastewater effluents are directly related to its organic load and microbial oxidation of the organic matter (Davies and Walker, 1986). Highly biodegradable wastes are rapidly oxidised and oxygen is rapidly depleted. Aquatic ecosystems are able to support a greater number of species of organisms when the dissolved oxygen concentration is high. Oxygen depletion due to waste discharge has the effect of increasing the numbers of decomposer organisms at the expense of others (Meertens *et al.*, 1995). Not only does the water then become devoid of aerobic organisms, but anaerobic decomposition also results in the formation of a variety of foul smelling volatile organic acids and gases such as hydrogen sulphide and methane, the stench of which can be quite unpleasant to residents in the vicinity.

4.3.3 Phosphates

Phosphates are an essential plant nutrient and are often the most limiting nutrient to plant growth in freshwater. If effluents containing high concentrations of phosphates are discharged into a river, algae and water weeds grow wildly as a result of eutrophication, choke the water way and use up large amounts of oxygen resulting in the death of aquatic organisms (Mosley *et al*., 2004), thus having negative impacts on nature conservation, recreation and drinking water production. It is, therefore, necessary to control the emission of phosphates from discharges of wastewater (van Larsdrecht, 2005).

4.3.4 Free Residual Chlorine

While chlorine is still the widely used disinfectant especially for wastewater treatment (Tchobanoglous *et al*., 2003), it does not only disinfect, but also rapidly reacts with contaminants such as NH^{4+}, NO^{2-}, H_2S, Fe^{2+} and other organic compounds, leading to the formation of compound called trihalomethanes, which are considered health hazards (Akpor, 2011). Besides, high concentrations of free residual chlorine is directly toxic and so has ecological consequences while very low (under-dose) may result in inadequate removal of pathogens in the wastewater matrix and effluents will tend to have high concentrations of pathogens, which may have public health consequences especially where people rely on untreated surface water for drinking or recreation.

4.3.5 Temperature

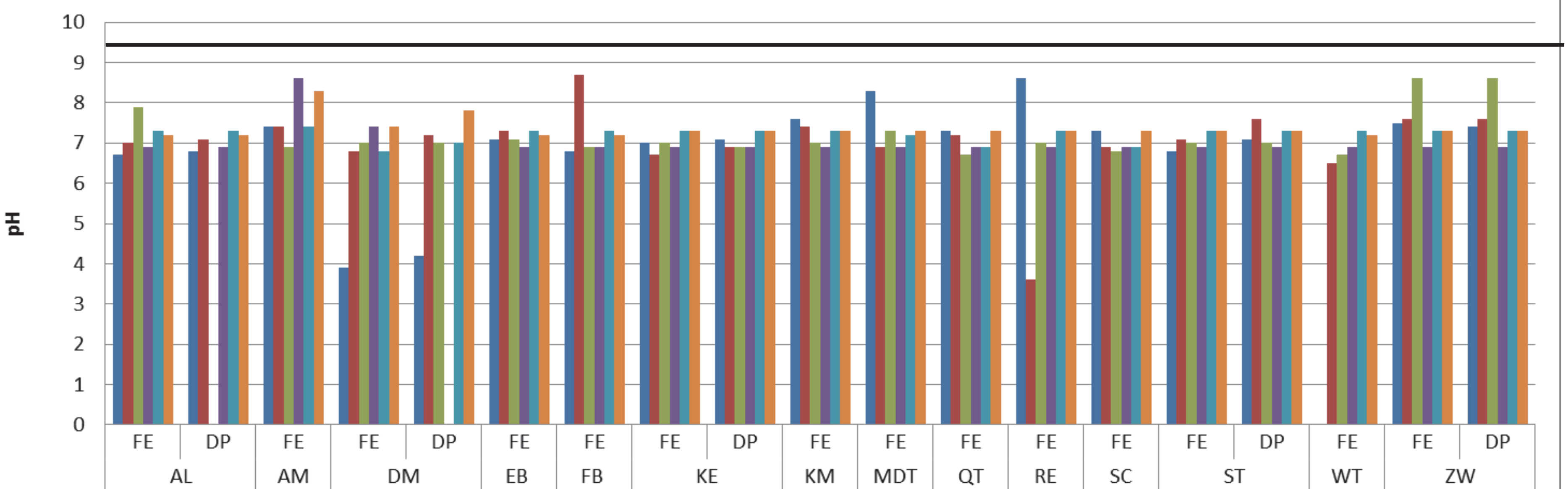
Temperature is the driver of all physicochemical processes that happen in the aquatic milieu and the discharge of effluents with a temperature of > 35°C can result in thermal pollution. This may result in lessening of the dissolved oxygen in water creating hypoxic conditions which are detrimental to the survival of aquatic organisms.

4.4 Results and Discussion

The physicochemical qualities of the effluents during the 12 month reporting period for the 14 WWTP under investigation are as summarised in Figures 4.2 to 4.25. Compliance to effluent quality guidelines varied with parameter and with WWTPs as shown in Table 4.2.

Table 4.2: Physicochemical parameter range of values and compliance levels

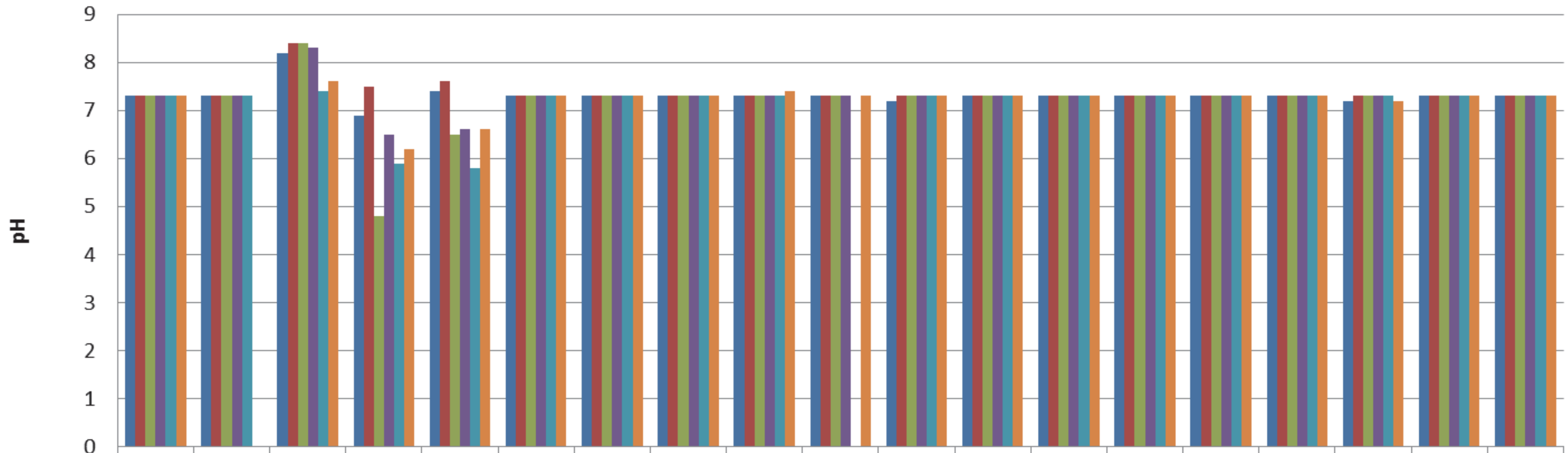
Parameter	Range	Guideline value	Compliance level
pH	3.6-8.7	5.5-9.5	
Turbidity	1-567 NTU	< 5 NTU	
EC	92-1429 µS/cm	70 mS/m	
TDS	27-915 mg/l	450 mg/l	
DO	0.7-17.9 mg/l	≥ 5 mg/l	
Temperature	12-31°C	≤ 35°C	
Free Residual Cl	0-8.8 mg/l	0.25 mg/l	
BOD	0.1-17.0 mg/l	3-6 mg/l	
COD	4.67-3283 mg/l	75 mg/l	
Nitrite	0-19.1 mg/l	15 mg/l	
Nitrate	0-21 mg/l	15 mg/l	
Phosphate	0-68 mg/l	10 mg/l	



	FE	DP	FE	FE	DP	FE	FE	FE	DP	FE	FE	FE	FE	FE	FE	DP	FE	FE	DP
	AL		AM	DM		EB	FB	KE		KM	MDT	QT	RE	SC	ST		WT	ZW	
Sep-12	6.7	6.8	7.4	3.9	4.2	7.1	6.8	7	7.1	7.6	8.3	7.3	8.6	7.3	6.8	7.1		7.5	7.4
Oct-12	7	7.1	7.4	6.8	7.2	7.3	8.7	6.7	6.9	7.4	6.9	7.2	3.6	6.9	7.1	7.6	6.5	7.6	7.6
Nov-12	7.9		6.9	7	7	7.1	6.9	7	6.9	7	7.3	6.7	7	6.8	7	7	6.7	8.6	8.6
Dec-12	6.9	6.9	8.6	7.4		6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
Jan-13	7.3	7.3	7.4	6.8	7	7.3	7.3	7.3	7.3	7.3	7.2	6.9	7.3	6.9	7.3	7.3	7.3	7.3	7.3
Feb-13	7.2	7.2	8.3	7.4	7.8	7.2	7.2	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.2	7.3	7.3

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point).

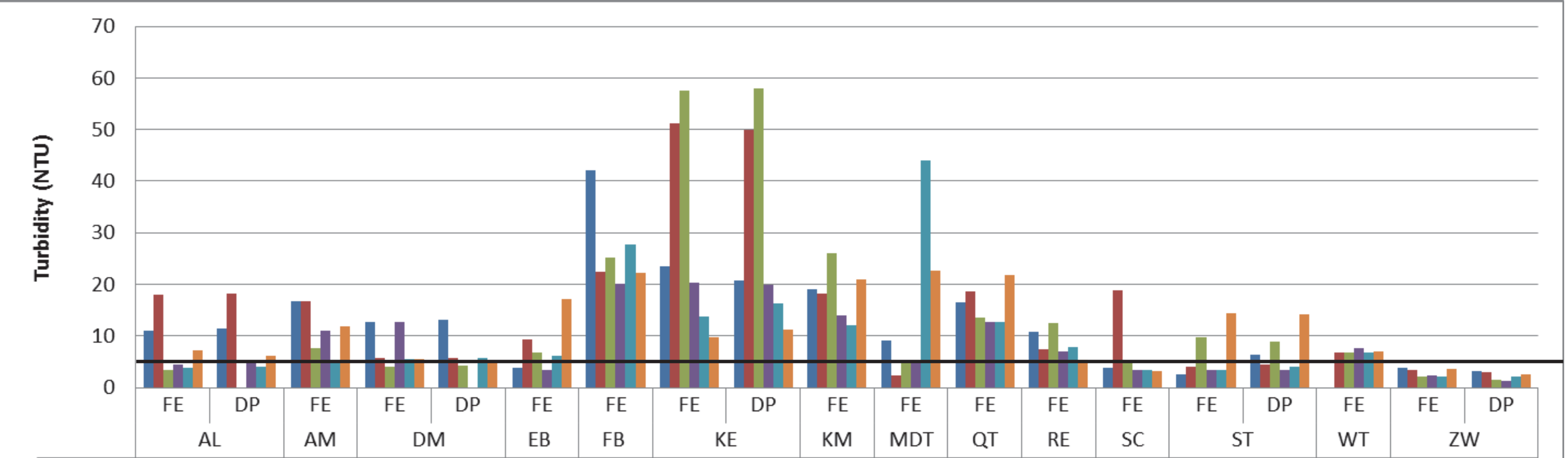
Figure 4.2: pH levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between September 2012 and February 2013



	FE (AL)	DP (AL)	FE (AM)	FE (DM)	DP (DM)	FE (EB)	FE (FB)	FE (KE)	DP (KE)	FE (KM)	FE (MDT)	FE (QT)	FE (RE)	FE (SC)	FE (ST)	DP (ST)	FE (WT)	FE (ZW)	DP (ZW)
Mar-13	7.3	7.3	8.2	6.9	7.4	7.3	7.3	7.3	7.3	7.3	7.2	7.3	7.3	7.3	7.3	7.3	7.2	7.3	7.3
Apr-13	7.3	7.3	8.4	7.5	7.6	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
May-13	7.3	7.3	8.4	4.8	6.5	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
Jun-13	7.3	7.3	8.3	6.5	6.6	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
Jul-13	7.3	7.3	7.4	5.9	5.8	7.3	7.3	7.3	7.3		7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
Aug-13	7.3		7.6	6.2	6.6	7.3	7.3	7.3	7.4	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.2	7.3	7.3

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point).

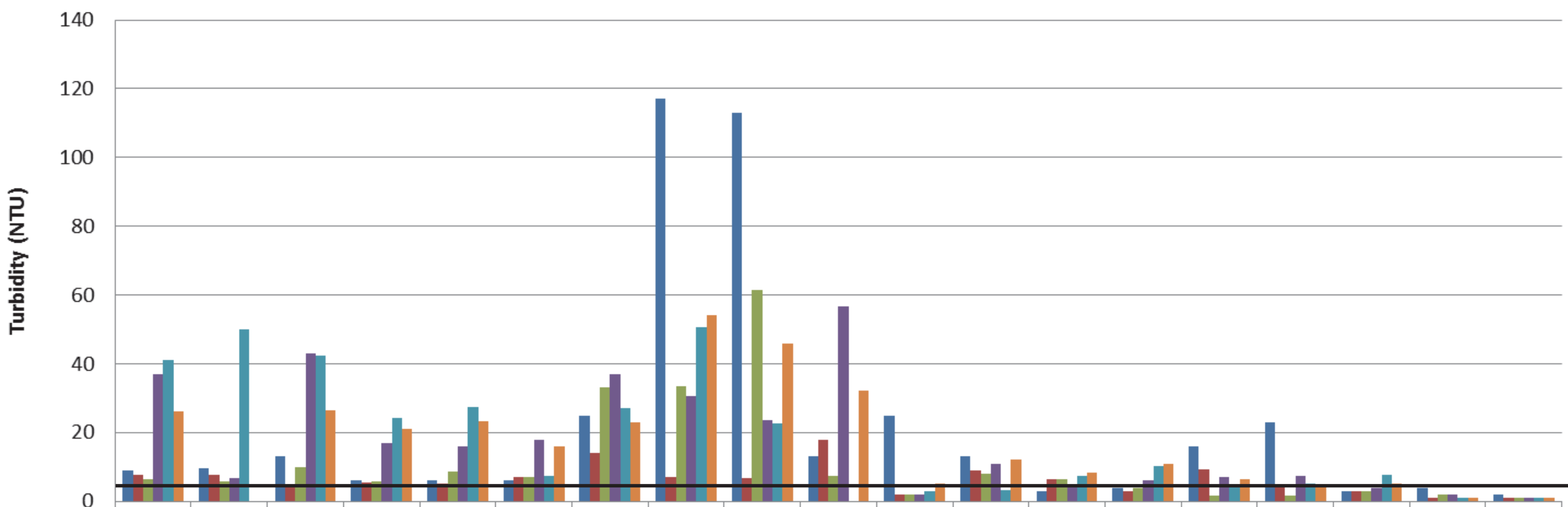
Figure 4.3: pH levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between March 2013 and August 2013



	AL FE	AL DP	AM FE	DM FE	DM DP	EB FE	FB FE	KE FE	KE DP	KM FE	MDT FE	QT FE	RE FE	SC FE	ST FE	ST DP	WT FE	ZW FE	ZW DP
Sep-12	11.1	11.4	16.7	12.8	13.1	3.9	42.2	23.5	20.7	19.1	9.1	16.5	10.9	3.8	2.6	6.4		3.8	3.1
Oct-12	18	18.3	16.7	5.8	5.8	9.3	22.5	51.3	49.9	18.2	2.3	18.6	7.4	18.9	4	4.4	6.8	3.3	3
Nov-12	3.5		7.6	4	4.2	6.7	25.1	57.5	58	26	5.2	13.6	12.4	5.2	9.8	8.8	6.7	2.1	1.6
Dec-12	4.4	5.2	11.1	12.8		3.5	20.1	20.4	20	14	4.8	12.7	7	3.4	3.5	3.3	7.7	2.3	1.2
Jan-13	3.9	4	5.4	5.5	5.7	6.1	27.8	13.7	16.3	12	44.1	12.7	7.8	3.4	3.5	4.1	6.7	2.2	2.1
Feb-13	7.3	6.1	11.9	5.5	5.1	17.1	22.2	9.8	11.2	21	22.7	21.7	5.3	3.2	14.3	14.1	6.9	3.7	2.5

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point). Values above the bold line at 5 mg/l indicate non-compliance.

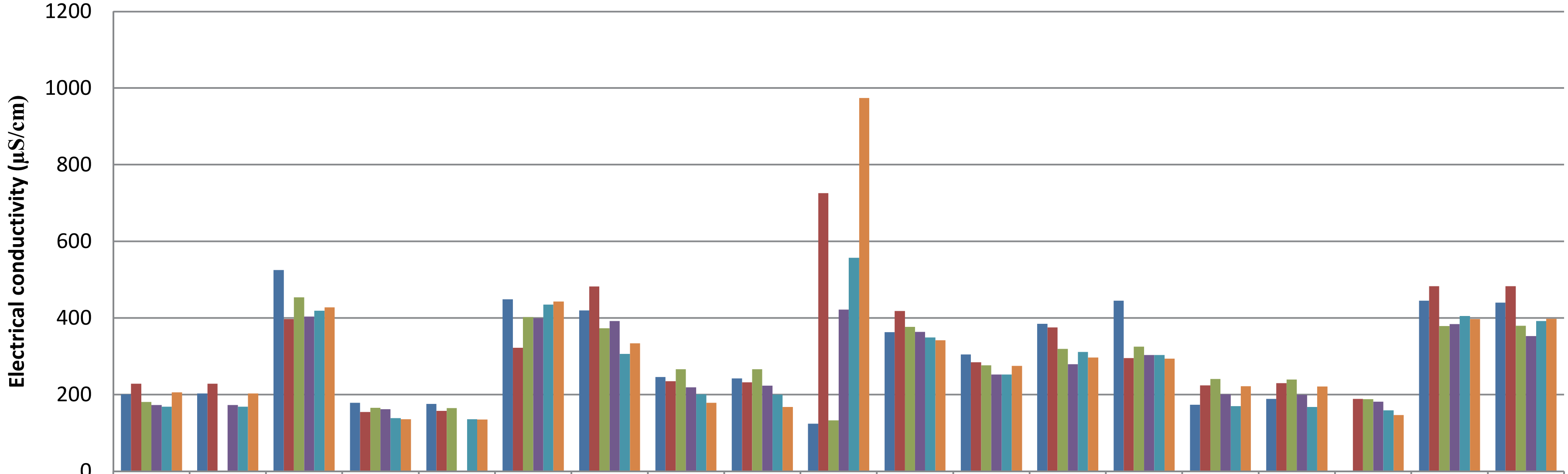
Figure 4.4: Turbidity levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between September 2012 and February 2013



	AL FE	AL DP	AM FE	DM FE	DM DP	EB FE	FB FE	KE FE	KE DP	KM FE	MDT FE	QT FE	RE FE	SC FE	ST FE	ST DP	WT FE	ZW FE	ZW DP
Mar-13	8.8	9.6	13	6	6	6	25	117	113	13	25	13	3	4	16	23	3	4	2
Apr-13	7.6	7.7	4.8	5.6	5	7	14	7.2	6.7	18	2	9	6.3	3	9.2	4.3	3	1	1
May-13	6.4	5.9	9.9	5.7	8.6	7	33	33.6	61.6	7.4	2	8	6.3	4	1.7	1.7	3	2	1
Jun-13	37	6.7	43	17	16	18	37	30.6	23.5	56.7	2	11	4.8	6	7.1	7.3	4	2	1
Jul-13	41	50	42.3	24.3	27.3	7.3	27	50.7	22.7		3	3.3	7.3	10.3	4.7	5	7.7	1	1
Aug-13	26		26.3	21	23.3	16	23	54	46	32.3	5	12	8.3	11	6.3	4.3	5	1	1

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point). Values above the bold line at 5 mg/l indicate non-compliance.

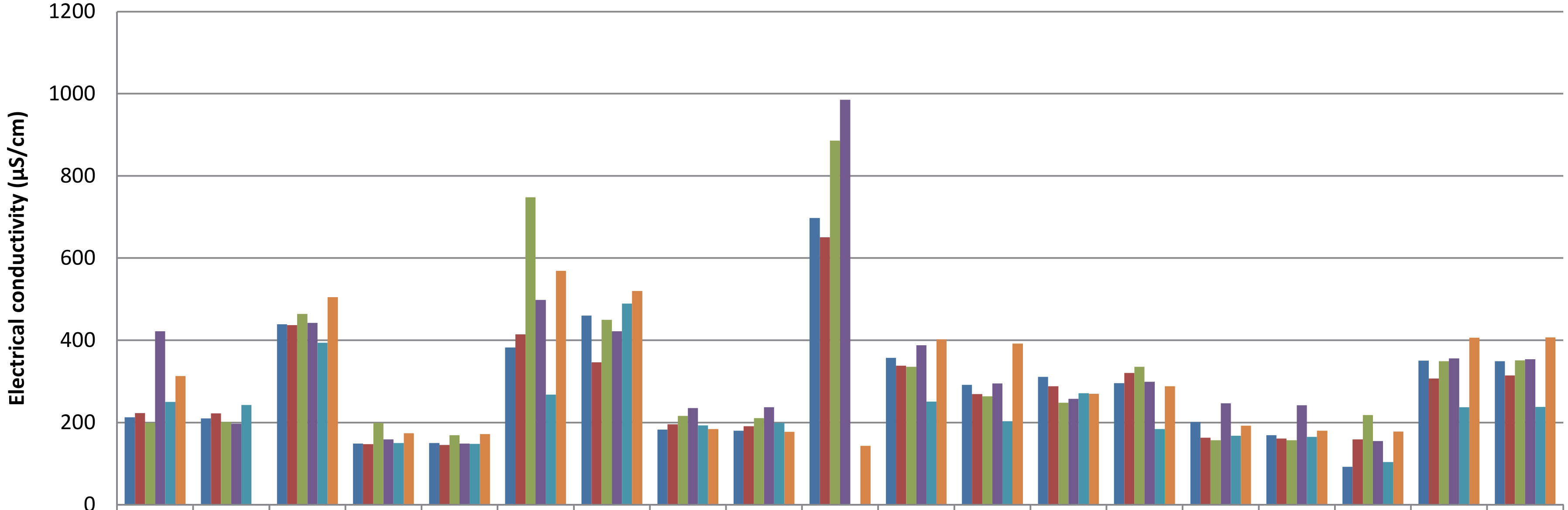
Figure 4.5: Turbidity levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between March 2013 and August 2013



	AL FE	AL DP	AM FE	DM FE	DM DP	EB FE	FB FE	KE FE	KE DP	KM FE	MDT FE	QT FE	RE FE	SC FE	ST FE	ST DP	WT FE	ZW FE	ZW DP
Sep-12	201	203	525	179	176	449	420	246	242	124	363	305	385	445	174	189		445	440
Oct-12	228	228	397	155	158	322	482	235	232	726	418	284	375	295	224	230	189	483	483
Nov-12	181		454	166	165	402	373	266	266	133	377	276	319	325	241	239	188	379	380
Dec-12	173	173	404	162		399	392	219	223	422	364	252	279	303	201	199	182	384	353
Jan-13	169	169	419	139	136	435	306	201	200	557	349	252	311	303	170	168	159	405	392
Feb-13	206	203	428	136	135	443	334	179	168	974	342	275	297	294	222	221	147	397	398

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point).

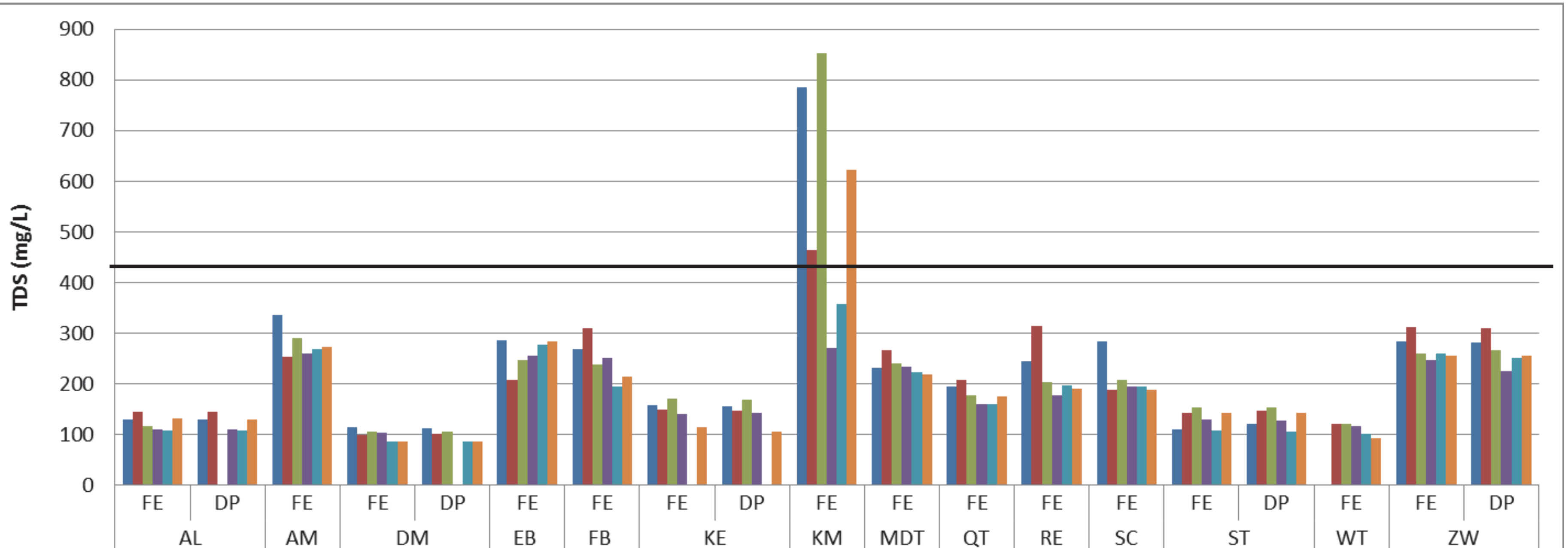
Figure 4.6: Electrical conductivity levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between September 2012 and February 2013



	AL FE	AL DP	AM FE	DM FE	DM DP	EB FE	FB FE	KE FE	KE DP	KM FE	MDT FE	QT FE	RE FE	SC FE	ST FE	ST DP	WT FE	ZW FE	ZW DP
Mar-13	213	210	439	149	150	382	460	183	180	698	357	292	311	296	201	169	92	350	349
Apr-13	223	222	437	147	145	414	346	196	191	651	338	269	288	320	163	161	159	307	314
May-13	199	201	464	199	169	748	450	216	211	886	335	264	248	335	157	157	218	349	351
Jun-13	422	197	442	159	149	498	422	235	237	985	388	295	258	299	247	242	155	356	354
Jul-13	250	243	394	150	148	268	489	193	199		251	203	271	184	168	165	104	237	238
Aug-13	313		505	174	172	569	520	184	177	143	402	392	270	288	192	180	178	406	407

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point).

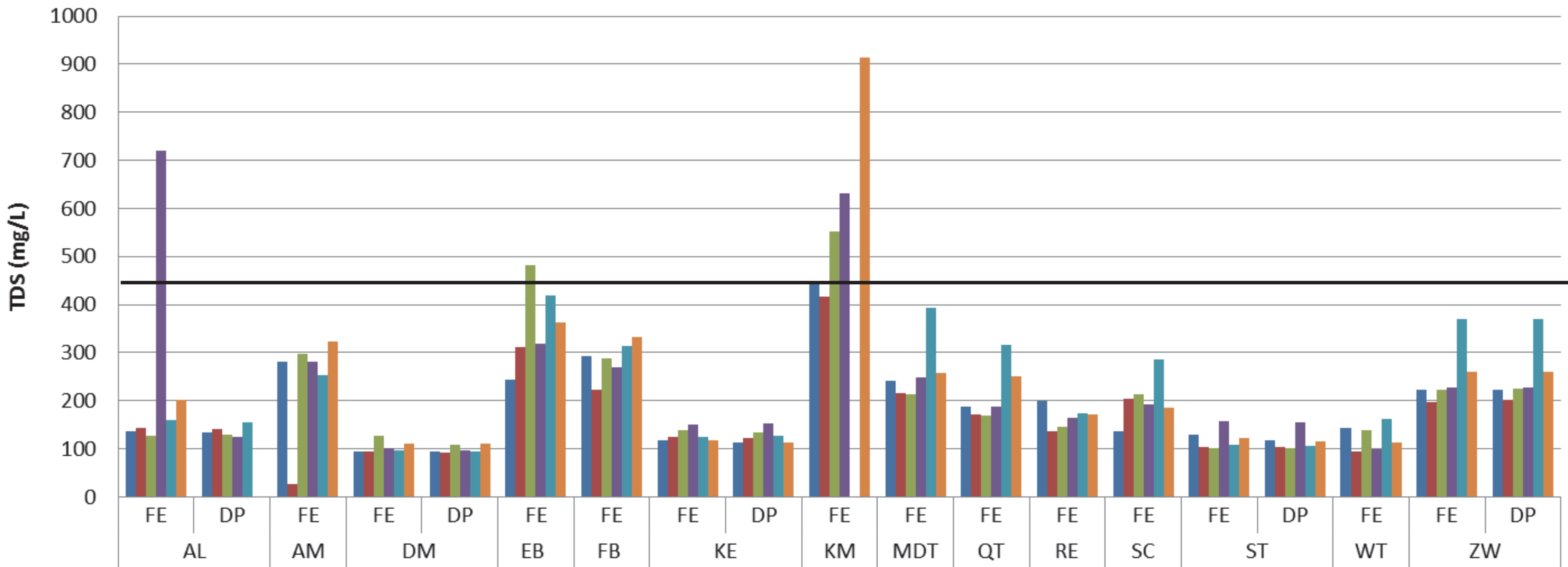
Figure 4.7: Electrical conductivity levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between March 2013 and August 2013



	AL		AM	DM		EB	FB	KE		KM	MDT	QT	RE	SC	ST		WT	ZW	
	FE	DP	FE	FE	DP	FE	FE	FE	DP	FE	FE	FE	FE	FE	FE	DP	FE	FE	DP
Sep-12	129	130	336	114	112	287	269	157	155	786	231	195	245	285	111	121		285	282
Oct-12	146	146	254	99	101	208	309	150	148	465	267	208	315	189	143	147	121	312	309
Nov-12	116		291	106	106	246	239	171	170	853	240	178	204	208	154	153	121	261	267
Dec-12	111	111	259	104		256	251	140	143	270	233	161	178	194	129	127	117	246	226
Jan-13	108	108	268	87	87	278	196			357	223	161	198	194	109	107	102	259	251
Feb-13	132	130	274	87	87	284	214	115	107	623	219	176	190	189	142	142	94	255	255

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point). Values above the bold line at 450 mg/l indicate non-compliance.

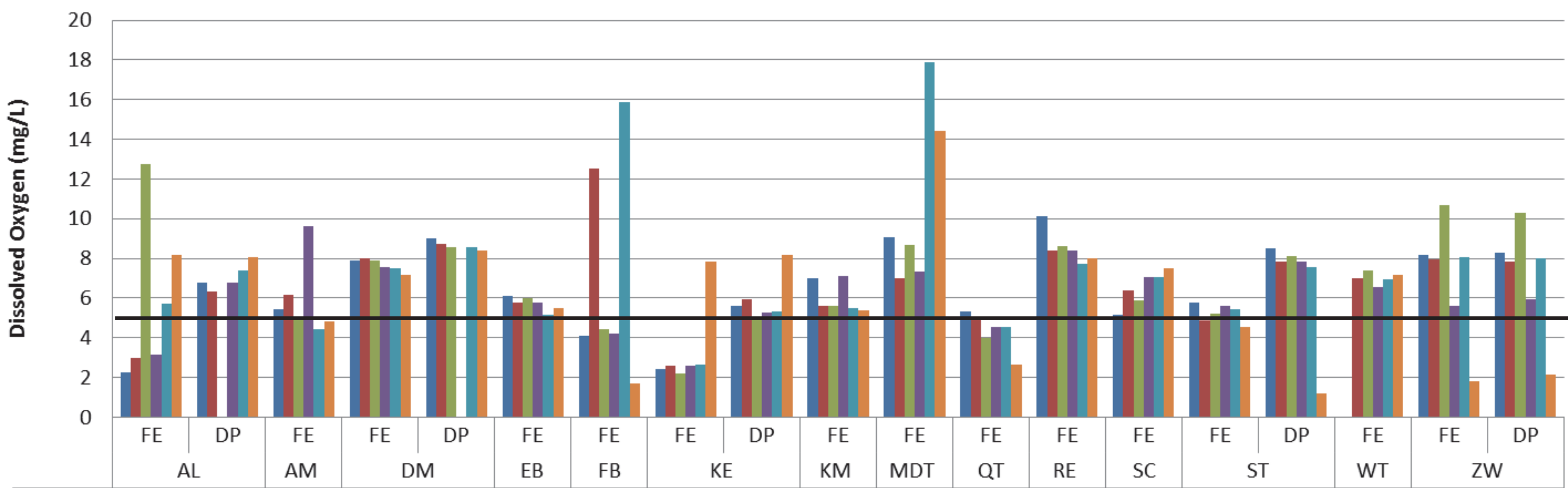
Figure 4.8: Total Dissolved Solids levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between September 2012 and February 2013



	AL FE	AL DP	AM FE	DM FE	DM DP	EB FE	FB FE	KE FE	KE DP	KM FE	MDT FE	QT FE	RE FE	SC FE	ST FE	ST DP	WT FE	ZW FE	ZW DP
Mar-13	136	135	282	95	94	245	294	117	113	447	242	187	199	136	129	119	144	224	223
Apr-13	143	142	27	94	93	312	222	126	122	417	216	172	136	205	104	103	95	197	201
May-13	127	129	298	127	108	481	288	138	135	551	214	169	145	214	101	101	140	223	225
Jun-13	720	126	282	102	96	319	270	151	152	631	249	188	165	192	158	155	99	228	227
Jul-13	159	156	253	96	95	419	313	124	127		393	317	173	287	108	106	162	370	371
Aug-13	202		323	111	110	364	332	117	114	915	258	251	172	185	123	115	114	260	261

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point). Values above the bold line at 450 mg/l indicate non-compliance.

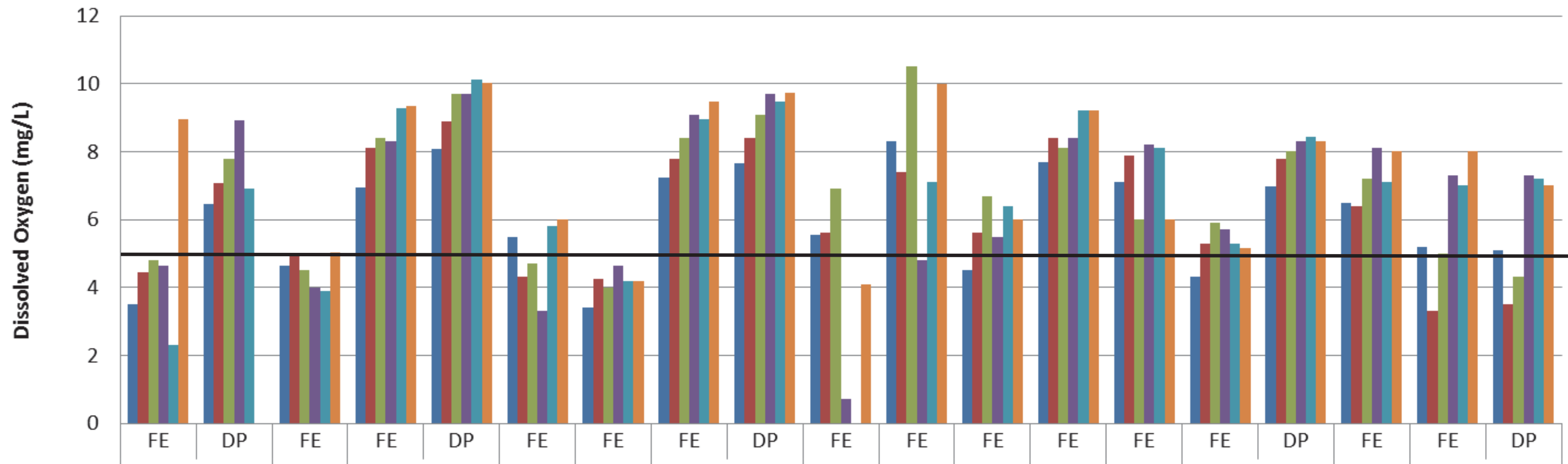
Figure 4.9: Total Dissolved Solids levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between March 2013 and August 2013



	AL FE	AL DP	AM FE	DM FE	DM DP	EB FE	FB FE	KE FE	KE DP	KM FE	MDT FE	QT FE	RE FE	SC FE	ST FE	ST DP	WT FE	ZW FE	ZW DP
Sep-12	2.25	6.76	5.46	7.88	9	6.11	4.1	2.41	5.58	6.98	9.05	5.3	10.14	5.14	5.78	8.48		8.16	8.27
Oct-12	2.99	6.32	6.17	8	8.74	5.78	12.53	2.6	5.94	5.61	6.99	4.97	8.41	6.38	4.88	7.81	6.99	7.97	7.82
Nov-12	12.75		4.98	7.9	8.54	6.02	4.42	2.2	5.01	5.6	8.7	4	8.6	5.9	5.24	8.1	7.4	10.7	10.3
Dec-12	3.13	6.77	9.6	7.54		5.77	4.19	2.57	5.26	7.1	7.33	4.57	8.4	7.04	5.58	7.82	6.56	5.61	5.93
Jan-13	5.73	7.37	4.43	7.5	8.59	5.15	15.9	2.64	5.3	5.5	17.9	4.57	7.7	7.04	5.43	7.57	6.94	8.08	8.02
Feb-13	8.19	8.05	4.82	7.14	8.4	5.5	1.72	7.85	8.19	5.4	14.4	2.67	8	7.5	4.56	1.18	7.16	1.83	2.16

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point). Values above the bold line at 5 mg/l indicate non-compliance.

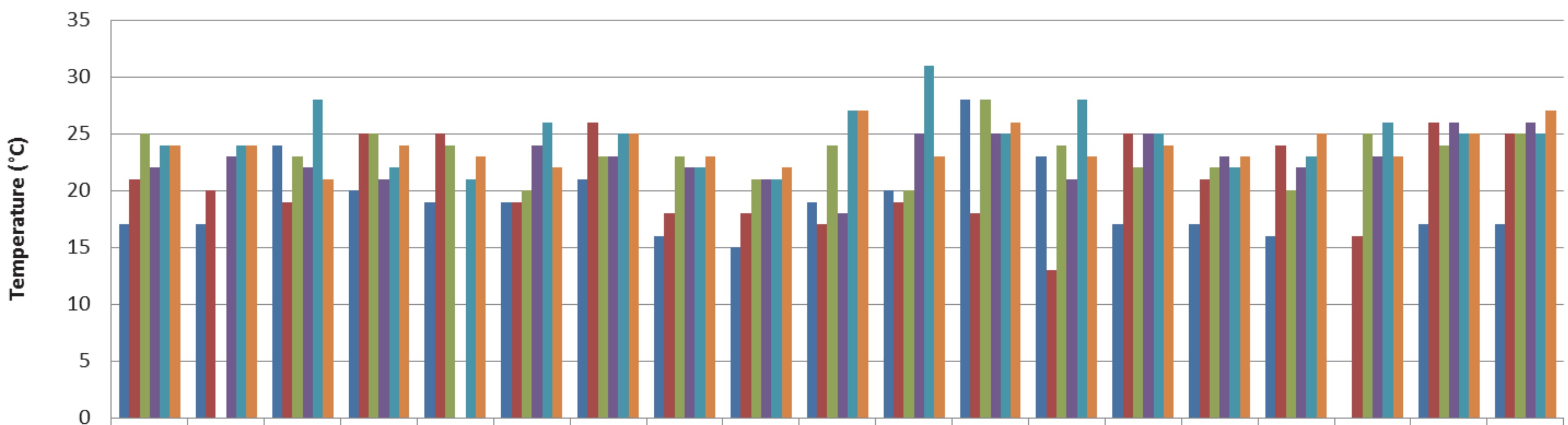
Figure 4.10: Dissolved Oxygen levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between September 2012 and February 2013



	AL FE	AL DP	AM FE	DM FE	DM DP	EB FE	FB FE	KE FE	KE DP	KM FE	MDT FE	QT FE	RE FE	SC FE	ST FE	ST DP	WT FE	ZW FE	ZW DP
Mar-13	3.51	6.47	4.65	6.93	8.09	5.5	3.4	7.25	7.66	5.54	8.3	4.5	7.7	7.1	4.33	6.96	6.5	5.2	5.1
Apr-13	4.43	7.06	5	8.1	8.9	4.3	4.25	7.8	8.4	5.6	7.4	5.6	8.4	7.9	5.3	7.8	6.4	3.3	3.5
May-13	4.81	7.8	4.5	8.4	9.7	4.7	4	8.4	9.1	6.9	10.5	6.7	8.1	6	5.9	8	7.2	5	4.3
Jun-13	4.64	8.91	4	8.3	9.7	3.3	4.64	9.1	9.7	0.7	4.8	5.5	8.4	8.2	5.7	8.3	8.1	7.3	7.3
Jul-13	2.29	6.91	3.91	9.27	10.11	5.8	4.2	8.97	9.47		7.1	6.4	9.2	8.1	5.29	8.44	7.1	7	7.2
Aug-13	8.94		5.04	9.36	10.04	6	4.18	9.47	9.74	4.1	10	6	9.2	6	5.16	8.31	8	8	7

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point). Values above the bold line at 5 mg/l indicate non-compliance.

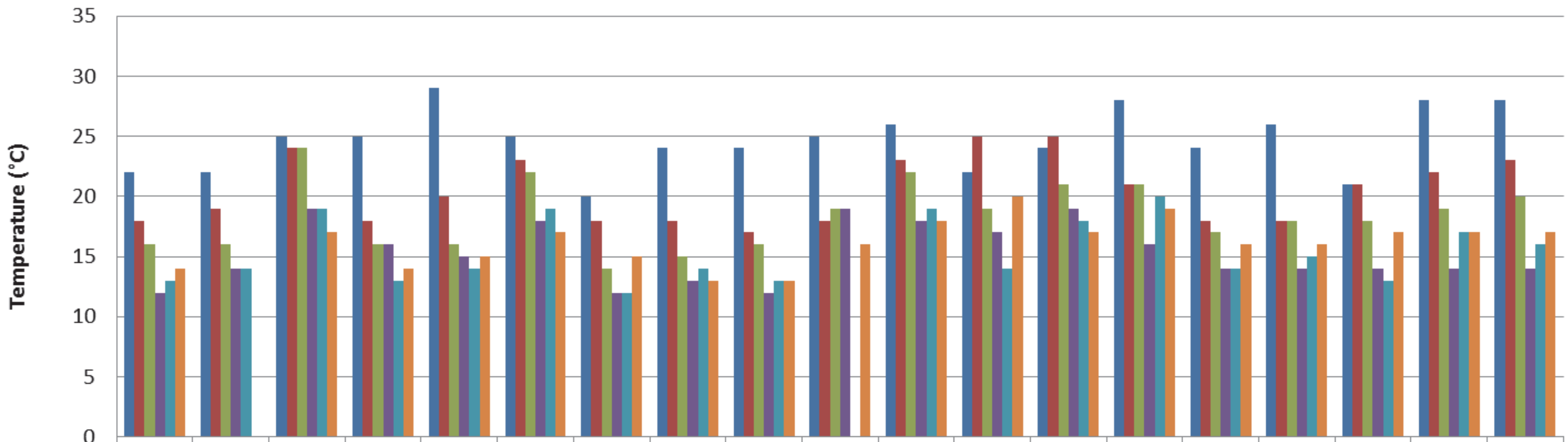
Figure 4.11: Dissolved Oxygen levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between March 2013 and August 2013



	FE	DP	FE	FE	DP	FE	FE	FE	DP	FE	FE	FE	FE	FE	FE	DP	FE	FE	DP
	AL		AM	DM		EB	FB	KE		KM	MDT	QT	RE	SC	ST		WT	ZW	
Sep-12	17	17	24	20	19	19	21	16	15	19	20	28	23	17	17	16		17	17
Oct-12	21	20	19	25	25	19	26	18	18	17	19	18	13	25	21	24	16	26	25
Nov-12	25		23	25	24	20	23	23	21	24	20	28	24	22	22	20	25	24	25
Dec-12	22	23	22	21		24	23	22	21	18	25	25	21	25	23	22	23	26	26
Jan-13	24	24	28	22	21	26	25	22	21	27	31	25	28	25	22	23	26	25	25
Feb-13	24	24	21	24	23	22	25	23	22	27	23	26	23	24	23	25	23	25	27

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point).

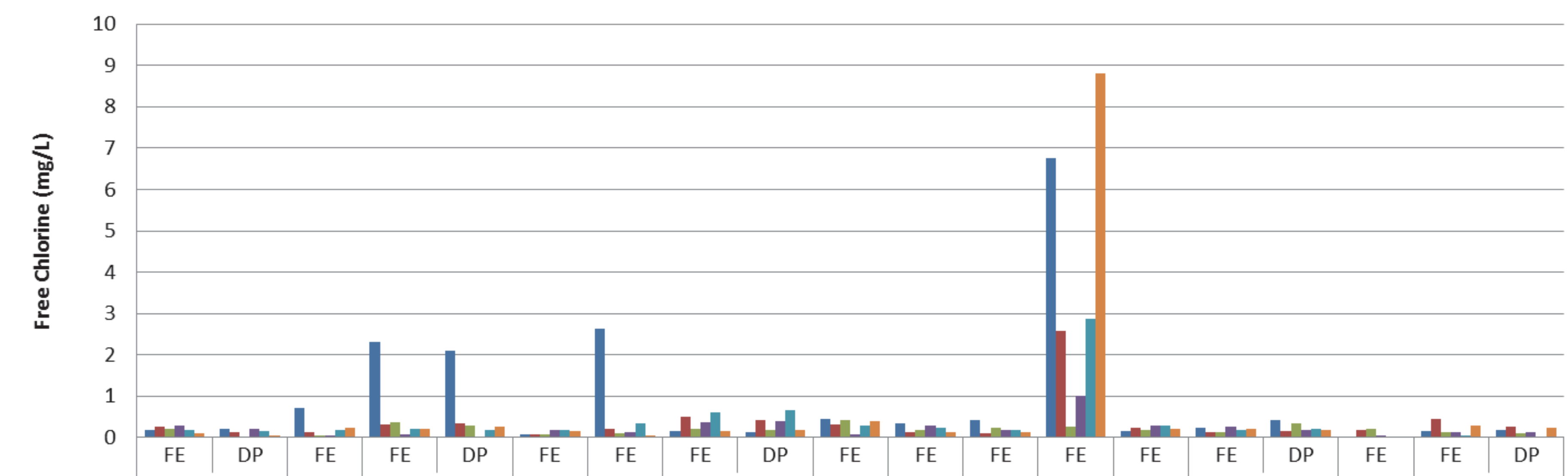
Figure 4.12: Temperature levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between September 2012 and February 2013



	AL FE	AL DP	AM FE	DM FE	DM DP	EB FE	FB FE	KE FE	KE DP	KM FE	MDT FE	QT FE	RE FE	SC FE	ST FE	ST DP	WT FE	ZW FE	ZW DP
Mar-13	22	22	25	25	29	25	20	24	24	25	26	22	24	28	24	26	21	28	28
Apr-13	18	19	24	18	20	23	18	18	17	18	23	25	25	21	18	18	21	22	23
May-13	16	16	24	16	16	22	14	15	16	19	22	19	21	21	17	18	18	19	20
Jun-13	12	14	19	16	15	18	12	13	12	19	18	17	19	16	14	14	14	14	14
Jul-13	13	14	19	13	14	19	12	14	13		19	14	18	20	14	15	13	17	16
Aug-13	14		17	14	15	17	15	13	13	16	18	20	17	19	16	16	17	17	17

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point).

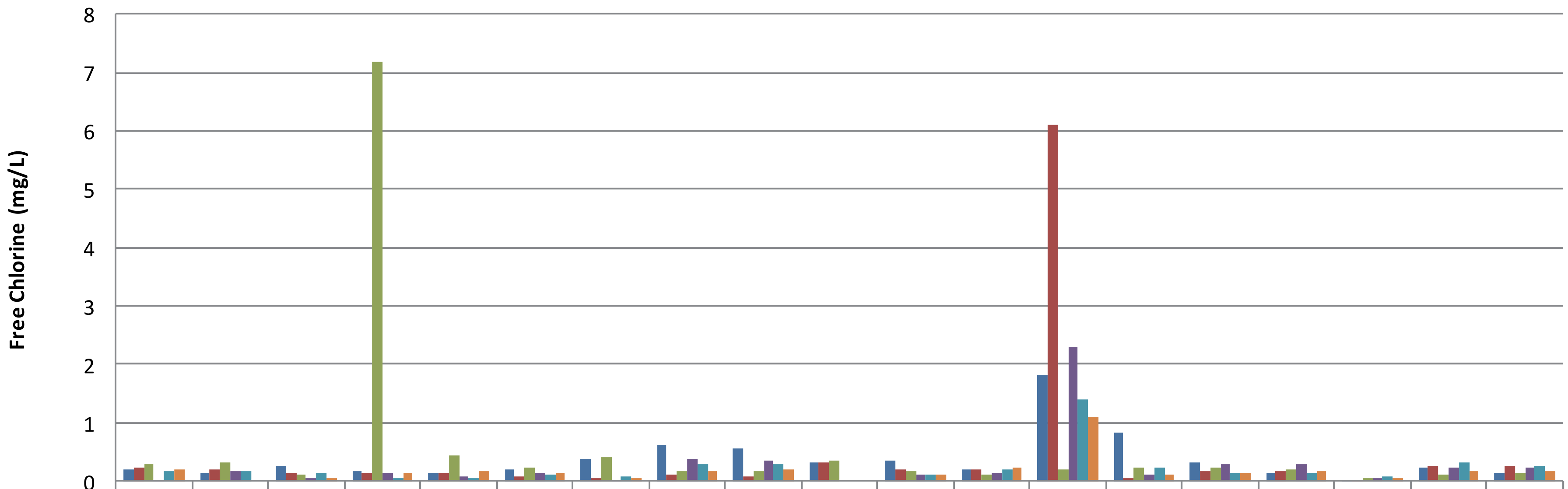
Figure 4.13: Temperature levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between March 2013 and August 2013



	FE	DP	FE	FE	DP	FE	FE	FE	DP	FE	FE	FE	FE	FE	FE	DP	FE	FE	DP
	AL		AM	DM		EB	FB	KE		KM	MDT	QT	RE	SC	ST		WT	ZW	
Sep-12	0.18	0.2	0.71	2.31	2.1	0.09	2.64	0.15	0.14	0.44	0.35	0.43	6.75	0.15	0.23	0.41		0.15	0.19
Oct-12	0.27	0.13	0.13	0.31	0.33	0.09	0.21	0.49	0.41	0.32	0.13	0.1	2.57	0.24	0.13	0.15	0.17	0.46	0.26
Nov-12	0.22		0.05	0.36	0.28	0.09	0.11	0.21	0.18	0.43	0.17	0.23	0.27	0.17	0.13	0.34	0.21	0.14	0.1
Dec-12	0.29	0.22	0.05	0.09		0.19	0.13	0.37	0.39	0.09	0.3	0.18	1	0.28	0.27	0.17	0.06	0.13	0.12
Jan-13	0.19	0.15	0.17	0.22	0.17	0.18	0.35	0.61	0.65	0.3	0.23	0.18	2.88	0.28	0.19	0.21		0.05	0.02
Feb-13	0.1	0.05	0.24	0.21	0.26	0.16	0.05	0.15	0.17	0.39	0.13	0.13	8.8	0.22	0.21	0.18	0.03	0.3	0.23

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point).

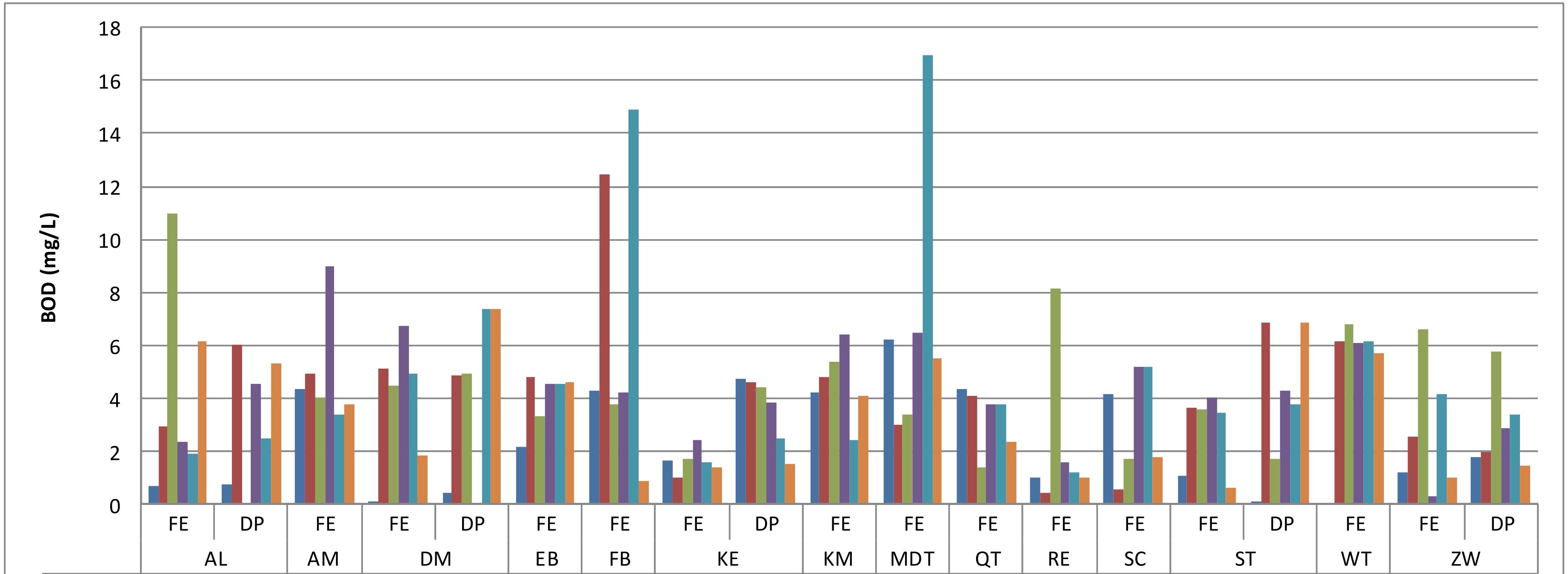
Figure 4.14: Free Chlorine levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between September 2012 and February 2013



	FE	DP	FE	FE	DP	FE	FE	FE	DP	FE	FE	FE	FE	FE	FE	DP	FE	FE	DP
	AL		AM	DM		EB	FB	KE		KM	MDT	QT	RE	SC	ST		WT	ZW	
Mar-13	0.19	0.13	0.25	0.16	0.15	0.19	0.38	0.61	0.57	0.33	0.34	0.21	1.82	0.84	0.33	0.13	0.01	0.23	0.15
Apr-13	0.24	0.2	0.13	0.14	0.13	0.08	0.06	0.1	0.09	0.33	0.2	0.21	6.1	0.04	0.16	0.17	0.03	0.27	0.25
May-13	0.3	0.31	0.1	7.18	0.43	0.22	0.4	0.16	0.18	0.35	0.16	0.12	0.2	0.23	0.23	0.2	0.05	0.12	0.15
Jun-13	0.02	0.17	0.05	0.14	0.09	0.15	0.02	0.37	0.35	0	0.1	0.14	2.3	0.1	0.3	0.28	0.06	0.22	0.22
Jul-13	0.16	0.16	0.14	0.06	0.06	0.12	0.09	0.29	0.28		0.12	0.21	1.4	0.24	0.13	0.15	0.09	0.31	0.26
Aug-13	0.19		0.05	0.13	0.16	0.15	0.05	0.17	0.2	0	0.12	0.23	1.1	0.12	0.14	0.16	0.05	0.18	0.18

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point).

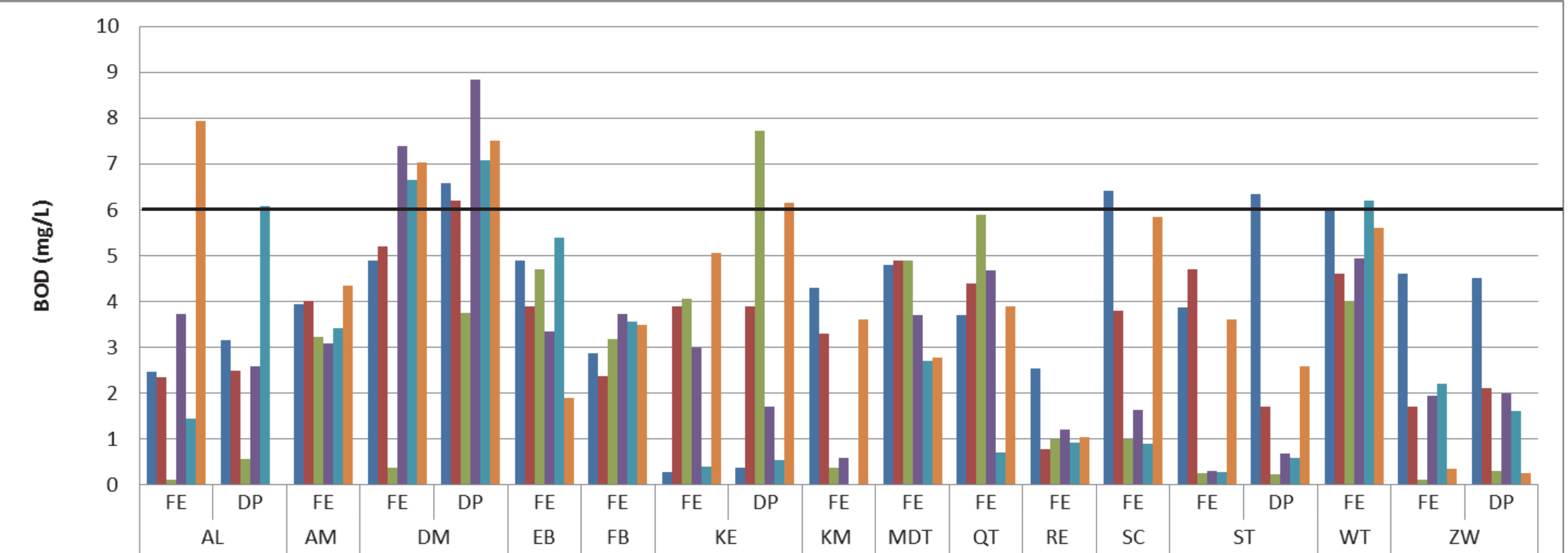
Figure 4.15: Free Chlorine levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between March 2013 and August 2013



	FE	DP	FE	FE	DP	FE	FE	FE	DP	FE	FE	FE	FE	FE	FE	DP	FE	FE	DP
	AL		AM	DM		EB	FB	KE		KM	MDT	QT	RE	SC	ST		WT	ZW	
Sep-12	0.66	0.77	4.36	0.13	0.43	2.17	4.3	1.67	4.76	4.22	6.24	4.36	1	4.17	1.08	0.13		1.19	1.81
Oct-12	2.92	6.03	4.95	5.12	4.84	4.8	12.43	1.04	4.62	4.8	3.02	4.08	0.46	0.53	3.63	6.85	6.18	2.58	1.97
Nov-12	11		4	4.48	4.9	3.3	3.76	1.71	4.39	5.38	3.4	1.42	8.13	1.7	3.6	1.7	6.8	6.6	5.8
Dec-12	2.35	4.57	8.99	6.76		4.56	4.24	2.43	3.86	6.4	6.5	3.76	1.6	5.18	4.02	4.26	6.12	0.32	2.88
Jan-13	1.89	2.5	3.4	4.96	7.36	4.57	14.91	1.57	2.52	2.4	16.96	3.76	1.21	5.18	3.48	3.77	6.18	4.18	3.41
Feb-13	6.17	5.32	3.76	1.84	7.35	4.6	0.86	1.41	1.5	4.1	5.51	2.39	1.02	1.8	0.65	6.85	5.69	1.03	1.43

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point). Values above the bold line at 6 mg/l indicate non-compliance.

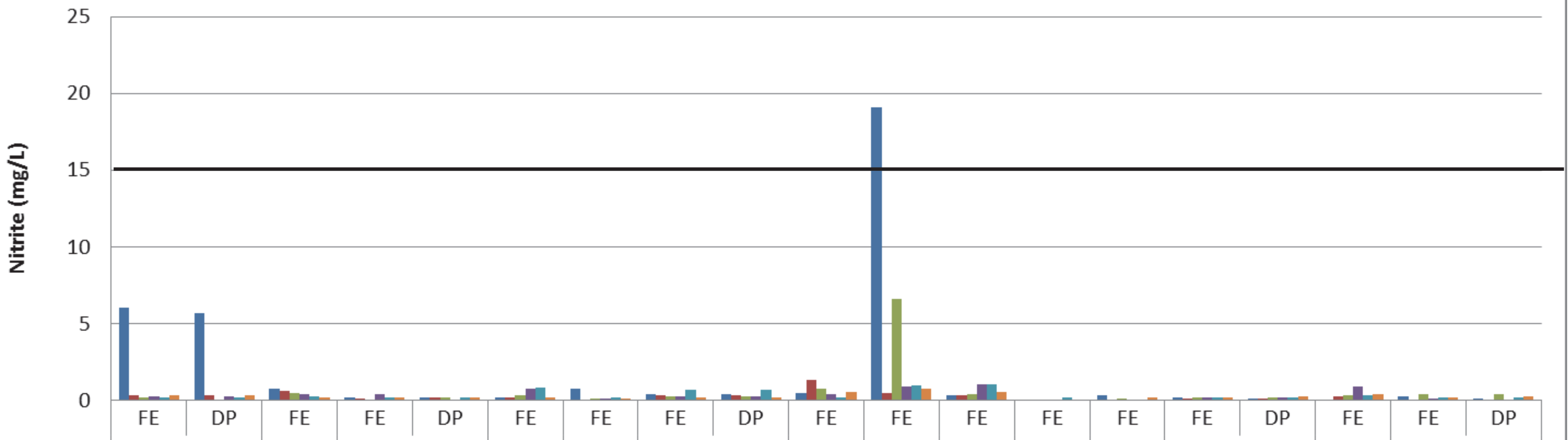
Figure 4.16: Biochemical Oxygen Demand levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between September 2012 and February 2013



	AL FE	AL DP	AM FE	DM FE	DM DP	EB FE	FB FE	KE FE	KE DP	KM FE	MDT FE	QT FE	RE FE	SC FE	ST FE	ST DP	WT FE	ZW FE	ZW DP
Mar-13	2.46	3.16	3.93	4.9	6.59	4.9	2.86	0.27	0.38	4.3	4.8	3.7	2.53	6.41	3.88	6.35	6	4.6	4.5
Apr-13	2.35	2.5	4	5.2	6.2	3.9	2.38	3.9	3.9	3.3	4.9	4.4	0.77	3.8	4.7	1.7	4.6	1.7	2.1
May-13	0.1	0.57	3.23	0.37	3.75	4.7	3.18	4.05	7.73	0.37	4.9	5.9	1	1	0.26	0.24	4	0.1	0.3
Jun-13	3.73	2.59	3.08	7.39	8.84	3.34	3.73	2.98	1.7	0.59	3.69	4.68	1.2	1.64	0.3	0.69	4.94	1.95	1.99
Jul-13	1.44	6.07	3.42	6.65	7.08	5.4	3.57	0.39	0.54		2.7	0.7	0.92	0.9	0.28	0.59	6.2	2.2	1.6
Aug-13	7.94		4.35	7.02	7.51	1.9	3.49	5.06	6.14	3.6	2.77	3.9	1.03	5.83	3.6	2.59	5.6	0.34	0.25

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point). Values above the bold line at 6 mg/l indicate non-compliance.

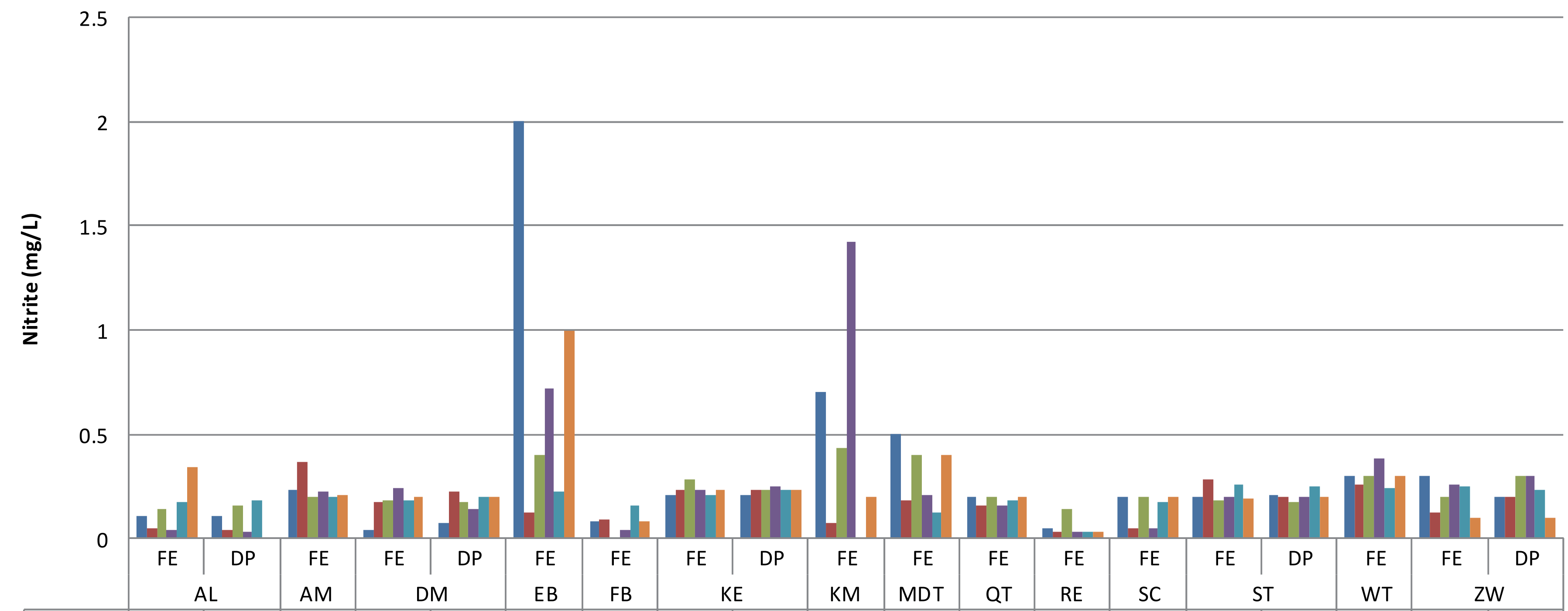
Figure 4.17: Biochemical Oxygen Demand levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between March 2013 and August 2013



	FE	DP	FE	FE	DP	FE	FE	FE	DP	FE	FE	FE	FE	FE	FE	DP	FE	FE	DP
	AL		AM	DM		EB	FB	KE		KM	MDT	QT	RE	SC	ST		WT	ZW	
Sep-12	6.03	5.67	0.77	0.18	0.18	0.19	0.77	0.41	0.43	0.47	19.1	0.34	0.05	0.32	0.16	0.13		0.27	0.14
Oct-12	0.32	0.35	0.6	0.09	0.17	0.18	0.05	0.34	0.35	1.34	0.49	0.33	0.07	0.04	0.14	0.14	0.24	0.06	0.05
Nov-12	0.21		0.46	0.01	0.15	0.36	0.09	0.23	0.23	0.72	6.6	0.41	0.04	0.13	0.19	0.2	0.32	0.42	0.43
Dec-12	0.24	0.26	0.4	0.43		0.75	0.09	0.23	0.24	0.37	0.87	1.04	0.04	0.04	0.2	0.2	0.89	0.1	0.06
Jan-13	0.16	0.19	0.25	0.16	0.16	0.86	0.16	0.7	0.71	0.2	0.98	1.04	0.17	0.04	0.19	0.18	0.33	0.17	0.17
Feb-13	0.35	0.34	0.2	0.18	0.19	0.21	0.1	0.2	0.18	0.52	0.73	0.57	0.04	0.16	0.21	0.22	0.37	0.18	0.24

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point). Values above the bold line at 15 mg/l indicate non-compliance.

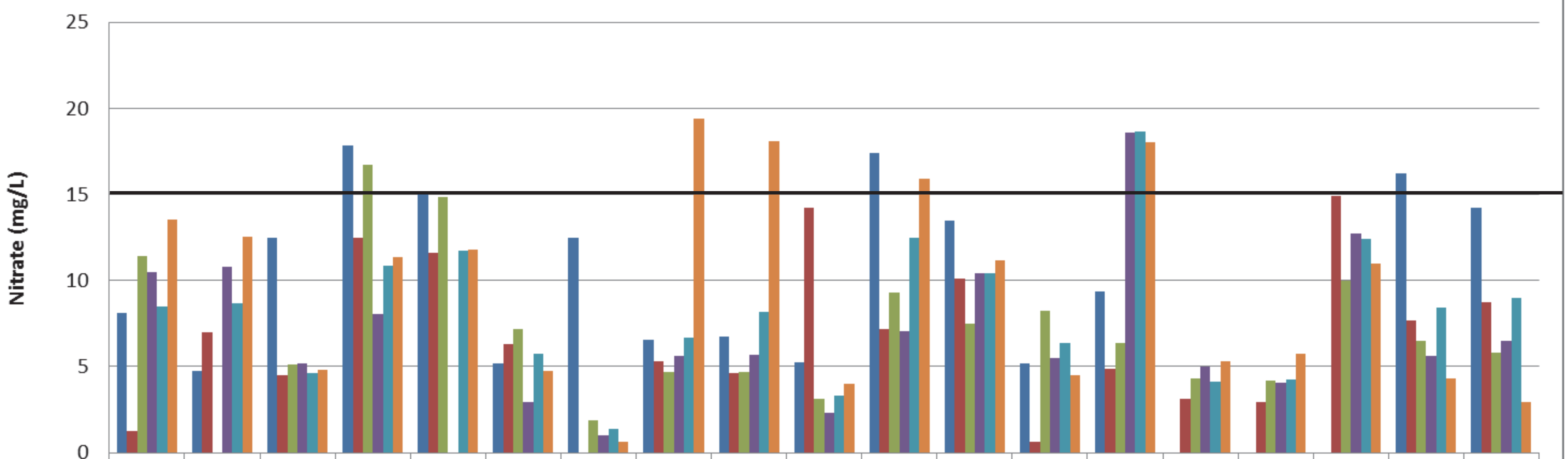
Figure 4.18: Nitrite levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between September 2012 and February 2013



	AL FE	AL DP	AM FE	DM FE	DM DP	EB FE	FB FE	KE FE	KE DP	KM FE	MDT FE	QT FE	RE FE	SC FE	ST FE	ST DP	WT FE	ZW FE	ZW DP
Mar-13	0.11	0.11	0.23	0.04	0.07	2	0.08	0.21	0.21	0.7	0.5	0.2	0.05	0.2	0.2	0.21	0.3	0.3	0.2
Apr-13	0.05	0.04	0.37	0.17	0.22	0.12	0.09	0.23	0.23	0.07	0.18	0.16	0.03	0.05	0.28	0.2	0.26	0.12	0.2
May-13	0.14	0.16	0.2	0.18	0.17	0.4	0	0.28	0.23	0.43	0.4	0.2	0.14	0.2	0.18	0.17	0.3	0.2	0.3
Jun-13	0.04	0.03	0.22	0.24	0.14	0.72	0.04	0.23	0.25	1.42	0.21	0.16	0.03	0.05	0.2	0.2	0.38	0.26	0.3
Jul-13	0.17	0.18	0.2	0.18	0.2	0.22	0.16	0.21	0.23		0.12	0.18	0.03	0.17	0.26	0.25	0.24	0.25	0.23
Aug-13	0.34		0.21	0.2	0.2	1	0.08	0.23	0.23	0.2	0.4	0.2	0.03	0.2	0.19	0.2	0.3	0.1	0.1

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point).

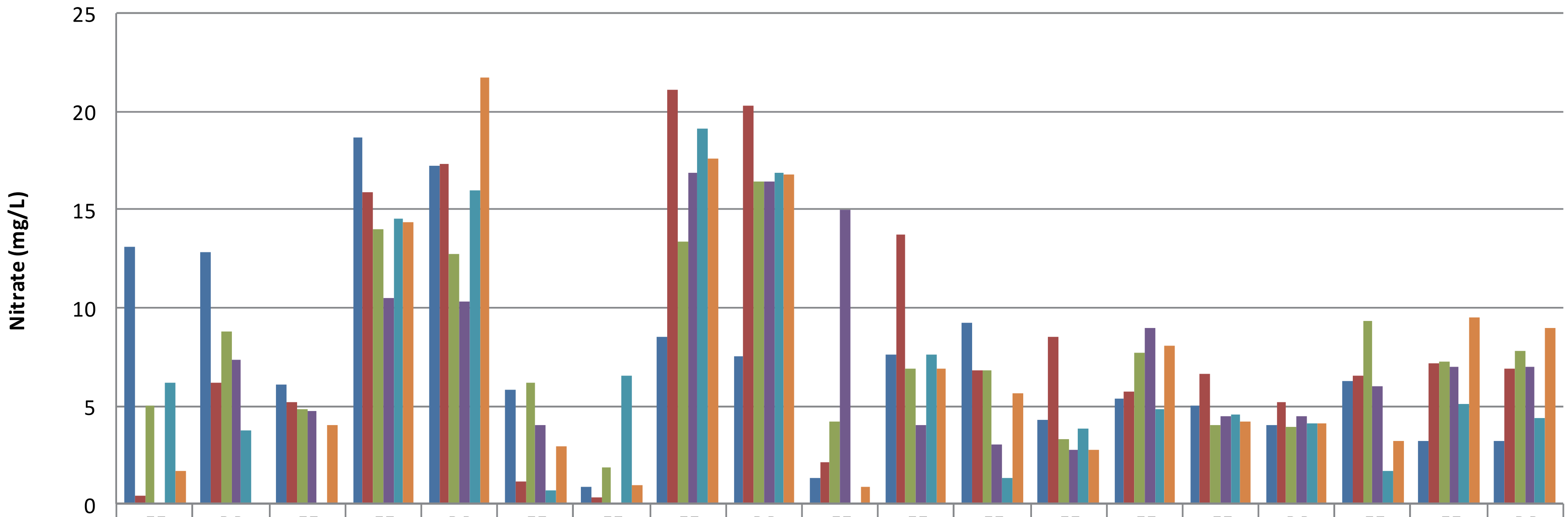
Figure 4.19: Nitrite levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between March 2013 and August 2013



	FE	DP	FE	FE	DP	FE	FE	FE	DP	FE	FE	FE	FE	FE	FE	DP	FE	FE	DP
	AL		AM	DM		EB	FB	KE		KM	MDT	QT	RE	SC	ST		WT	ZW	
Sep-12	8.13	4.77	12.5	17.85	15.12	5.17	12.5	6.57	6.77	5.25	17.4	13.5	5.2	9.35	0	0		16.25	14.25
Oct-12	1.29	7	4.47	12.5	11.63	6.33	0	5.33	4.63	14.2	7.2	10.1	0.63	4.9	3.1	2.97	14.9	7.7	8.76
Nov-12	11.43		5.1	16.7	14.87	7.2	1.9	4.67	4.7	3.1	9.3	7.51	8.27	6.4	4.3	4.2	9.97	6.5	5.8
Dec-12	10.47	10.8	5.17	8.03		2.93	1	5.6	5.7	2.33	7.07	10.4	5.5	18.6	5.03	4.06	12.7	5.6	6.5
Jan-13	8.47	8.67	4.6	10.87	11.7	5.77	1.4	6.7	8.2	3.3	12.47	10.4	6.4	18.63	4.1	4.23	12.4	8.43	8.97
Feb-13	13.53	12.53	4.83	11.37	11.8	4.73	0.63	19.4	18.1	4.03	15.9	11.2	4.5	18	5.3	5.76	11	4.3	2.93

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point). Values above the bold line at 15 mg/l indicate non-compliance.

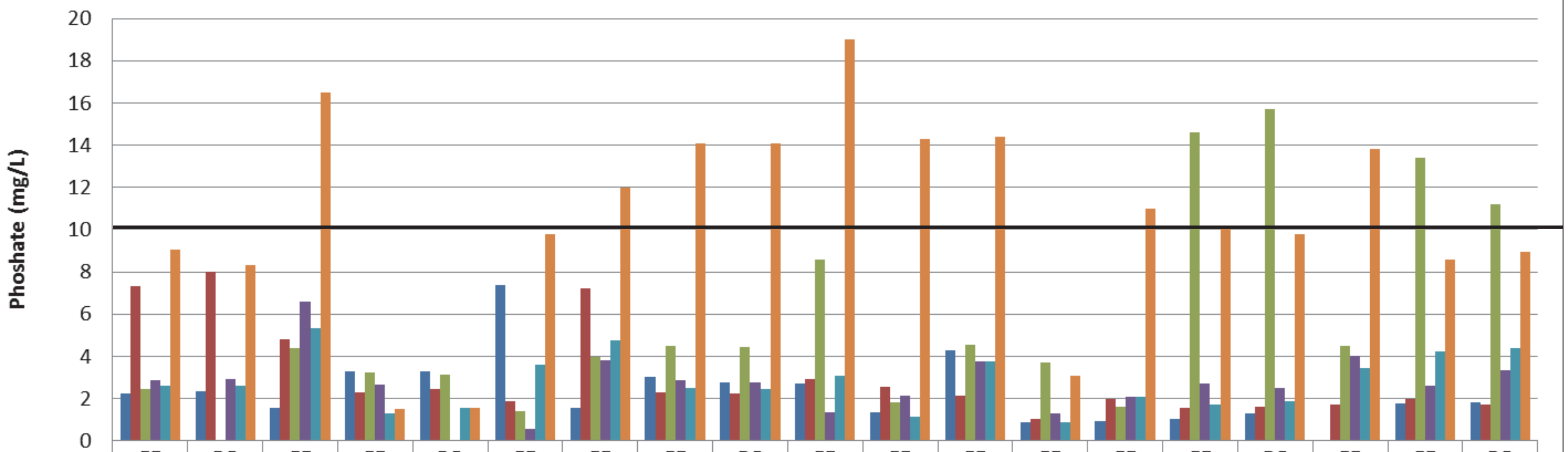
Figure 4.20: Nitrate levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between September 2012 and February 2013



	AL FE	AL DP	AM FE	DM FE	DM DP	EB FE	FB FE	KE FE	KE DP	KM FE	MDT FE	QT FE	RE FE	SC FE	ST FE	ST DP	WT FE	ZW FE	ZW DP
Mar-13	13.1	12.8	6.13	18.7	17.23	5.8	0.87	8.5	7.57	1.3	7.6	9.27	4.3	5.37	5.03	4	6.23	3.17	3.17
Apr-13	0.41	6.2	5.2	15.87	17.37	1.17	0.29	21.1	20.3	2.1	13.7	6.8	8.5	5.7	6.6	5.2	6.5	7.2	6.9
May-13	5.04	8.77	4.8	13.97	12.77	6.2	1.9	13.4	16.4	4.2	6.9	6.8	3.3	7.7	4	3.93	9.3	7.23	7.77
Jun-13	0.07	7.38	4.77	10.47	10.3	4	0.07	16.9	16.4	15	4	3	2.8	9	4.43	4.43	6	7	7
Jul-13	6.17	3.79	0	14.53	15.97	0.7	6.5	19.1	16.9		7.6	1.3	3.8	4.8	4.6	4.1	1.7	5.1	4.4
Aug-13	1.67		4	14.37	21.73	2.97	1	17.6	16.8	0.9	6.87	5.63	2.8	8.1	4.2	4.1	3.2	9.5	9

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point). Values above the bold line at 15 mg/l indicate non-compliance.

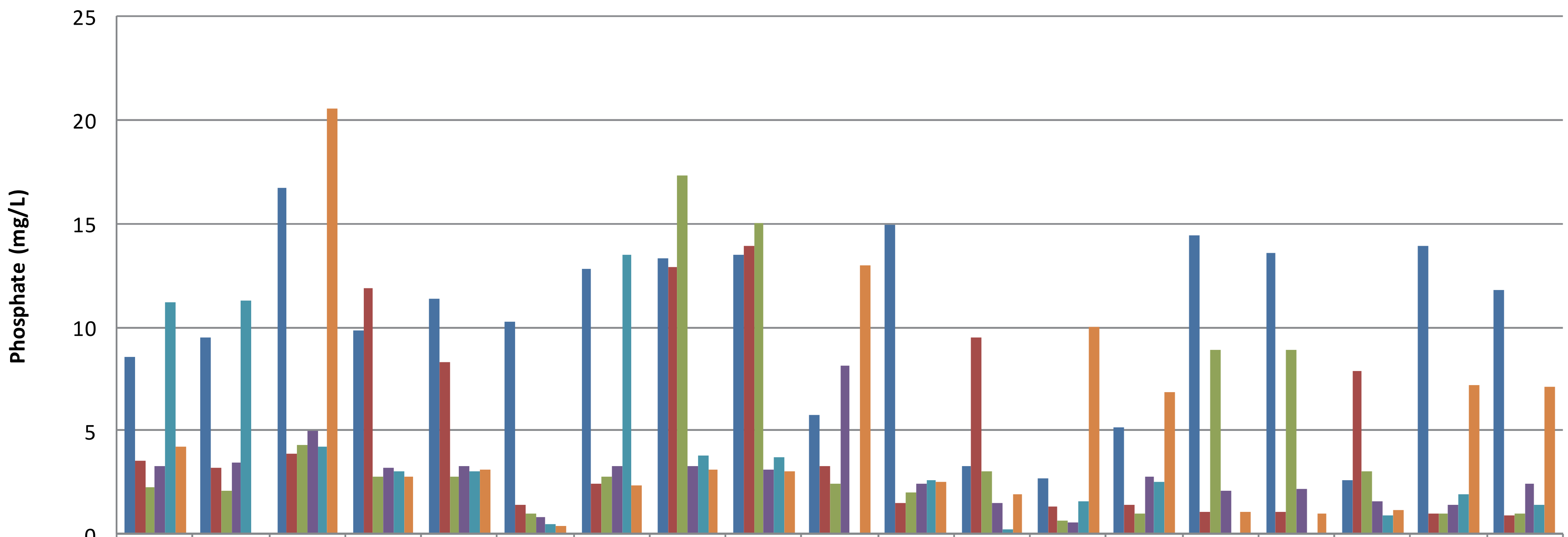
Figure 4.21: Nitrate levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between March 2013 and August 2013



	AL FE	AL DP	AM FE	DM FE	DM DP	EB FE	FB FE	KE FE	KE DP	KM FE	MDT FE	QT FE	RE FE	SC FE	ST FE	ST DP	WT FE	ZW FE	ZW DP
Sep-12	2.26	2.33	1.56	3.3	3.31	7.39	1.56	3.04	2.75	2.7	1.36	4.29	0.9	0.94	1.04	1.31		1.76	1.84
Oct-12	7.3	8.03	4.8	2.29	2.43	1.89	7.23	2.27	2.26	2.9	2.55	2.15	1.05	1.98	1.58	1.59	1.7	1.97	1.72
Nov-12	2.43		4.4	3.22	3.15	1.39	3.96	4.5	4.44	8.6	1.81	4.57	3.7	1.6	14.6	15.7	4.5	13.4	11.2
Dec-12	2.89	2.91	6.57	2.66		0.57	3.79	2.86	2.74	1.36	2.13	3.74	1.31	2.09	2.72	2.52	4.04	2.6	3.35
Jan-13	2.61	2.61	5.35	1.29	1.55	3.6	4.73	2.48	2.46	3.1	1.15	3.74	0.89	2.09	1.73	1.85	3.46	4.22	4.39
Feb-13	9.07	8.33	16.47	1.52	1.54	9.77	12	14.1	14.1	19	14.3	14.4	3.1	11	10.1	9.8	13.8	8.6	8.97

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point). Values above the bold line at 10 mg/l indicate non-compliance.

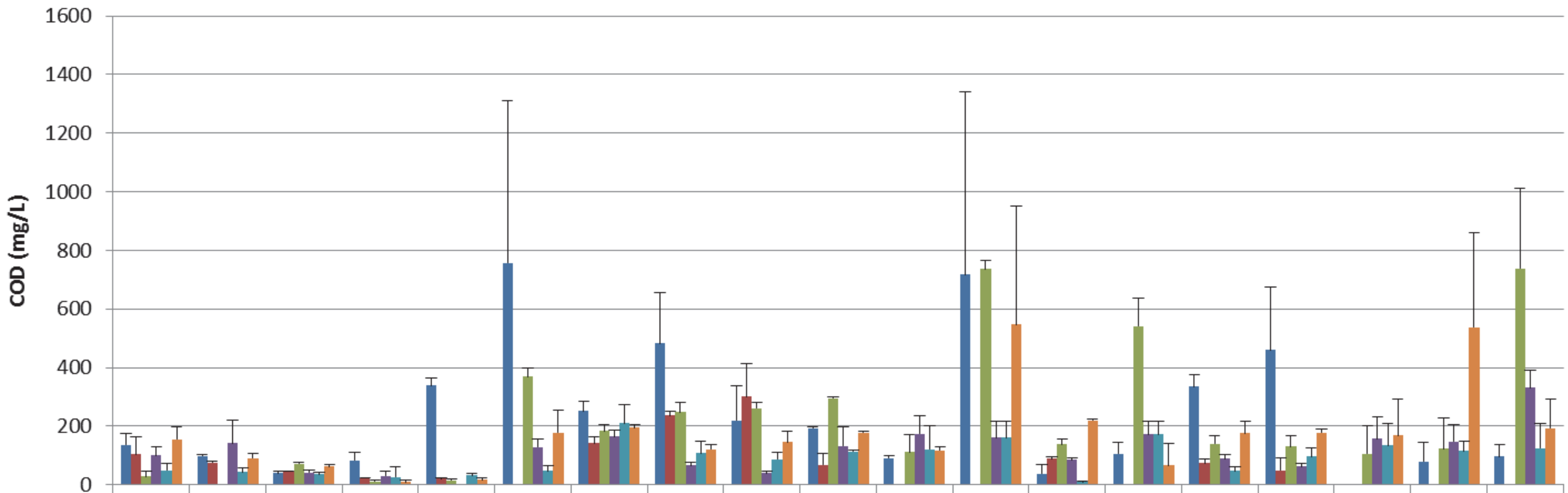
Figure 4.22: Phosphate levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between September 2012 and February 2013



	FE	DP	FE	FE	DP	FE	FE	FE	DP	FE	FE	FE	FE	FE	FE	DP	FE	FE	DP
	AL		AM	DM		EB	FB	KE		KM	MDT	QT	RE	SC	ST		WT	ZW	
Mar-13	8.57	9.47	16.77	9.8	11.33	10.23	12.8	13.3	13.5	5.8	14.93	3.31	2.7	5.2	14.4	13.6	2.64	13.9	11.83
Apr-13	3.52	3.22	3.89	11.89	8.33	1.4	2.45	12.9	13.9	3.3	1.5	9.5	1.3	1.42	1.1	1.1	7.9	1.01	0.95
May-13	2.29	2.12	4.29	2.8	2.74	1	2.78	17.3	15	2.4	2	3	0.67	1	8.9	8.9	3	1	1
Jun-13	3.28	3.45	4.97	3.19	3.29	0.8	3.28	3.26	3.14	8.1	2.4	1.5	0.6	2.8	2.13	2.2	1.6	1.4	2.4
Jul-13	11.2	11.3	4.21	3.02	3.02	0.5	13.5	3.78	3.71		2.6	0.25	1.55	2.5	0	0	0.9	1.9	1.4
Aug-13	4.22		20.57	2.79	3.11	0.4	2.33	3.08	3.05	13	2.5	1.9	10	6.9	1.07	0.99	1.2	7.2	7.1

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point). Values above the bold line at 10 mg/l indicate non-compliance.

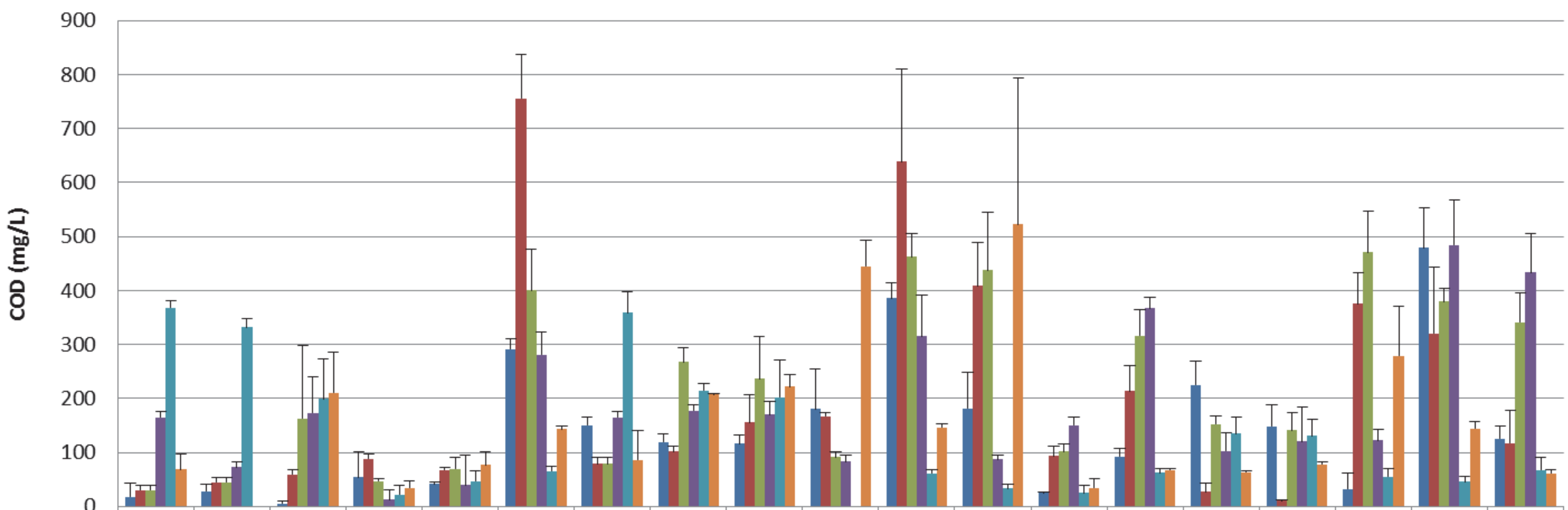
Figure 4.23: Phosphate levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between March 2013 and August 2013



	AL FE	AL DP	AM FE	DM FE	DM DP	EB FE	FB FE	KE FE	KE DP	KM FE	MDT FE	QT FE	RE FE	SC FE	ST FE	ST DP	WT FE	ZW FE	ZW DP
Sep-12	136	99	39	82	339	757	252	482	219	192	90	717	37	105	335	460		79	96
Oct-12	103	76	45	23	20		142	237	301	67			89		73	49			
Nov-12	28		71	11	15	368	183	247	261	295	111	737	137	541	140	131	105	122	739
Dec-12	100	141	42	30		129	165	66	40	133	173	160	84	171	89	62	157	148	331
Jan-13	49	43	36	27	32	47	209	107	86	113	121	160	10	171	47	96	135	116	125
Feb-13	154	90	62	10	17	178	195	121	145	177	117	546	220	66	176	177	169	537	193

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point).

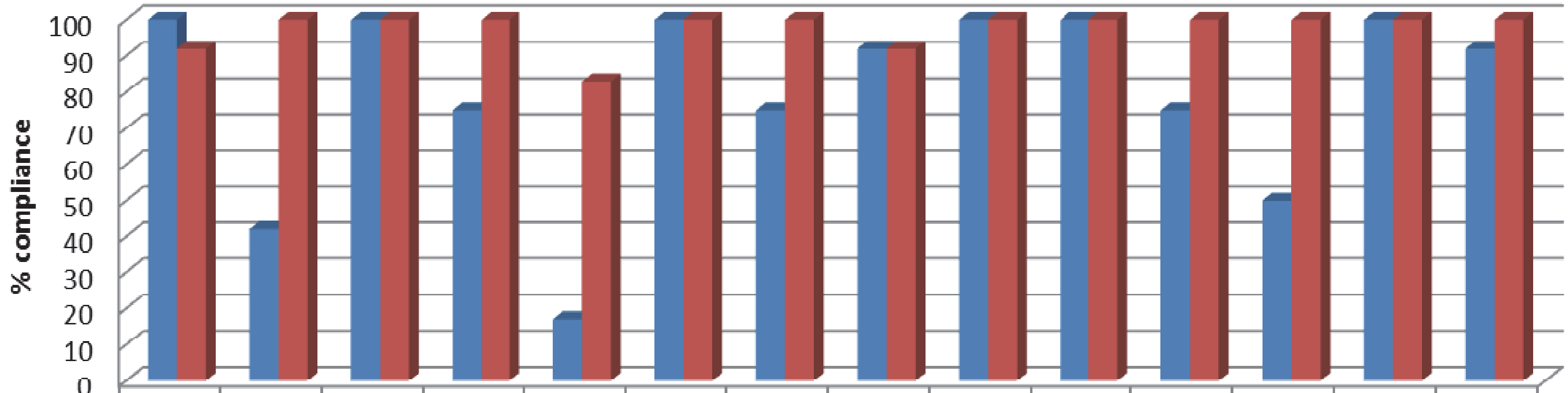
Figure 4.24: Chemical Oxygen Demand levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between September 2012 and February 2013



	FE	DP	FE	FE	DP	FE	FE	FE	DP	FE	FE	FE	FE	FE	FE	DP	FE	FE	DP
	AL		AM	DM		EB	FB	KE		KM	MDT	QT	RE	SC	ST		WT	ZW	
Mar-13	18	28	5	54	42	291	150	119	116	180	386	181	25	92	225	148	32	479	126
Apr-13	30	45	59	88	68	756	79	102	156	167	639	409	93	214	27	11	375	320	117
May-13	30	45	163	47	70	400	79	268	236	91	462	437	102	316	153	141	471	379	341
Jun-13	165	73	173	13	40	281	165	177	170	83	315	87	150	367	103	120	122	484	433
Jul-13	368	332	199	22	47	65	359	215	202		61	33	25	62	135	131	55	46	68
Aug-13	69		211	34	77	143	86	207	222	445	146	523	34	68	63	78	279	144	61

Key: AL (Alice); AM (Amalinda); DM (Dimbaza); EB (East Bank); FB (Fort Beaufort); KE (Keiskammahoek); KM (Komga); MDT (Mdantsane); QT (Queenstown); RE (Reeston); SC (Schornville); ST (Stutterheim); WT (Whittlesea); ZW (Zwelitsha); FE (final effluent); DP (discharge point).

Figure 4.25: Chemical Oxygen Demand levels of wastewater effluents of 14 selected WWTP in the Eastern Cape Province between March 2013 and August 2013



	Alice	Amalinda	Dimbaza	East Bank	Fort Beaufort	Keiskammahoek	Komga	Mdantsane	Reeston	Whittlesea	Zwelitsha	Queenstown	Schorneville	stutterrheim
DO	100	42	100	75	17	100	75	92	100	100	75	50	100	92
BOD	92	100	100	100	83	100	100	92	100	100	100	100	100	100

Figure 4.26: Level of compliance of the WWTP' effluents to set guideline for DO and BOD

4.4.1 pH

The 14 WWTP effluents were compliant with the set regulatory guideline values for pH (Table 4.1).

4.4.2 DO and BOD

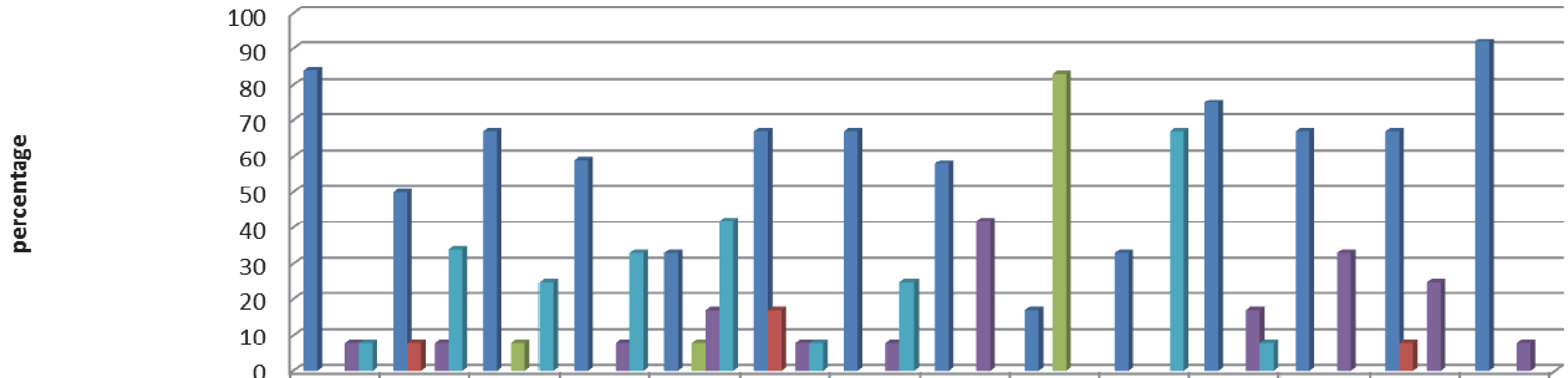
With the exception of effluents from Amalinda, Fort Beaufort and Queenstown WWTPs, effluents compliance to set guidelines for both DO and BOD was higher than 50% indicating the efficiency of the WWTPs in removing organic matter from the effluents. The oxygen balances of the receiving water bodies are therefore unlikely to be altered by the discharge of these effluents. This is further helped by the fact that nutrient concentrations, especially nitrates and nitrites were also compliant to set guidelines for the prevention of eutrophication. While the set guideline for phosphate concentrations in wastewater effluents is ≤ 10 mg/l, concentrations of between 10 and 15 mg/l were treated as marginally non-compliant (in this study) and unlikely to cause eutrophication owing to dilution, especially in rivers with high flow volumes.

4.4.3 Phosphate

Taking phosphate concentrations of > 15 mg/l to be overtly non-compliant, effluents from Amalinda WWTP showed a compliance quotient of 75%; those from Keiskammahoek and Komga WWTPs were 92% compliant while the rest were 100% compliant (Figure 4.27). Judging from these findings, these WWTPs are efficient in nutrient removal and the discharged effluents are unlikely to cause eutrophication in receiving water bodies.

4.4.4 Free Residual Chlorine

The concentrations of free residual chlorine for the 12 months sampling period for all the 14 WWTPs were categorised into compliant (0.15-0.4 mg/l), marginal under-dose (0.1 to ≤ 0.14 mg/l), extreme under-dose (< 0.1 mg/l), marginal overdose (0.5-0.9 mg/l) and extreme overdose (≥ 1 mg/l) in relation to the regulation guideline of 0.25 mg/l (Table 4.1). Results were expressed as percentages and are presented in Figure 4.26. With the exception of the Fort Beaufort, Reeston and Whittlesea WWTPs, the rest of the plants chlorinated their effluents to compliant levels for ≥ 50% of the times. Reeston WWTP recorded the highest incidences (83%) of extreme chlorine overdose in its effluents, a result that mirrors on the results of the bacteriological analysis of its effluents (Figures 5.4, 5.6, 5.8 and 5.9). While the results of bacteriological analysis for this plant were "outstanding", the effects of chlorine overdosing have both ecological and economic implications as already discussed above. Cases of chlorine extreme under-dosing were also rampant with 50% of the plants applying substandard dosages of chlorine for > 20% of the times. Fort Beaufort WWTP (42%) and Amalinda WWTP (33%) are some of the plants whose under-dosing regimens had a counter effect in their bacteriological results (Chapter 5). However, cases of chlorine overdose were not as rampant as those of chlorine under-dose. When workers at these WWTPs were asked about their chlorine dosing methods they indicated that the amount they put depends on the volume of influent and most importantly, that they use manual methods of chlorine application, basing on experience.



	Alice	Amalinda	Dimbaza	East Bank	Fort Beaufort	Keiskammahoek	Komga	Mdantsane	Reeston	Whittlesea	Zwelitsha	Queenstown	Schornville	stutterheim
% compliant	84	50	67	59	33	67	67	58	17	33	75	67	67	92
% marginal overdose		8				17							8	
% extreme overdose			8		8				83					
% marginal underdose	8	8		8	17	8	8	42			17	33	25	8
% extreme underdose	8	34	25	33	42	8	25			67	8			

Figure 4.27: Percentage chlorine compliance of the effluents from the 14 WWTPs under study

4.4.5 Temperature

The effluents of all 14 WWTP were compliant with the set regulatory guidance Value (Table 4.1).

4.6 Conclusion

The levels of temperature, nitrates, nitrites, phosphates BOD, EC, pH and TDS were largely compliant to set guidelines (shown in Table 4.1), and where non-compliance was observed, the overshoot was mostly marginal. WWTP with high margins of non-compliance with respect to DO may need to make sure that their aerators are optimally functional. The observed cases of extreme chlorine over-dosing, the most severe of which were observed at Reeston WWTP, and under-dosing, recorded in 50% of the plants, suggests the need to put in place automated mechanisms for chlorination of effluents as part of the disinfection process. We conclude therefore, that with respect to physicochemical parameters, the WWTPs performed satisfactorily and produced effluents of acceptable standards for discharge into freshwater ecosystems without upsetting their nutrient balance. These effluents can potentially be used for irrigation without increasing the salinity of the soils.

CHAPTER FIVE : BACTERIOLOGICAL ANALYSIS

5.1 Introduction

Wastewater treatment plants (WWTPs) can, depending on their treatment technologies, maintenances and technical abilities of the workers, be very effective in removal or reduction of microbial loads. However, it is not possible for the microbial quality of the effluents to match the microbial quality of the water in the receiving water bodies. Discharge of effluents will therefore, despite the level of treatment, potentially alter the microbial content of the receiving water bodies (Drury *et al*., 2013). This is especially the case in highly urbanised societies which tend to have numerous large WWTPs whose effluent discharges end up contributing significant portions of the flow of receiving riverine systems. One good example is the Chicago Area Waterway System (which includes all segments of the Chicago River as well as the North Shore Channel) whose annual flow comprises more than 70% of treated municipal wastewater effluent (Illinois Department of Natural Resources, 2011). Routine analysis of the microbial quality of treated wastewater effluents is therefore warranted in order to maintain the microbial load of receiving water bodies within acceptable limits for both human use and lotic ecosystems survival. In this chapter we report on the analysis and findings of the bacteriological qualities of the final effluents of 14 WWTPs around the Eastern Cape Province.

5.2 Sampling and Analytical Procedures

Wastewater samples for bacteriological analysis were collected in sterile 2 litre polypropylene bottles in which 0.1% of a 3% (w/v) solution of sodium thiosulphate had been added. Samples were collected from the final effluent tanks and discharge points (where accessible) and transported to the Applied and Environmental Microbiology Research Group (AEMREG) laboratory in cooler boxes for analysis within a period of 6 h after collection. During analysis for all groups of bacteria studied, water samples were serially diluted and concentrated on nitrocellulose membrane filters (0.45 um pore size, Millipore) by passing 100 ml[6] of each dilution through the filter using the membrane filtration method (Figure 5.1) as recommended by Standard Methods (2005).

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[6]WHO(1997) recommended 100 ml and 10-100 ml as typical volumes for microbiological analyses for treated water and partially treated water when using the membrane filtration technique. The final effluents under study are disinfected and as such considered as treated.
WHO (1997). Water sampling and analysis. In: Surveillance and control of community supply. Guidelines for drinking water quality.

a b

Figure 5.1: (a) Equipment setup for the membrane filtration assay and (b) A student uses the membrane filtration technique to process wastewater samples for bacteriological analysis

For the enumeration of faecal coliforms, the filters were then placed on mFC agar using sterile forceps and incubated at 44.5^{o}C for 24 h. Colonies that exhibited any shades of blue were counted and reported as CFU/100 ml of water. For *E. coli* counts, the filters were placed onto *E. coli*-Coliform chromogenic agar and incubated at 35^{o}C for 18-24 h. Blue-dark violet colonies were enumerated and reported as CFU/100 ml of water. Presumptive *E. coli* isolates were recovered from these plates, purified and preserved in 20% glycerol at -80^{o}C until ready for pathotypes characterisation. Presumptive *E. coli* O157:H7 counts were done using *E. coli* O157:H7 chromogenic agar and confirmation was done using the *E. coli* O157 latex test reagent kit (Pro-Lab Diagnostics). For *Vibrio* counts, the filters were placed onto thiosulphate citrate bile salts sucrose agar (TCBS agar). For the purposes of quality control, the spread plate technique was also employed where known (100 µl) volumes of effluent samples were spread on TCBS agar as previously described by Igbinosa *et al*. (2011). Presumptive Vibrio were isolated from the plates, purified and subjected to Gram staining and oxidase test. Only Gram-negative, oxidase positive isolates were selected and preserved in 20% glycerol at -80^{o}C until ready for further analysis.

5.3 Results and Discussion

Counts for faecal coliforms, presumptive *E. coli* and presumptive *Vibrio* ranged variably from 0 to 10^{5} cfu/100 ml. Figure 5.2 shows the appearance of the colonies of each of these groups of bacteria on their respective differential media.

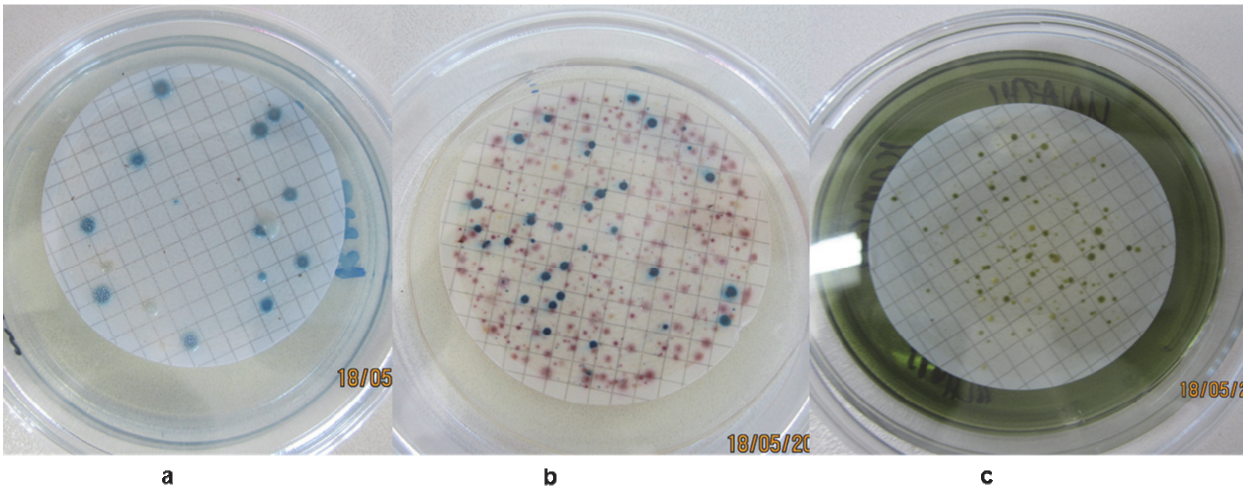

Figure 5.2: Appearance of colonies of (a) faecal coliforms on mFC agar (b) presumptive *E. coli* (blue colonies) on *E. coli*-Coliform chromogenic agar and (c) presumptive *Vibrio* on TCBS agar

Only 46% of the plants were positive for presumptive *E. coli* O157:H7 at densities of zero to the order of 10^5 cfu/100 ml. However, confirmation tests done using the *E. coli* O157 latex test reagent kit (Pro-Lab Diagnostics) revealed that the presumptions were wrong as none of the presumptive *E. coli* O157:H7 were positive for the test.

Figures 5.3-5.8 present the detailed results for mean faecal coliform counts (cfu/100 ml), mean presumptive *E. coli* counts, and mean presumptive *Vibrio* counts respectively, for all the fourteen WWTPs studied

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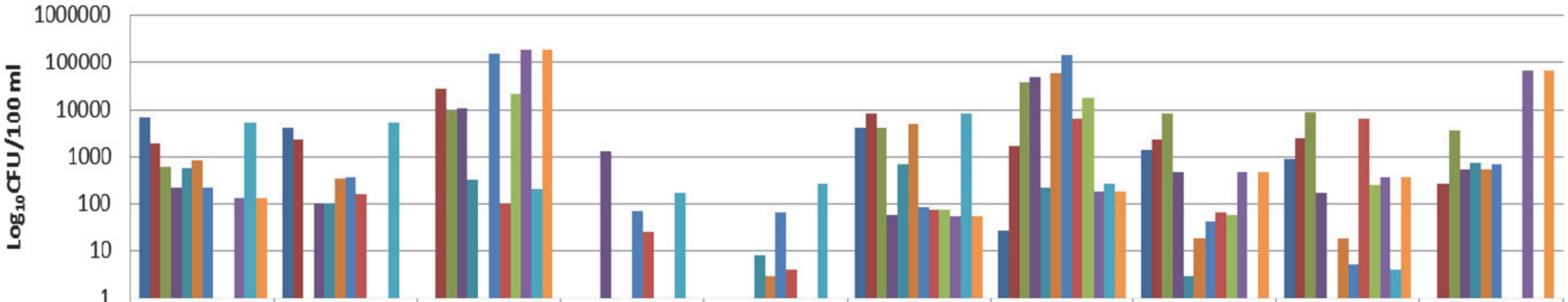
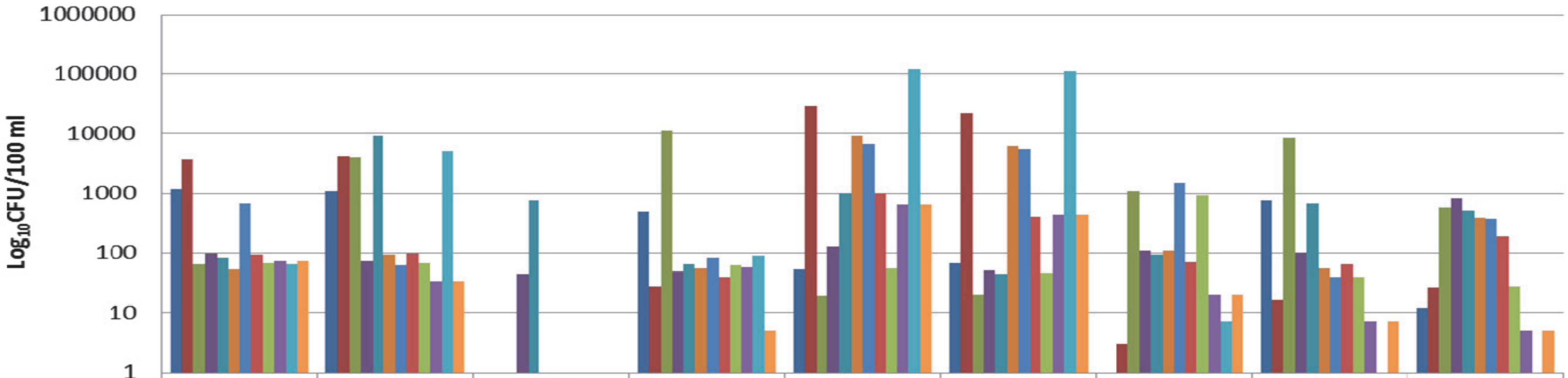


	Alice FE	Alice DP	Amalinda FE	Dimbaza FE	Dimbaza DP	EastBank FE	FortBeaufort FE	Keiskammahoek FE	Keiskammahoek DP	Komga FE
Sep-12	7100	3900	0	0	0	4000	27	1400	880	0
Oct-12	1900	2300	28000	1	0	8400	1700	2300	2500	260
Nov-12	600	0	9300	0	0	4000	37000	8500	9200	3500
Dec-12	217	103	11000	1310	0	58	46700	490	170	550
Jan-13	567	100	333	0	8	720	217	3	0	750
Feb-13	833	363	0	0	3	4800	56700	19	18	550
Mar-13	217	387	160000	71	66	82	148000	44	5	680
Apr-13	0	157	100	26	4	75	6630	68	6400	0
May-13	0	0	20900	0	0	74	17300	60	240	0
Jun-13	127	1	185000	0	0	55	175	480	380	64000
Jul-13	5070	4970	205	167	267	8200	253	1	4	0
Aug-13	127	1	185000	0	0	55	175	480	380	64000

Key: FE (final effluent); DP (discharge point).

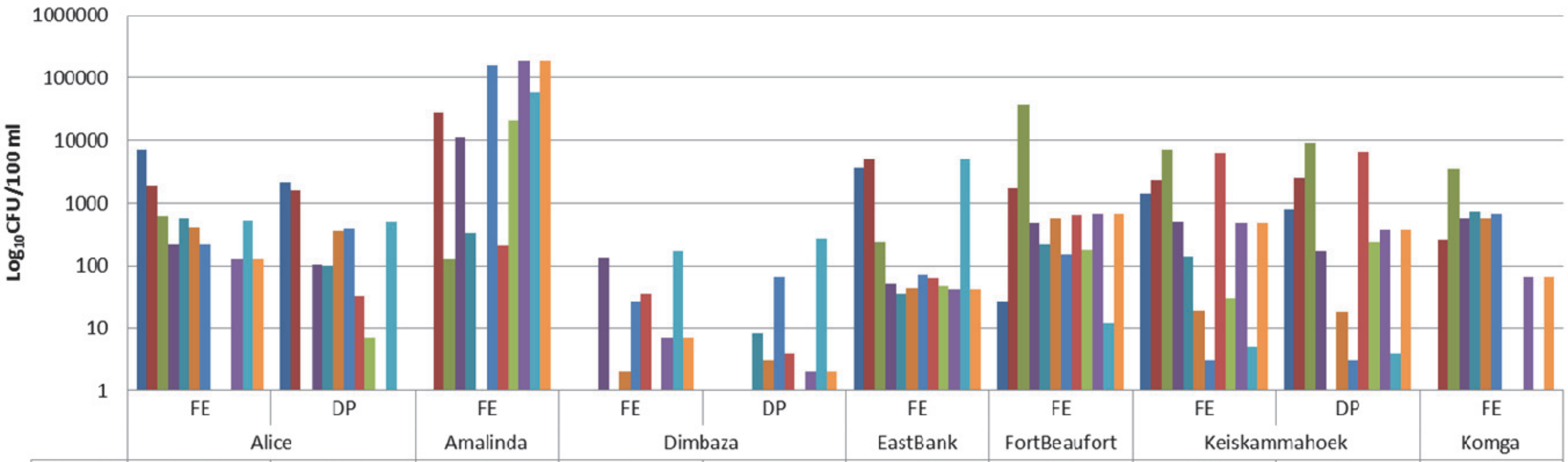
Figure 5.3: Mean faecal coliform counts for seven of the WWTP between September 2012 and August 2013



	FE Mdantsane	FE Queenstown	FE Reeston	FE Schorneville	FE Stutterrheim	DP Stutterrheim	FE Wittlesea	FE Zwelitsha	DP Zwelitsha
Sep-12	1200	1100	0	500	52	68	0	750	12
Oct-12	3700	4200	0	28	29000	22000	3	16	26
Nov-12	64	4000	0	11000	19	20	1100	8400	580
Dec-12	97	74	44	48	130	50	110	100	810
Jan-13	82	9000	740	64	1000	44	93	670	530
Feb-13	52	95	0	56	9100	6400	110	55	390
Mar-13	660	61	0	84	6900	5600	1500	40	370
Apr-13	93	96	0	39	1000	410	71	64	190
May-13	68	68	0	62	56	47	930	40	27
Jun-13	72	34	0	57	650	440	20	7	5
Jul-13	65	5200	0	91	120000	110000	7	0	0
Aug-13	72	34	0	5	650	440	20	7	5

Key: FE = final effluent, DP = discharge point

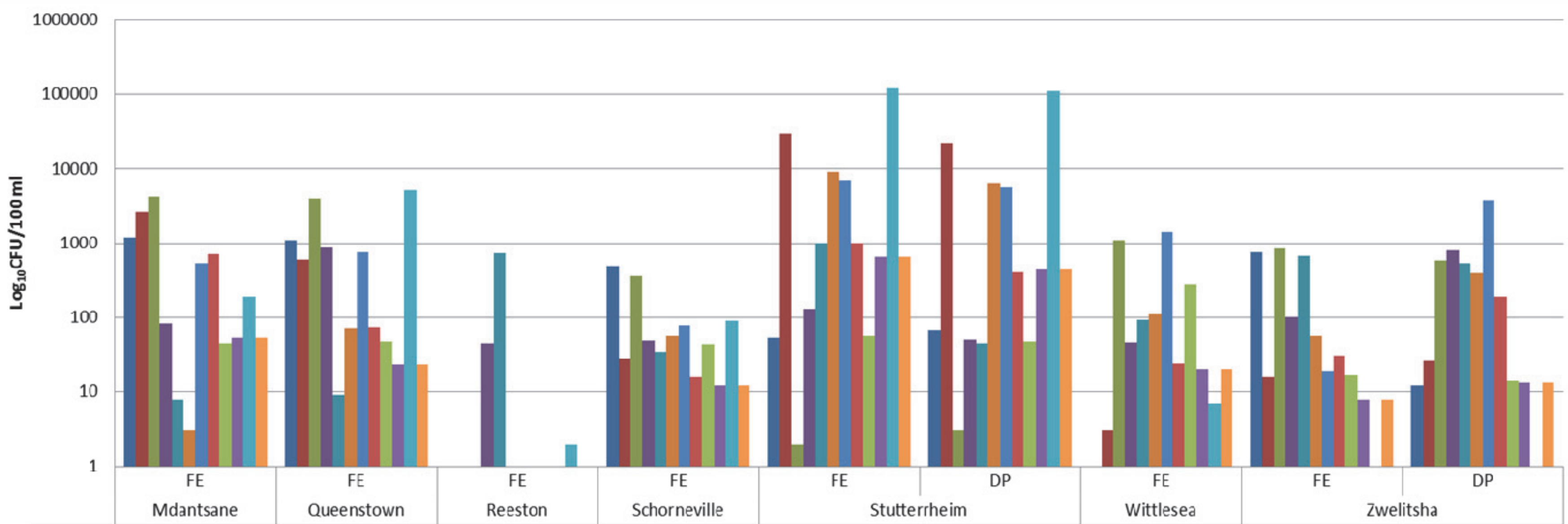
Figure 5.4: Mean faecal coliform counts for seven other WWTP between September 2012 and August 2013



	Alice FE	Alice DP	Amalinda FE	Dimbaza FE	Dimbaza DP	EastBank FE	FortBeaufort FE	Keiskammahoek FE	Keiskammahoek DP	Komga FE
Sep-12	7100	2200	0	1	0	3600	27	1400	810	0
Oct-12	1900	1600	28000	0	0	5100	1700	2300	2500	260
Nov-12	600	0	130	0	0	240	37000	7100	9200	3500
Dec-12	217	103	11000	131	0	52	467	490	170	550
Jan-13	567	100	333	0	8	35	217	140	1	750
Feb-13	403	363	0	2	3	43	567	19	18	550
Mar-13	217	387	160000	27	66	71	148	3	3	680
Apr-13	1	33	210	36	4	62	663	6400	6600	0
May-13	0	7	20900	0	1	47	173	30	240	0
Jun-13	127	1	185000	7	2	41	677	480	380	64
Jul-13	507	497	60000	167	267	5000	12	5	4	0
Aug-13	127	1	185000	7	2	41	677	480	380	64

Key: FE = final effluent, DP = discharge point

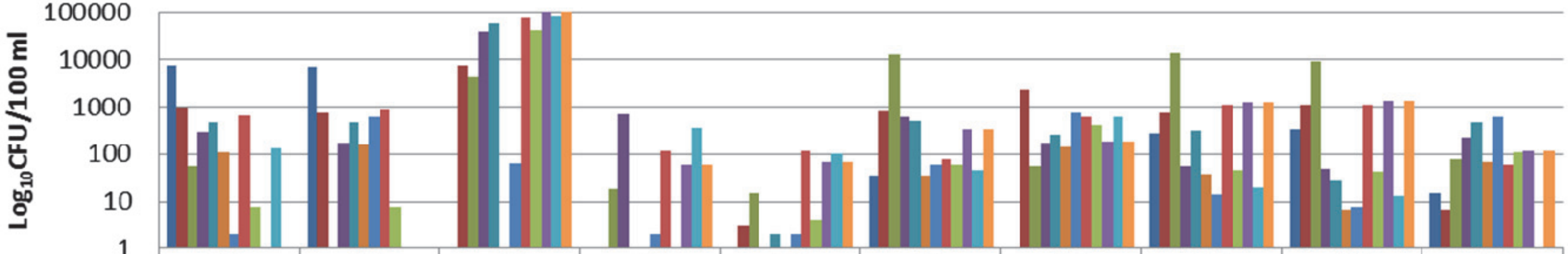
Figure 5.5: Presumptive *E. coli* counts for seven of the WWTP between September 2012 and August 2013



	FE Mdantsane	FE Queenstown	FE Reeston	FE Schorneville	FE Stutterrheim	DP Stutterrheim	FE Wittlesea	FE Zwelitsha	DP Zwelitsha
Sep-12	1200	1100	0	500	52	68	0	750	12
Oct-12	2600	600	0	28	29000	22000	3	16	26
Nov-12	4300	4000	0	360	2	3	1100	840	580
Dec-12	83	860	44	48	130	50	46	100	810
Jan-13	8	9	740	34	1000	44	93	670	530
Feb-13	3	72	0	56	9100	6400	110	55	390
Mar-13	530	750	0	78	6900	5600	1400	19	3700
Apr-13	720	73	0	16	1000	410	24	31	190
May-13	44	47	0	43	56	47	280	17	14
Jun-13	52	23	0	12	650	440	20	8	13
Jul-13	190	5200	2	91	120000	110000	7	0	0
Aug-13	52	23	0	12	650	440	20	8	13

Key: FE = final effluent, DP = discharge point

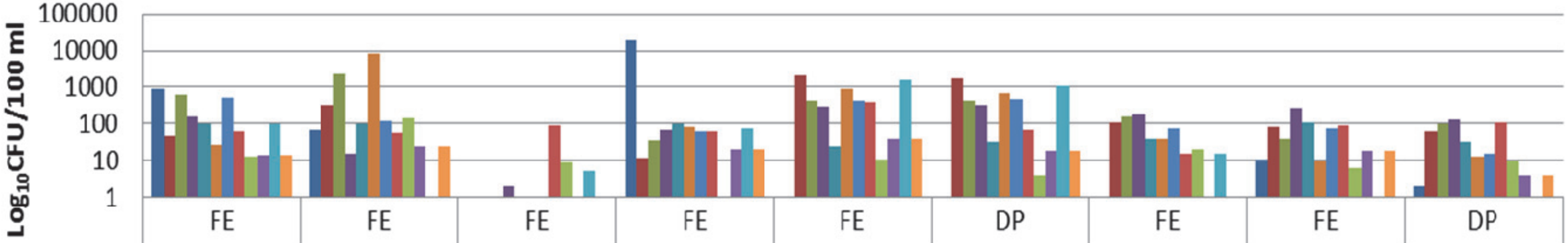
Figure 5.6: Presumptive *E. coli* counts for seven other WWTP between September 2012 and August 2013



	Alice FE	Alice DP	Amalinda FE	Dimbaza FE	Dimbaza DP	EastBank FE	FortBeaufort FE	Keiskammahoek FE	Keiskammahoek DP	Komga FE
Sep-12	7700	7300	0	1	1	36	0	270	350	15
Oct-12	950	730	7700	1	3	850	2300	770	1100	7
Nov-12	57	0	4600	18	15	13000	57	14000	9300	80
Dec-12	297	170	41000	700	0	610	177	56	51	223
Jan-13	467	467	60000	1	2	520	257	310	28	479
Feb-13	113	167	0	0	0	35	143	37	7	70
Mar-13	2	600	65	2	2	62	730	14	8	620
Apr-13	667	900	78300	118	121	77	600	1100	1100	62
May-13	8	8	43000	0	4	60	403	48	43	107
Jun-13	0	0	99300	59	68	350	190	1300	1400	114
Jul-13	130	0	84000	367	100	45	597	20	13	0
Aug-13	0	0	99300	59	68	350	190	1300	1400	114

Key: FE = final effluent, DP = discharge point

Figure 5.7: Presumptive *Vibrio* counts for seven of the WWTP between September 2012 and August 2013



	Mdantsane FE	Queenstown FE	Reeston FE	Schorneville FE	Stutterrheim FE	Stutterrheim DP	Wittlesea FE	Zwelitsha FE	Zwelitsha DP
Sep-12	920	68	0	18400	0	0	0	11	2
Oct-12	48	340	0	12	2100	1700	110	84	65
Nov-12	620	2300	1	35	450	450	160	38	100
Dec-12	160	15	2	70	310	320	190	263	134
Jan-13	100	96	1	99	24	33	40	107	32
Feb-13	26	8300	0	84	930	720	39	10	13
Mar-13	520	124	0	61	440	470	72	74	16
Apr-13	60	58	87	61	400	66	16	92	107
May-13	13	148	9	0	11	4	21	6	10
Jun-13	14	25	0	21	41	18	0	19	4
Jul-13	95	1	5	74	1600	1100	16	0	0
Aug-13	14	25	0	21	41	18	0	19	4

Key: FE = faecal indicator, DP = discharge point

Figure 5.8: Presumptive *Vibrio* counts for seven other WWTP between September 2012 and August 2013

The proportion of WWTPs whose samples exceeded the 1000 cfu/100 ml set guideline for faecal coliforms in the final effluent of WWTP as set by DWAF (1984) was 35% in September 2012, 50% in October and November 2012 respectively, 36% in December 2012, 21% in January 2013, 36% in February and March 2013 respectively, 29% in April 2013, 36% in May 2013, 29% in June 2013, 36% in July and 57% in August 2013. Figure 5.9 gives a summary of the percentage compliance of each WWTP to the 1000 CFU/100 ml guideline for faecal coliforms in wastewater effluents as obtained in this study and further compares those values with the Green Drop 2012 values for microbiological compliance.

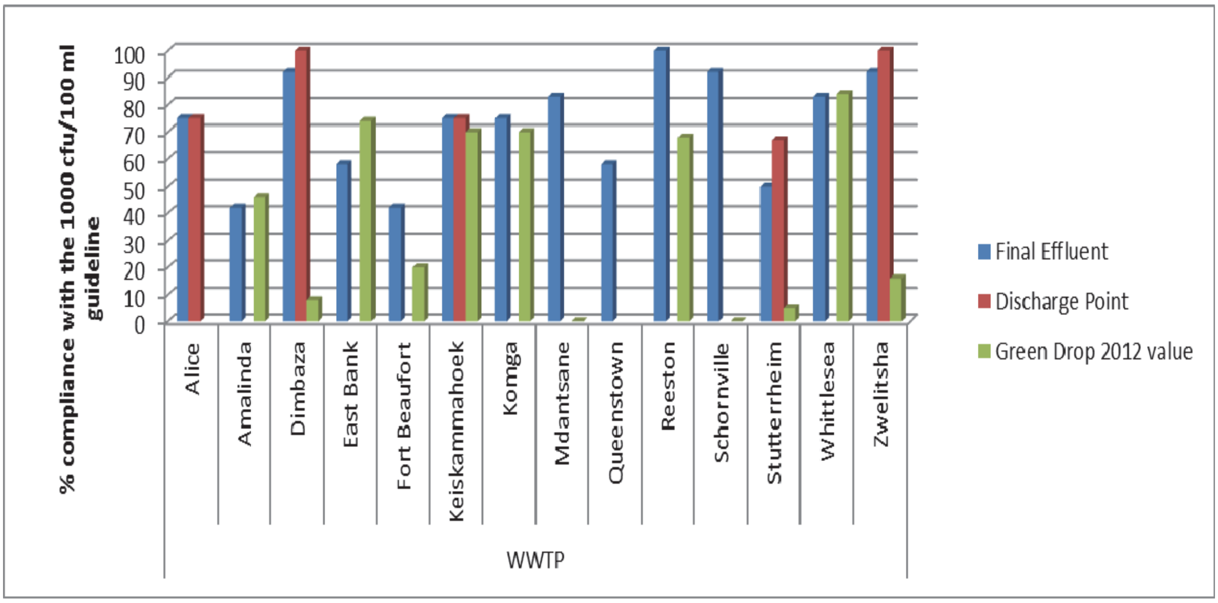


Figure 5.9: Effluents percentage compliance to the 1000 CFU/100 ml guideline for faecal coliforms

On the average, 86% of the WWTPs had a compliance rate of ≥ 50% to the faecal coliform guideline of 1000 CFU/100 ml in their effluents. This contrasts with the Green Drop 2012 average compliance value of 36% for the selected WWTPs. This could be indicative of either an improvement in the microbiological compliance of these WWTPs or the differences in the methodologies employed by the project team (this study; Membrane Filtration Method) versus those of the municipalities in their microbiological analysis. The Amathole District Municipality has enlisted the services of Amatola Water for the microbiological analysis of their wastewater effluent samples and this service provider uses the Colilert Method (which uses the most probable number [MPN] method) for microbiological analysis. It is, however, difficult to judge, basing on the separate findings of Amatola Water and the project team, which were equally done in separate time frames and on different sets of samples, to make a conclusion as to which of the two methods is preferred. There is need, therefore, for the two methods to be used to simultaneously analyse samples to improve reliability of data. The following were of particular note: Schornville WWTP had a compliance score of 92% (this study) against a score of 0% (Green Drop 2012); Dimbaza WWTP 100% (this study) against 8% (Green Drop 2012); Stutterheim WWTP 67% (this study) against 5% (Green Drop 2012); Zwelitsha WWTP 100% (this study) against 16% (Green Drop 2012) and Mdantsane WWTP 83% (this study) against 0% (Green Drop 2012). With the exception of Alice and Queenstown WWTPs for which information was not submitted to WISA for the compilation of the Green Drop 2012 report, comparable results were obtained for the remainder of the WWTPs.

Cases of chlorine under-dosing were observed among the 14% poorly performing WWTPs, with free chlorine levels as low as 0.09 mg/ml being recorded at the East Bank (EB) WWTP for three months running (September 2012-November 2012). Very low chlorine levels (0.05 mg/ml) were also recorded at the Amalinda (AM) WWTP for two months running (November-December 2012). It was also observed that WWTPs which had performed poorly during the first six month sampling period (September 2012-February 2013) had a dramatic turnaround in the last six months of the sampling where some plants with previously high coliform counts came down as low as zero counts for consecutive months running. The high incidences of indicator bacteria in the final effluents of most of the plants are worrisome as it suggests the possible presence of enteric pathogens. Of significant note was the high prevalence of presumptive *Vibrio* and *E. coli* in the final effluents and in some cases in effluents with adequate chlorine dosing.

In the face of climatic change and increasing water scarcity, issues of both water quantity and quality are of concern. The reuse of wastewater is one of the main options being considered as a new source of water in regions where water is scarce (Blumenthal *et al*., 2000). Guidelines have therefore been set to regulate use of wastewater for various purposes which might expose the public or even workers, to health risks. For restricted irrigation for example, guidelines for exposure to faecal coliform bacteria have been put in place to protect farmworkers, their children and nearby populations from enteric viral and bacterial infections (Blumenthal *et al*., 2000). In setting the appropriate guidelines, irrigation methods used and exposed groups of people, is taken into consideration. Data from epidemiological studies in Israel (Shuval *et al*., 1989) and the USA (Camann *et al*., 1986) on situations in which spray or sprinkler irrigation was used suggest that a level of $\leq 10^5$ faecal coliform bacteria/100 ml would protect both farmworkers and the nearby population from infection transmitted through direct contact or aerosols from wastewater. If this guideline ($\leq 10^5$) were to be applied to effluents of WWTPs in this study, then almost all effluents, except those from the Amalinda WWTP, would be fit for sprinkler irrigation use.

However, a reduced guideline of $\leq 10^3$ faecal coliform bacteria/100 ml has been set (WHO, 1989) when adult farmworkers are engaged in flood or furrow irrigation and when children under age 15 are regularly exposed through work or play. Again, if this guideline were to be used to determine the suitability of effluents in this study for irrigation use, effluents from all WWTPs under this study, with the exception of the Amalinda and Fort Beaufort WWTPs, would qualify for use on ≥ 60% of the cases. WWTPs whose performance surpassed others in this regard were Reeston WWTP (whose final effluents had less than 10^3 faecal coliform bacteria/100 ml throughout the study period), Dimbaza (both final effluent and discharge point samples had less than 10^3 faecal coliform bacteria/100 ml throughout the study period) as well as Zwelitsha final effluent samples which also had less than 10^3 faecal coliform bacteria/100 ml throughout the study period. DWAF (1984) set a guideline of 0 *E. coli* counts/100 ml for treated wastewater effluents. Using this guideline, only effluents from the Reeston and Dimbaza WWTPs make the grade, implying that of all the WWTPs under this study, these two surpass the rest. While this guideline uses a no detectable risk approach (total protection of the public from any risk arising from exposure to wastewater effluents), it is in many cases unachievable and makes wastewater treatment technology quiet expensive, especially for developing countries whose resources are thin. Also, effluents from WWTPs which meet this guideline tend to be high in salinity and therefore less preferred by farmers for use in irrigation.

Besides faecal coliform bacteria or bacterial pathogens such as *Vibrio*, *E. coli* and *Salmonella* among others, wastewater plays a major role in the environmental transmission of protozoan pathogens such as *Giardia*, *Cryptosporidium* and *Cyclospora* (Robertson, 1999). Research shows that protozoan (oo)cysts are not effectively removed by conventional wastewater treatment processes, with reported efficiencies varying from 26-100% (Robertson, 1999; Bukhari *et al*., 1997). In addition, human oral challenge studies have shown that the median infectious dose for *Giardia* is between 10 and 100 cysts and for *Cryptosporidium* between 30 and 1000 (oo)cysts (Cooper, 1998).

Statistical analysis using Minitab 16 shows that effluents from the Amalinda WWTP had significantly higher faecal coliform counts than the rest of the WWTPs (P < 0.05). The statistical output is displayed below.

```
                              Individual 95% CIs For Mean Based on
                                 Pooled StDev
Level          N   Mean  StDev   ----+---------+---------+--------+-----
Alice         12    866   2174   (----*---)
Amalinda      12  43105  40251                               (---*---)
Dimbaza       12    111    213   (---*---)
East Bank     12   1333   3684    (---*---)
Fort Beaufort 12    470    621   (---*----)
Keiskammahoek 12   1602   3938    (---*---)
Komga         11    172    198   (---*----)
Mdantsane     12    216    301   (---*---)
Queenstown    12    958   2399   (----*---)
Reeston       12      9     25   (---*---)
Schornville   12   1578   5298    (---*---)
Stutterheim   12    529    683   (---*----)
Whittlesea    11     60     66   (---*---)
Zwelitsha     12     60     74   (---*---)
                                 ----+---------+---------+--------+-----
                                     0     15000     30000     45000
```

As with faecal coliforms, statistical (Minitab 16) comparisons of the mean presumptive *Vibrio* and presumptive *E. coli* densities in the wastewater effluents also showed that effluents from the Amalinda WWTP had significantly higher counts (P = 0.00) than those from the rest of the WWTPs, for both groups of bacteria.

Results for presumptive *E. coli* O157:H7 were not subjected to statistical scrutiny owing to the fact that none of the presumptive isolates was confirmed to be O157:H7 when subjected to serum agglutination tests. These results point to the inadequacy of the methodologies that were employed for isolation of *E. coli* O157:H7 in this study. Much more accurate methods are required. This also highlights the importance of not making any conclusions based on presumptive microbiological results as it may cause undue panic to the public.

An independent samples T Test (IBM SPSS version 20) comparison of mean faecal coliform bacteria counts from the discharge point samples with the mean faecal coliform bacteria counts from the final effluent samples (of all WWTPs) showed no significant differences ($P > 0.05$) between the bacteriological qualities of the final effluent and discharge point samples. This is despite observed higher compliance percentages in discharge point samples as compared to final effluent samples (Figure 5.9).

5.3.1 Bacteriological characterisation: confirmation, pathotyping and antibiogram analyses.

Three hundred confirmed *Vibrio* isolates, randomly selected from a pool of 668 confirmed Vibrio isolates taken from across all sampling sites, were characterised into three pathotypes. Of these, 11.6% (35) were confirmed to be *V. parahaemolyticus*, 28.6% (86) were confirmed to be *V. fluvialis*, 28% (84) were confirmed to be *V. vulnificus* while 31.8% (95) belonged to other *Vibrio* spp. not assayed for in this study. When these confirmed pathotypes were subjected to antibiogram profiling, the outcome was as articulated in Table 5.1 below.

Table 5.1: Antibiogram profiling results for three *Vibrio* spp.

Antibiotic	***V. fluvialis* (n = 35)**			***V. parahaemolyticus* (n = 86)**			***V. vulnificus* (n = 84)**		
	S (%)	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**
imipenem	100	0	0	100	0	0	100	0	0
nalidic acid	40	40	20	90	0	10	71	14	14.1
erythromycin	10	0	90	0	0	100	15	14	71
sulfamethazole	0	0	100	0	12.5	87.5	14.2	0	85.7
cefuroxime	60	0	40	75	0	25	64	7.1	28.5
penicillin g	0	10	90	12.5	0	87.5	0	0	100
chloramphenicol	10	0	90	75	0	2.5	92	0	7.1
polymixin b	0	0	100	12.5	0	87.5	0	0	100
Trimethroprim & sulfamethazole	40	0	60	100	0	0	90	0	10
tetracycline	0	0	100	0	0	100	0	0	100
gentamicin	100	0	0	100	0	0	100	0	0
meropenem	100	0	0	100	0	0	100	0	0
trimethoprim	10	0	90	86	0	100	10	0	90

About 50% and above of the all three *Vibrio* species categories showed susceptibilities against gentamycin, cerufoxime and imipenem. Multiple antibiotic resistance patterns were also evident especially against such antibiotics as Tetracyclin, Polymixin B, Chloramphenicol, Penicillin B, Sulfamethazole and Erythromycin against which prevalence of over 60% of the bacteria were resistant.

Equally, a randomised sample of 600 isolates confirmed to be *E. coli* by PCR was first characterised into *E. coli* pathotypes which were subsequently subjected to antibiogram profiling. Of the seven *E. coli* pathotypes assayed for, only three were detected and these include enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC) and uropathogenic *E. coli* (UPEC). Antibiogram results are shown in Table 5.2.

Table 5.2: Antibiogram profiling results for *E. coli* pathotypes

Antibiotic	Pathotype								
	EPEC (n = 7)			EAEC (n = 16)			UPEC (n =23)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Streptomycin 10 µg	80	0	20	100	0	0	100	0	0
Ciprofloxacin 5 µg	80	0	20	100	0	0	100	0	0
Ampicillin 25 µg	14	14	72	44	0	56	22	8	70
Chloramphenicol 10 µg	60	0	40	40	60	0	100	0	0
Meropenem 10 µg	100	0	0	100	0	0	100	0	0
Cefuroxime 30 µg	100	0	0	60	40	0	100	0	0
Norfloxacin 10 µg	80	0	20	100	0	0	100	0	0
Gentamycin 120 µg	100	0	0	100	0	0	83	0	17
Imipenem 10 µg	100	0	0	100	0	0	100	0	0
Amikacin 30 µg	100	0	0	100	0	0	100	0	0
Tetracycline 30 µg	30	0	70	44	0	56	30	0	70
Cefatoxime 30 µg	100	0	0	100	0	0	100	0	0
Nalidixic acid 30 µg	60	20	20	80	20	0	100	0	0
Polymyxin B 300 Units	100	0	0	80	0	20	100	0	0
Sulphamethoxazole 25 µg	0	0	100	20	0	80	0	0	100
Colistin Sulphate 10 µg	100	0	0	80	0	20	100	0	0
Cephalexin 30 µg	20	80	20	40	20	40	100	0	0
Nitrofurantoin 300 µg	100	0	0	80	0	20	100	0	0

While prevalence of resistance of *E. coli* pathotypes to the test antimicrobials was remarkably lower than what was observed for *Vibrio* pathotypes, over 50% of the *E. coli* pathotypes were resistance against sulfamethazol, tetracycline and ampicillin. These findings concur with the findings of Byarugaba (2004) who reported that water is an important source for human infections with antimicrobial resistant bacteria. Also, James *et al*. (2003) and Okoh *et al*. (2007) reported that that wastewater effluents, treated or untreated, are a veritable source of enteric bacteria in aquatic environments. The release of pathogenic enteric micro-organisms into aquatic environments can be a source of disease when water is used for drinking, recreational activities or irrigation (Atieno *et al*., 2013). The public health risk is increased if the pathogenic enteric bacteria present in wastewater effluents (and hence in receiving water sources) are antibiotic resistant because of the reduced efficacy of antibiotic treatment against human diseases caused by such bacteria (Tendencia and De la Pena, 2002; Wenzel and Edmond, 2009). Baine *et al*. (1977) reported that a large waterborne outbreak involving R+ bacteria (bacteria with R factors for antibiotic resistant gene transfer) led to a large number of deaths in Mexico, partly due to the failure of the patients to respond to antibiotics of choice. The New York Times (Tue 17 Sep 2013) quoted Centre for Disease Control (CDC) officials as having reported that at least 2 million Americans fall ill from antimicrobial-resistant bacteria every year and that at least 23 000 die from those infections. The paper reported that one particularly lethal type of drug-resistant bacteria, known as CRE (carbapenem resistant *Enterobacteriacea*), has become resistant to nearly all antimicrobials on the US market, further stating that though still relatively rare, CRE causes about 600 deaths a year in the US alone. Should the proliferation of antimicrobial resistant organisms be allowed to go unchecked, society will return to a time when people died from ordinary infections. This point is further buttressed by Torrice (Undated), in an article entitled “Multidrug Resistance Gene Released by Chinese WWTP”, where he wrote;

"*In recent years, increasing numbers of patients worldwide have contracted severe bacterial infections that are untreatable by most available antibiotics. Some of the gravest of these infections are caused by bacteria carrying genes that confer resistance to a broad class of antibiotics called beta-lactams, many of which are treatments of last resort. Now a research team reports that some wastewater treatment plants in China discharge one of these potent resistance genes into the environment. Environmental and public health experts worry that this discharge could promote the spread of resistance*."

There is also the possibility of antibiotic resistance genes being transmitted to autochthonous bacteria if such genes are carried by transferable and mobile genetic elements such as plasmids, thus contributing to the spread of antimicrobial resistance (Sayah *et al*., 2005). Development of drug resistance may be caused by the occurrence of antimicrobial agents at low concentration both in human bodies by continued usage and also in the wastewater matrix via leaching. The correlation between antimicrobial use and antibiotic resistance of commensal bacteria has been documented (Van den Bogaard and Stobberingh, 2000). We can assume therefore, that the extent to which bacterial isolates are exposed to antibiotics before their release in the environment could be one of the reasons for the levels of antibiotic resistance shown by *Vibrio* isolates in this study. While hospitals and other health care facilities have taken steps to prevent drug-resistant infections (New York Times, Tue 17 Sep 2013), less is known about preventing infections outside hospitals, especially once those organisms find their ways into the environment.

5.4 Conclusion

Based on the outcome of this and other studies (Silva *et al*., 2006; Andersen *et al*., 1993; Mezrioui and Baleux, 1994), we conclude that WWTPs constitute important reservoirs of enteric bacteria which carry potentially transferable resistance genes which are aided by a large concentration of possible donor and recipient bacteria of transferable antibiotic resistance determinants and availability of nutrients in the wastewater matrix. In view of the fact that antibiotic resistance is to a greater extent mediated by genetic determinants, and considering that there are no guidelines for antibiotic resistance determinants in water, we propose the need for establishing set guideline at least for free nucleic acids in water.

CHAPTER SIX : VIROLOGICAL ANALYSIS

6.1 Introduction

Infectious enteric viruses originating from human faeces can be spread to rivers, lakes and oceans via the discharge of wastewater effluents because of their resistance to most wastewater treatment technologies (Hot *et al*., 2003; Gerba *et al*., 1996). Therefore, monitoring the occurrence of enteric viruses in wastewater effluents may prove a suitable tool to study the circulating viruses in certain communities and the persistence of such viruses in treated effluents (La Rosa *et al*., 2010), of which information can be useful for epidemiological surveys and microbial risk assessments. While studies elsewhere (Haramoto *et al*., 2006; van den Berg *et al*., 2005) have demonstrated that enteric viruses persist in high levels in treated wastewater effluents, no such data exists for wastewater effluents in the Eastern Cape Province of South Africa. We hereby report on the quantitative detection of adenoviruses, hepatitis A virus, rotaviruses and enteroviruses in wastewater effluents from 14 WWTP in the Eastern Cape Province.

6.2 Concentration of Viruses in Water

Viruses in the effluent samples were concentrated following the adsorption-elution method as described by Haramoto *et al*. (2005), with some modifications. This method is based on electrostatic interactions. Under neutral pH conditions, viruses are negatively charged but are positively charged under acidic conditions. Multivalent cations (Mg^{2+}, Al^{3+}) can change the surface charge of viruses thereby allowing adsorption to negatively charged membranes. Five millilitres of 250 mM $AlCl_3$ was passed through an HA filter (0.45 µm pore size and 47 mm diameter, Millipore) to form a cation (Al^{3+})-coated filter. Then, a 500 ml aliquot of the water sample was passed through the filter. A volume of 200 ml of 0.5 mM H_2SO_4 was then passed through the membrane to wash off the multivalent cations, leaving viral particles (now positively charged due to the addition of the acid) attached to the HA filter. Viral particles were then eluted by addition of 10 ml of 1 mM NaOH. Eluates were kept in tubes containing 0.1 ml of 50 mM H_2SO_4 and 0.1 ml of 100x Tris-EDTA (TE) buffer for neutralisation before further concentration. The concentrates/eluates were subjected to further concentration using a Centriprep YM-50 ultrafiltration device (Millipore) to obtain a final volume of approximately 700 µl. Further filtration and concentration of more water samples was done to have a final concentrate volume of about 2 ml. The concentrates were then pooled together per sample and stored at -80°C until ready for use.

6.3 Nucleic acid extraction and real-time PCR assays

Extraction of adenovirus (AdV) DNA was performed using commercial kits (Quick-*g*DNA MiniPrep, Zymo Research) according to the manufacturer's instructions. Purified viral DNA was eluted in 60 μL of DNase-free water. Quantification of AdV genomes was done using a StepOne Plus Real time PCR System (OPTIPLEX 755, Applied Biosystems) in a one-step reaction using 96-well microtiter plates. The wells were loaded with 20 μL of a reaction buffer containing 12.5 μL of 2 × *Taq*Man universal PCR MasterMix, 400nM forward primer, 400nM reverse primer, and 250nM TaqMan probe (Applied Biosystems) and PCR grade water. Five microliter (5 μL) aliquots of sample DNA were then added to the wells with mixing to give 25 μL total reaction mixtures. The amplification protocol included one cycle of 15 min at 95°C for *Taq* polymerase activation followed by 45 cycles of denaturation at 95°C for 10 s, annealing at 55°C for 30 s, and extension at 72°C for 20 s using the primers sets JTVX 5'-GGA CGC CTC GGA GTA CCT GAG-3' (forward primer), JTVX 5'-ACI GTG GGG GTT TCT GAA CTT GTT-3' (reverse primer) and 5'-FAM-CTG GTG CAG TTC GCC CGT GCC A-MGBNFQ -3' (probe). Data was collected at the extension step. To enable absolute quantification of the viral genomes, a standard curve was formulated as follows; DNA was extracted from an adenovirus ATCC positive strain (ATCC VR-930), the DNA was then quantified using a Qubit fluorometer (Invitrogen) and diluted by serial tenfold dilution. The sample extracts and standards' samples were subjected to real-time PCR simultaneously, followed by analysis using SDS software (Applied Biosystems) to obtain quantitative data on the titre of viral DNA in each well. Three wells each were used for the standard, no template control and samples, and the average used for subsequent calculations. The total number of viral genomes in the samples was calculated by multiplying the titre of viruses per 5 μL by the volumes of the samples.

6.4 Risk characterisation

Microbial risk assessment (MRA) procedure contains four steps which are: hazard identification, exposure assessment, dose-response assessment (probability of infection) and the risk characterisation step (Haas, 1996).

6.4.1 Hazard Identification

This study focuses on wastewater as a possible transmission route of waterborne disease. In that regard, the faecal-oral transmission of gastrointestinal pathogens is the main hazard emanating from inhalation of irrigation aerosol, ingestion of effluent irrigated crops, surface or sub-surface water that has been polluted by wastewater or its effluents or even the accidental ingestion of the wastewater itself. A major part of waterborne disease outbreak with unknown etiological agents is believed to be viral (Schwartzbrod, 1995). Gastrointestinal viral diseases usually have a shorter duration compared to bacterial and parasitic ones (Hedberg and Osterholm, 1993). Noroviruses are the most infectious causative agents of epidemic gastroenteritis (De Wit *et al*., 2001) with concentrations as low as 10 to $<10^4$ norovirus PCR-detectable units (PDU) being sufficient to cause infection, leading to gastrointestinal disease in two-thirds of the individuals infected (Lindesmith *et al*., 2003). They have previously been detected in raw urban sewage (Loisy *et al*., 2000; Lodder *et al*., 1999). Rotaviruses are the most common cause of diarrhoea in children though adults can also be infected, depending on their immune status (AWWA, 1999). The incubation period for rotaviral gastroenteritis is less than 48 hours with duration of illness of 5 to 8 days. Its symptoms usually include vomiting, diarrhoea and dehydration, but fever and respiratory problems can also occur (AWWA, 1999). Rotavirus has the highest infectivity of the waterborne viruses (Gerba *et al*., 1996). Maximum shedding of virions coincides with the third to fourth day of disease with excretion of as much as 10^{11} virions g^{-1} faeces (Faechem *et al*., 1983).

Adenovirus comes second, after rotavirus, as the most important viral pathogen of infantile gastroenteritis (Fong *et al*., 2010). Additionally, adenoviruses are associated with respiratory, urinary tract and eye infections (Fong and Lipp, 2005; WHO, 2011). At present, there are 51 known adenovirus serotypes which are classified into six species, species A to F (Metzgar *et al*., 2005; Fong and Lipp, 2005). Species F contains two fastidious enteric serotypes, 40 and 41, which constitute the majority of waterborne isolates and are among the leading causes of childhood diarrhoea (Tiemessen and Nel, 1996; WHO, 2011), although older children and adults may also be infected (Logan *et al*., 2006). Various research works have concluded that primary and secondary sewage treatment processes do not efficiently reduce the concentration of viruses (Fleischer *et al*., 2000; Hovi *et al*., 2001). Therefore, depending on the applied processes, treated sewage discharged onto surface waters may significantly enhance virus concentrations in receiving water bodies. In instances where the impacted water bodies serve as sources for drinking water, inadequate or failing treatment processes have led to the insufficient removal of viruses from source waters (Boccia *et al*., 2002). Adenovirus was used for the risk analysis assay in this study because of its ability to be transmitted via aerosols, in addition to the faecal oral route.

6.4.2 Exposure Assessment

The exposure assessment step determines the exposure routes, concentrations and distribution of the microorganisms. The dose of a pathogen is calculated from the density of the organism in the water times the volume ingested. Table 6.1 presents some estimated exposure volumes involving wastewater.

Table 6.1: Examples of treated wastewater end-uses, human exposure pathways and approximate exposure volumes

Exposure route	**Exposure type**	**Approx. exposure volume**	**reference**
Toilet flushing	Inhalation of aerosol	0.01 ml	(Dowd *et al*., 2000)
Crop irrigation	Ingestion of crop	10 ml	(Asano *et al*., 1992, Shuval *et al*., 1997)
	Inhalation of aerosol	0.05 ml	(Dowd *et al*., 2000, Kincaid *et al*., 1996)
Pond (as part of treatment)	Accidental ingestion	1 ml	(Ashbolt, 1999)

6.4.3 Dose-Response Assessment

Baseline information to establish a relationship between the dose of a microbial agent and the rate of infection in a population has been compiled by Haas *et al*. (1999) and Teunis *et al*. (1996) from human volunteer studies. Two main equations have been used to describe the relationship; exponential (1) and Beta-Poisson (2). When organisms are distributed randomly and the probability of infection for any organism equals r, then probability of infection is calculated using:

$P_{inf} = 1 – e^{-rDose}$ (1)

However, when “r” is not constant, then two parameters, α and β, describe the relation as:

$P_{inf} \sim 1- (1 + Dose/\beta)^{-\alpha}$ (2)

Compared to the exponential model, the Beta-Poisson model fits well with many dose-response datasets and adds plausibility to the assumption that ingestion of a single organism is sufficient to cause infection. It is also conservative when extrapolating to low doses (Teunis *et al*., 1996).

6.4.4 Risk Characterisation

The information from the hazard identification, exposure assessment and dose-response relationship steps is then integrated in the risk characterisation in order to estimate the magnitude of the public health problem. Most often the microbial risk is presented as the total number of infections per annum or system lifetime (Fane *et al*., 2002) which takes into consideration the infected/exposed number of people.

6.4.5 Acceptable Microbial Risk

It is important to define acceptable risk, as well as to know when to take preventive measures as when integrating information from the different aspects of sustainability. The most widely used acceptable microbial risk level is 1 infection per 10 000 people per annum, as proposed by the US EPA and Dutch regulation for microbial risks from treated drinking water (Anonymous, 2001; Regli *et al*., 1991) although Haas (1996) argues that it should be lowered to 1:1 000.

6.5 Results and Discussion

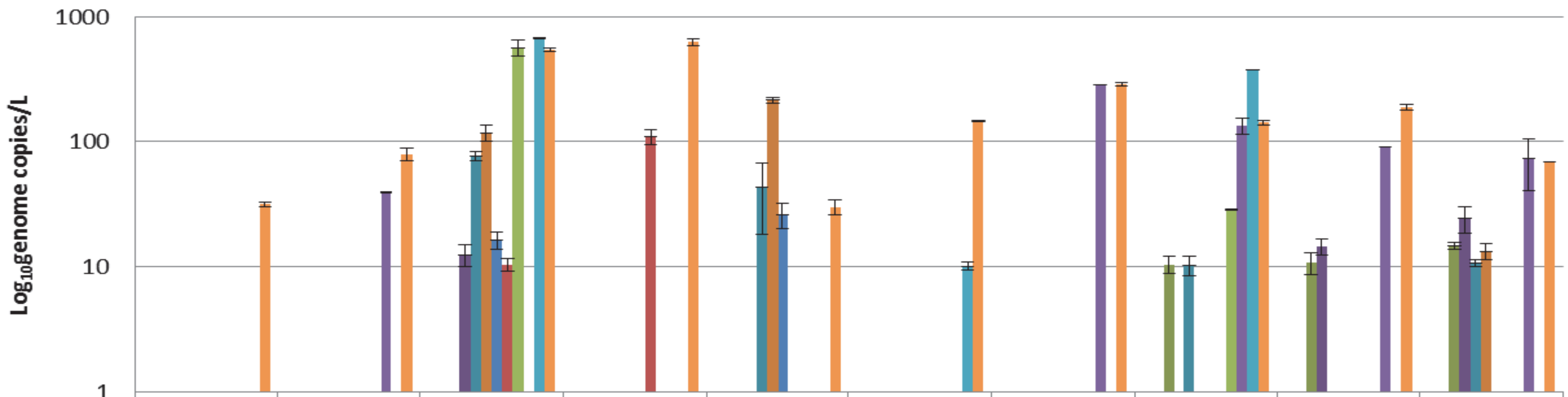
6.5.1 Viral Detection in Wastewater Samples

Table 6.2 shows the frequency of AdV detection as well as the detection ranges at each of the 14 WWTPs over the 12 month study period. Results for AdV detection are comprehensively presented in Figures 6.1 and 6.2.

Table 6.2: Detection frequency and detection range of AdV in discharge point* samples of the 14 WWTPs over a 1-year period

WWTP	Detection frequency (%)	Detection range (gc/l)	WWTP	Detection frequency (%)	Detection range (gc/l)
Alice	67	6.88×10^2 to 3.11×10^3	**Amalinda**	67	1.0×10^1 to 6.75×10^2
Fort Beaufort	42	2.11×10^2 to 5.92×10^2	**East Bank**	17	1.09×10^2 to 6.27×10^2
Mdantsane	50	3.67×10^2 to 6.28×10^2	**Queenstown**	33	2.6×10^1 to 2.13×10^2
Komga	17	4.4×10^1 to 2.75×10^2	**Whittlesea**	17	1.0×10^1 to 1.47×10^2
Zwelitsha	25	1.7×10^1 to 4.94×10^2	**Stutterheim**	50	1.0×10^1 to 3.8×10^2
Reeston	0	-	**Dimbaza**	17	3.9×10^1 to 7.9×10^1
Schornville	33	2.5×10^1 to 6.62×10^2	**Keiskammahoek**	50	1.0×10^1 to 7.2×10^1

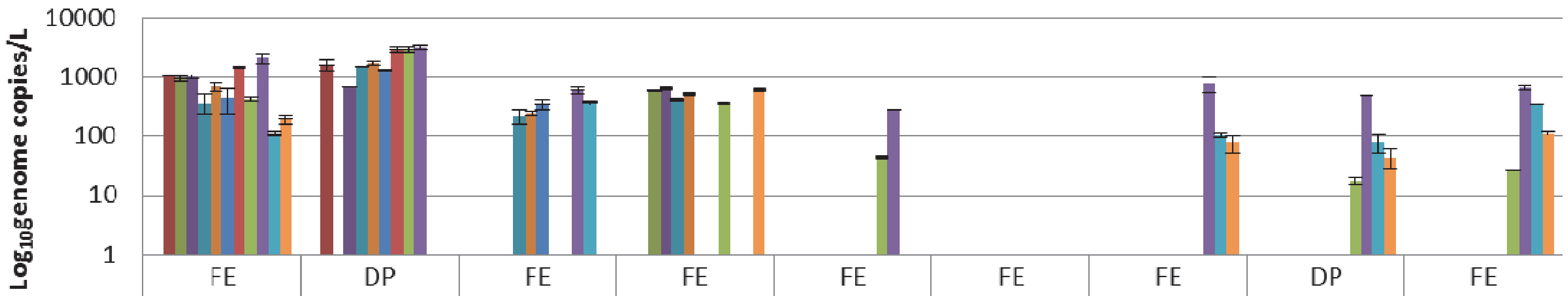
*Discharge point samples where available and reachable, otherwise data is for final effluent samples.



	Dimbaza FE	Dimbaza DP	Amalinda FE	East Bank FE	Queenstown FE	Whittlesea FE	Stutterheim FE	Stutterheim DP	Keiskammahoek FE	Keiskammahoek DP
Sep-12	0	0	0	0	0	0	0	0	0	0
Oct-12	0	0	0	0	0	0	0	0	0	0
Nov-12	0	0	0	0	0	0	0	10.4214	10.7316	14.7222
Dec-12	0	0	12.4436841	0	0	0	0	0	14.4816	24.2310001
Jan-13	0	0	76.8041255	0	42.7888916	0	0	10.3164	0	10.6494
Feb-13	0	0	117.875621	0	213.730288	0	0	0	0	13.3143
Mar-13	0	0	16.4354325	0	26.0520548	0	0	0	0	0
Apr-13	0	0	10.3632041	109.718818	0	0	0	0	0	0
May-13	0	0	562.331916	0	0	0	0	28.3404001	0	0
Jun-13	0	39.4693614	0	0	0	0	284.321429	133.892857	89.8214288	72.607143
Jul-13	0	0	675.94737	0	0	10.1210065	0	378.267858	0	0
Aug-13	31.5264837	79.689719	539.571763	627.891184	30.0557912	147.443295	287.589286	141.410715	188.214286	68.2142859

Key: FE = final effluent, DP = discharge point

Figure 6.1: Adenovirus detection data for seven of the WWTPs between September 2012 and August 2013



	FE	DP	FE	FE	FE	FE	FE	DP	FE
	Alice		Fort Beaufort	Mdantsane	Komga	Reeston	Zwelitsha		Schorneville
Sep-12	0	0		0	0	0	0	0	0
Oct-12	1042.49896	1623.74838	0	0	0	0	0	0	0
Nov-12	932.499068	0	0	599.9994	0	0	0	0	0
Dec 12	998.75043	688.747883	0	628.749371	0	0	0	0	0
Jan-13	371.251057	1494.99851	211.249789	421.249579	0	0	0	0	0
Feb-13	689.99931	1722.49828	237.499763	513.749486	0	0	0	0	0
Mar-13	443.750985	1332.49867	349.99965	0	0	0	0	0	0
Apr-13	1472.49853	2914.99709	0	0	0	0	0	0	0
May-13	437.499563	2871.24856	0	367.499633	44.5970983	0	0	17.3300827	25.7825742
Jun-13	2084.99792	3114.99689	592.499408	0	275.891153	0	754.963531	494.231649	662.177909
Jul-13	109.99989	0	376.249624	0	0	0	105.660609	79.6784918	353.892503
Aug-13	193.751235	0	0	601.249399	0	0	76.3213523	43.6785278	111.589174

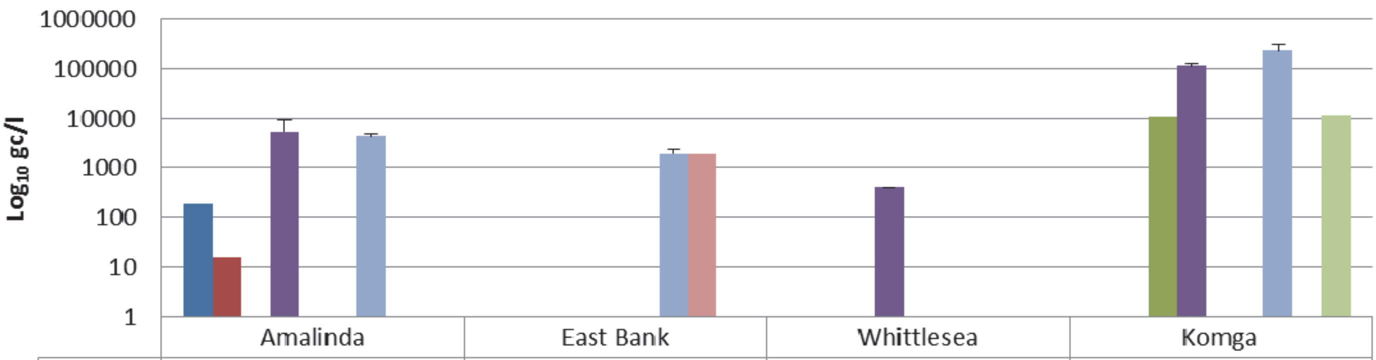
Key: FE = final effluent, DP = discharge point

Figure 6.2: Adenovirus detection data for seven other WWTPs between September 2012 and August 2013

The Alice WWTP had by far the most prevalence of AdV in both its final effluent and discharge point samples compared to any other WWTP under this study. Statistical analysis (One-way ANOVA, SPSS Version 20) also showed that effluents from the Alice WWTP had significantly higher (P = 0.00) concentrations of AdV genomes compared to effluents from the rest of the other WWTP. Although less discharge point samples were positive for AdV compared to the final effluent samples, there was an uncharacteristic increase in AdV concentrations in the discharge point samples compared to the final effluent samples from the Alice WWTP, similar to the trend which was also observed in the final effluent and discharge point for faecal coliform densities from the same plant. Could this indicate recontamination of the effluent between the final effluent tank and the discharge point at the Alice WWTP? While this may be a possibility, it has not been investigated. The second highest prevalence of AdV genomes in wastewater effluents was observed at the Amalinda WWTP, although it was not statistically significantly different from the other WWTPs (with the exception of Alice WWTP).

The dynamics of RNA viruses (hepatitis A virus, enterovirus and rotavirus) in wastewater effluents were acutely different from those of AdV, the only DNA virus in this study. Enterovirus and hepatitis A virus were not detected in any of the 14 WWTPs while rotavirus was detected in 4 of the 14 WWTPs. Detection frequencies of rotavirus were, by WWTP, Amalinda (33%), East Bank (17%), Komga (33%) and Whittlesea (8%). Comprehensive results for rotavirus detection are shown in Figure 6.3.

But what could be the main reason for the differences in the mean viral concentrations in effluent samples of different WWTPs? The answer could lie in the differences in health conditions of people living in the different communities which cause the pathogen content of wastewater effluents to be notably different as reported by Jiménez (2003). The evaluation of viruses occurring in sewage samples can therefore be used as an indicator of viruses circulating in a community (Katayama *et al*., 2008). Asked in another way, could the observed pattern of RNA viruses distribution imply that the wastewater treatment technologies employed by these WWTPs were more effective in removing RNA viruses as compared to DNA viruses? The answer could be a “No” considering that, with the exception of Reeston WWTP, bacterial pathogens were still detected in these WWTPs effluents, and we know from literature that viruses (whether RNA or DNA) are more resistant to wastewater treatment technologies than are bacteria (La Rosa *et al*., 2010; Okoh *et al*., 2010; da Silver *et al*., 2007). The only tangible explanation still goes back to the dynamics of these viruses in the human populations contributing to the wastes that are treated in these plants.



	Amalinda	East Bank	Whittlesea	Komga
Sep-12	190			
Oct-12	16			
Nov-12				10678
Dec-12	5235		401	111580
Jan-13				
Feb-13				
Mar-13	4374	1945		225336
Apr-13		1870		
May-13				11214

Figure 6.3: Rotavirus detection data for the four WWTPs where it was detected

This explanation is more befitting considering that viruses are not a normal gut flora, and mostly occur in sick individuals who thus release them in their stools. In addition to the probable fluctuating occurrences of enteric viruses in human populations and hence in sewage effluents, rotavirus (among the RNA viruses) exhibits greater resistance to common disinfection strategies than most other enteric RNA viruses (Li *et al*., 2011; Clark and Graz, 2010; Lia *et al*., 2009), probably because of its double stranded genome. This could be another reason why, despite the non-detection of hepatitis A virus and enterovirus, rotavirus was still detected, as also was AdV. This finding is similar to that of Lodder and de Roda Husman (2005) in the Netherlands in which one of their objectives was to compare the concentration of RNA viruses in raw and treated sewage samples. They found out that the average virus concentrations in treated sewage were lower than in raw sewage, except for rotaviruses. Similarly, in that same study they also found out that the concentration of enteroviruses were the lowest of all viruses studied, both in raw and treated sewage samples.

The virtual absence, at Reeston, of both AdV and RNA viruses must be treated with caution considering the high frequency of acute chlorine over-dosing at that plant. However, the findings as they stand still raise an important question, “Can chlorine over-dosing totally eliminate viruses from effluent samples?” If so, “Can the chlorine dosing regimens in wastewater treatment be upped with an option to de-chlorinate before discharge? Will the attended public health gains justify/offset the cost implication of such an action?” This question can only be answered if carefully planned epidemiological, environmental impact and cost-benefit surveys are carried out.

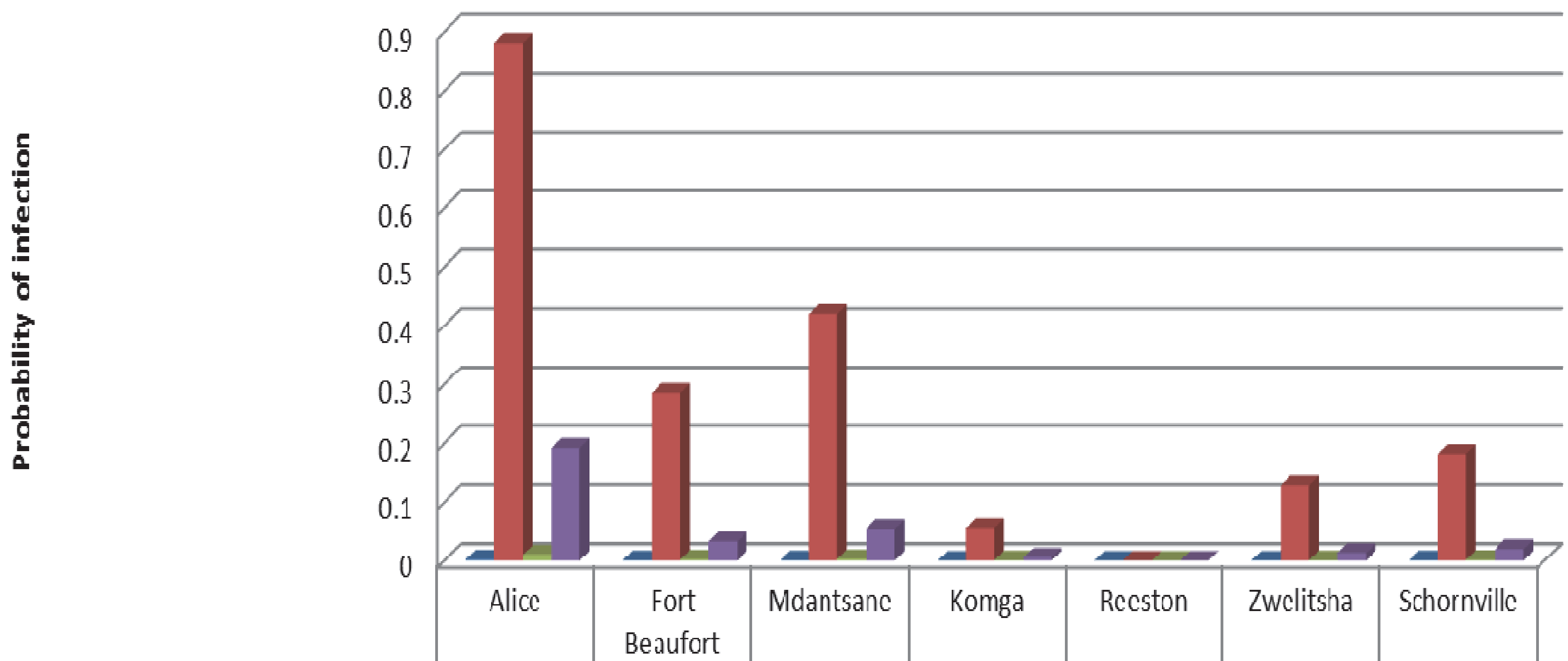
While detection of enteric viruses by molecular methods does not distinguish infective virions from inactivated viral nucleic acids, enteric viruses have been known to be able to survive wastewater treatment (Carter, 2005; Baggi and Peduzzi, 2000). Such viruses have the potential to pollute water sources of socio-economic importance (Gerba, 2007; Pinto´ and Saiz, 2007; Carter, 2005) and the low infectious dose of enteric viruses makes their presence in water sources to be of public health concern (Teunis *et al*., 2008). Human illnesses caused by waterborne viruses range from severe infections such as myocarditis, hepatitis, diabetes, and paralysis to relatively mild conditions such as self-limiting gastroenteritis (Banks *et al*., 2001).

6.5.2 Probability of Infection (P_{inf}) with Adenovirus

Using a ratio was 1:2 to represent the fraction of infectious adenovirus to total adenovirus particles (van Heerden *et al*., 2005) recovered by real-time PCR and the equation $P_i = 1\text{-}e^{(-rd)}$ to calculate the daily risk of infection from adenovirus; Table 6.2 below shows the calculated infectious doses for the various categories of wastewater end-uses. Figure 6.3 and 6.4 represent the probability of infection from adenovirus. For human adenovirus, the probability distribution, r, has been calculated to be 0.4172 (USEPA/USDA/FSIS, 2012; van Heerden *et al*., 2005).

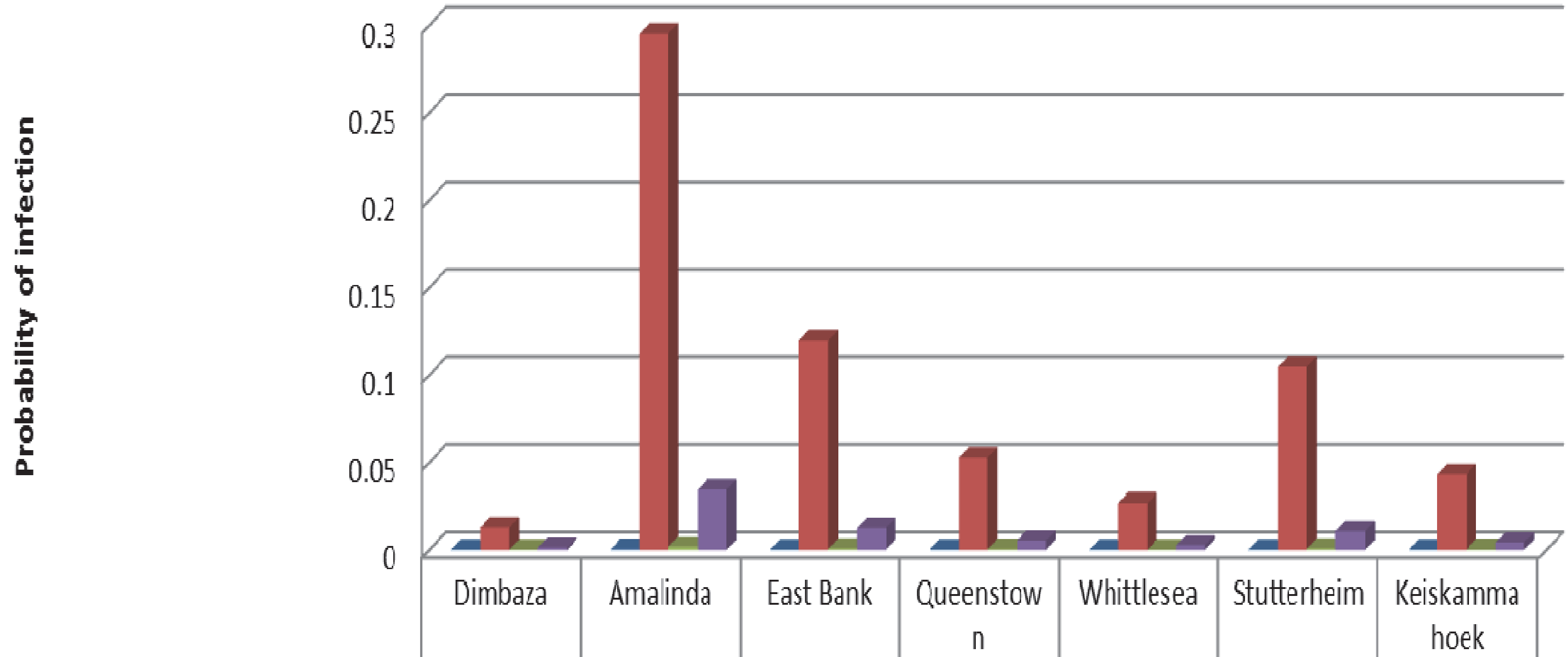
Table 6.3: Mean viral infectious doses (d) for different wastewater end-use categories

WWTP	Mean viral concentration (gc/l)	Mean infectious dose (gc/L, assuming a 1:2 infectivity ratio)	Mean infectious dose, toilet flush aerosol inhalation (assuming 0.01 ml exposure volume)	Mean infectious dose, irrigated crop ingestion (assuming 10 ml exposure volume)	Mean infectious dose, irrigation aerosol inhalation (assuming 0.05 ml exposure volume)	Mean infectious dose, pond water ingestion (assuming 1 ml exposure volume)
Alice	1022.551	511.2756	0.005113	5.112756	0.025564	0.511276
Amalinda	167.6478	83.82388	0.000838	0.838239	0.004191	0.083824
Dimbaza	6.278565	3.139283	3.14E-05	0.031393	0.000157	0.003139
East Bank	61.4675	30.73375	0.000307	0.307338	0.001537	0.030734
Fort Beaufort	160.6817	80.34083	0.000803	0.803408	0.004017	0.080341
Keiskammahoek	21.12447	10.56223	0.000106	0.105622	0.000528	0.010562
Komga	26.70735	13.35368	0.000134	0.133537	0.000668	0.013354
Mdantsane	261.0414	130.5207	0.001305	1.305207	0.006526	0.130521
Queenstown	26.05225	13.02613	0.00013	0.130261	0.000651	0.013026
Reeston	0	0	0	0	0	0
Schornville	96.12018	48.06009	0.000481	0.480601	0.002403	0.04806
Stutterheim	53.10668	26.55334	0.000266	0.265533	0.001328	0.026553
Whittlesea	13.13036	6.565179	6.57E-05	0.065652	0.000328	0.006565
Zwelitsha	65.49434	32.74717	0.000327	0.327472	0.001637	0.032747



	Alice	Fort Beaufort	Mdantsane	Komga	Reeston	Zwelitsha	Schornville
Pinf, Toilet flush aerosol inhalation	0.002130769	0.000335126	0.000544384	5.571E-05	0	0.000136612	0.000200487
Pinf, Irrigated crop consumption	0.881523661	0.284792049	0.419887003	0.054188075	0	0.127699409	0.181683989
Pinf, Irrigation aerosol inhalation	0.010608538	0.001674506	0.002718959	0.000278519	0	0.000682873	0.001002031
Pinf, Pond water ingestion	0.192089667	0.032962683	0.052997208	0.005555664	0	0.013569217	0.019850992

Figure 6.4: Probability of infection with adenovirus from various categories of wastewater end-uses



	Dimbaza	Amalinda	East Bank	Queenstown	Whittlesea	Stutterheim	Keiskammahoek
Pinf, Toilet aerosol inhalation	1.3097E-05	0.000349652	0.000128213	5.43435E-05	2.73896E-05	0.000110774	4.40647E-05
Pinf, Irrigated crop consumption	0.013011693	0.295109796	0.120341228	0.052894699	0.027018225	0.104864823	0.043108856
Pinf, Irrigation aerosol inhalation	6.54833E-05	0.001747038	0.000640901	0.000271688	0.00013694	0.000553749	0.000220304
Pinf, Pond water ingestion	0.001308851	0.034366892	0.012740267	0.00541976	0.002735245	0.011016918	0.004396869

Figure 6.5: Probability of infection with adenovirus from various categories of wastewater end-uses

Effluents from the Alice WWTP presented the highest risk of infection values for irrigated crop consumption and accidental ingestion of pond water. Other WWTPs whose effluents presented substantial risk of infection when irrigated crop is consumed fresh and wet (with irrigation water) included Mdantsane, Fort Beaufort and Amalinda. Some leafy salad vegetables can absorb pathogens from the environment (e.g., lettuce, sprouts), leading to prolonged survival of some pathogens (Strauss, 1985). Effluents from the Reeston WWTP presented a zero risk level for all categories of wastewater end-uses while effluents from the Dimbaza, Whittlesea, Queenstown, Keiskammahoek and Komga WWTPs also presented negligible risk of infection for all categories of wastewater end-uses.

Also, the calculated risk arising from inhalation of aerosol during irrigation using wastewater effluents is negligible even though the risk presented by ingestion of fruit or salad crop irrigated with wastewater is quite substantial for some WWTPs. Results from this risk calculation, as also were those from the bacteriological analysis, revealed that treated wastewater from these WWTPs may be used to irrigate cereal and fodder crops or any other crop which will need to be dried and/or processed before utilisation, with negligible or minimal risk of infection to either the consumer or the farm worker. At the most, enteroviruses have been known to survive for up to 59 days on crop surfaces (all conditions being favourable), otherwise they usually die off within two weeks (Feachem *et al*., 1983). Direct sunlight onto crop surfaces leads to rapid pathogen inactivation through desiccation and exposure to UV radiation while high temperatures and dry environments lead to rapid die-off (Strauss, 1985). Use of wastewater for irrigation facilitates convenient disposal of waste, adds valuable plant nutrients and organic matter to the soil (van der Hoek *et al*., 2002), provides reliable irrigation water supplies and, generally improves food security (Abaidoo *et al*., 2010). Should municipal authorities, farmers and the relevant stakeholders in the Eastern Cape Province decide to channel wastewater effluents for irrigation, it is our opinion that such a step would boost agriculture production in the Eastern Cape Province and raise its GDP.

Inhalation of aerosol during toilet flushing presented the least (almost negligible) risk of infection for all WWTPs. Basing on these results therefore, and in the face of a looming water crisis due to climate change, municipalities could also consider diverting wastewater final effluents for possible use in toilet flushing, with minimal risk to the user. Although this would require establishment of dual water supply systems, with potable and wastewater systems running parallel to each other, the expense would in the long term diminish compared to the millions of gallons of potable water that would be saved for other purposes for which use of potable water is non-negotiable. This however will require strict compliance to set guidelines for effluents to be used for this purpose.

With the exception of the Alice WWTP which had a substantial risk of infection from accidental ingestion of pond water, it seems workers at other WWTPs are faced with negligible risk of infection with AdV. However, we still maintain that utmost care should be taken when working with effluents, and workers should always have protective clothing on to avoid contamination and subsequent infections.

6.6 Conclusion

The presence of enteric viruses in wastewater final effluents suggests that a significant portion of the human population contributing wastes to these WWTP are infected with these viruses. Still, their detection in final effluents implies that the faecal-oral cycle of infection is likely to repeat itself especially if these communities draw drinking water from the same rivers/streams into which the WWTPs discharge their effluents. While subjecting the water to treatment before drinking may reduce or completely eradicate the chances of re-infection, the drinking water treatment plants and distribution systems will have to be at their optimum performance to eliminate chances of re-infection.

CHAPTER SEVEN : CONCLUSION AND RECOMMENDATIONS

South Africa is a semi-arid country and water is becoming an increasingly scarce resource. Municipalities may soon be forced to consider alternative sources of water in order to meet consumption requirements as well as to promote further development. At the same time, with South Africa's population expanding at a high rate, the need for increased food production is apparent. The potential for irrigation to raise both agricultural productivity and the living standards of the rural poor is a looming reality. Growing urban populations and increased domestic water usage result in greater quantities of municipal wastewater discharges which are spewed onto the environment, chiefly rivers. Paradoxically, these are the same rivers from which municipalities again draw water for potable water production purposes. With the current emphasis on public health, environmental health and water pollution issues, there is an increasing awareness of the need to dispose of these wastewaters safely and beneficially. Results of this study indicate that wastewater effluents in the Eastern Cape Province are contaminated with both bacterial and viral pathogens and that disposing of these effluents into public water courses may expose the public to risk of infections. We conclude that 24% of the WWTPs did not comply to set faecal coliform guidelines while *Vibrio* and *E. coli* were also detected in all of the WWTPs under this study. The release of pathogenic enteric microorganisms into aquatic environments can be a source of disease when water is used for drinking, recreational activities or irrigation. Greater than 50% of both the *Vibrio* and *E. coli* pathotypes exhibited multiple antibiotic resistance and we conclude that WWTPs constitute important reservoirs of enteric bacteria which carry potentially transferable resistance genes which are aided by a large concentration of donor and recipient bacteria of transferable genes and availability of nutrients in the wastewater matrix. Presence of viruses in treated sewage (noted in 93% of the WWTPs effluents in the case of adenovirus) will considerably contribute to the virus burden of the receiving water bodies. Consumption of even treated drinking water may result in infection if it coincides with failed water treatment while exposure to recreational activities and shellfish consumption may present a public health risk. However, risk characterisation of these wastewater effluents for different categories of water use indicated that the use of effluents for irrigation purposes presents negligible risk of infection to either the workers or the consumers.

We therefore recommend the following as possible future interventions by municipalities:

- For as long as municipal wastewater treatment facilities still discharge effluents into public water courses, national and local governments should prioritises the provision of potable water services to their residents, regardless of whether they are in urban or rural areas so as to protect public health and people's dignity.
- Municipalities may need to consider installing influent flow meters and automated chlorine dosing systems to curb cases of irregular chlorine dosing regimens. This will result in economic, public health and ecological gains.

- Municipal managers may also need to conduct refresher courses for their technical staff to keep them up-to-date with the latest operating and maintenance procedures for optimal WWTPs performance.
- Detection of bacterial and viral pathogens in sewage effluents points to large pockets of infected individuals in the communities, most of which go unreported and untreated. Health awareness campaigns may need to be carried out to educate people on the benefits of hygiene and seeking early treatment in cases of illness.
- The design of some WWTPs may have to be modified to allow for the minimum stipulated chlorine contact time before effluent discharge.
- Other pathogens such as *Salmonella*, *Shigella* and *Vibrio* may need to be included in routine monitoring of wastewater final effluent quality to complement general faecal indicator bacteria.
- Use of wastewater for irrigation purposes as this will result in the conservation of higher quality water in rivers and streams and make it more available for uses other than irrigation. The properly planned use of municipal wastewater for irrigation purposes will alleviate surface water pollution problems and not only conserve valuable water resources but also take advantage of the nitrogen and phosphorus content of sewage to grow crops with reduced requirements for commercial fertilizers. For WWTPs yet to be built, it will be advantageous to consider effluent reuse at the same time as wastewater collection, treatment and disposal are planned so that sewerage system designs can be optimized in terms of effluent transport and treatment methods as the cost of transmission of effluent from inappropriately sited WWTPs to distant agricultural land can be prohibitive. In the case that municipalities are able to completely divert effluents from WWTPs that have for long been discharging into rivers, to agricultural land, a staggered withdrawal may have to be done to avoid unforeseen ecological consequences.

Compared to the microbiological and physicochemical compliance levels contained in the Green Drop Report 2012, there was a significant improvement in compliance levels in this study, especially microbiological compliance. Whilst that may suggest an improvement in the functional performance of these WWTPs to produce effluents of acceptable standards, it also raises a question about the analytical procedures used by the municipalities against those that were used in this study. Our observations and recommendations for future studies are, therefore, as follows:

- Enteric viral and bacterial pathogens have been detected in sewage final effluents, implying that they are in circulation in the communities concerned. These findings provide a strong link with the findings of our previous study (Assessment of the incidence of faecal indicator bacteria and human enteric viruses in some rivers and dams in the Amathole District Municipality of the Eastern Cape Province of South Africa WRC Report No. K5/1968) where viruses were also detected in surface water sources noted to receiving effluents from some WWTP along its course. As the previous study and this current study only evaluated viral nucleic acids, there is need for a large scale investigation on the prevalence of infectious enteric viruses including epidemiological survey of diarrheal infections in the catchment.
- An interesting observation was made with regards to chlorine dosing regimens and prevalence of viral pathogens at the Reeston WWTP. Was it that there were no

viruses in the influent sewage for the whole year or the high chlorine concentrations completely eradicated intact viruses from wastewater effluents? How about the resultant nucleic acids that could not be detected by PCR? We recommend a detailed investigation into the effects of different chlorine dosing regimens on the survival and detectability of viral particles in water. Also, the isolation of some bacterial from effluents with high chlorine dose supports previous reports on increasing incidence of chlorine resistant bacteria. There is need for future in-depth study on this subject pursuant to coming up with probably new guidelines for chlorine dosing.

- Huge disparities were observed between the faecal coliform based microbiological compliance of the WWTPs in this study compared to the Green Drop Report 2012 results. While the results might suggest that the WWTPs have an improved performance since 2012, the conclusion is hard to make because of the different analytical methods used. We recommend that the Colilert Method (used by the municipalities) and the Membrane Filtration Method (used in this study) be evaluated against samples containing standardised inoculum and the best performing method be adopted for the Green Drop requirements
- Multiple antibiotic resistant bacterial pathogens (*Vibrio* and *E. coli*) were also isolated from sewage effluents in this study; the general assumption is that these pathogens acquired this resistance either by lateral gene transfer or from repeated exposures to antibiotics in human or animal bodies. But, what role(s) could antibiotic residues contribute to the multiple antibiotic resistances observed? We recommend a future in-depth investigation of the role of final effluents of WWTP as reservoirs of antibiotic resistance determinants in the watershed, to also include development of biosensors for the detection and quantification of relevant antibiotic resistance genetic elements in final effluents, and probably results in development of set guidelines for nucleic acids in final effluents.

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