ANTHROPOGENIC IMPACTS ON THE INTEGRITY OF THE BLESBOKSPRUIT CATCHMENT: A CASE STUDY OF SURFACE WATER POLLUTION

BY

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DECLARATION

I, Dipitseng Maropeng Phaleng, hereby declare that the research reported herewith on the topic "**Anthropogenic Impacts on the Integrity of the Blesbokspruit Catchment: A Case Study of Surface Water Pollution"**, is my own work and that all the sources used or quoted in this dissertation have been indicated and acknowledged by means of complete referencing.

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ABSTRACT

Water Quality Management is one of the critical challenges currently facing South Africa. The triad of water resource management, socio- economic development and environmental sustainability are key issues that require balance and compromise. The effects of anthropogenic activities on the Blesbokspruit catchment were examined. Water samples were collected from nine strategically selected sites along the stream for a period of ten months in six weekly intervals and analysed for physio-chemical, selected trace metals and microbial entities. Results revealed that variables of concern were Electrical Conductivity (EC), Total Suspended Solids (TSS), Nitrates, Phosphates, Sulphates and Chemical Oxygen Demand (COD). Mean levels of these parameters in this order ranged from 93.0-146.63mS/m; 11.25-39mg/L; 0.16-2.01mg/L; 0.5-0.96mg/L; 118.63-379.5mg/L and 15.0-34.0mg/L respectively. Levels of *E. coli* and F. coliforms also ranged from 19.13-43999.125 cfu/100mL and 20.63-16878.5 cfu/100mL respectively which were of concern. Levels of analysed trace metals were tolerable except for Fe with a range of 0.04-0.73mg/L. Generally, the results from this study indicate that the river is contaminated and therefore not suitable for direct human consumption as well as for irrigation purposes.

Keywords: Water Quality Management, Anthropogenic, Water pollution, Metals, Microbial, Surface water, South Africa

LIST OF ABBREVIATIONS

AMD	Acid Mine Drainage
ARD	Acid Rock Drainage
BB	Blesbokspruit [sample monitoring point]
DEAT	Department of Environmental Affairs and Tourism
DWAF	Department of Water Affairs and Forestry
EMF	Environmental Management Framework
EMM	Ekurhuleni Metropolitan Municipality
FAO	Food and Agriculture Organisation
INC	National Insurance Contributions
IWQO	Instream Water Quality Objectives
NEMA	National Environmental Management Act
NWA	National Water Act
PROPER	Programme for Pollution Control Evaluation and Rating
STW	Sewage Treatment Works
WCW	Water Care Works
WDCS	Waste Discharge Charge System
WHO	World Health Organisation

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CHAPTER 1

1.1 Introduction / background information

Water resources, especially surface waters are crucial to the livelihood of human beings, remarkably in developing countries including South Africa which has been described as a semi-arid country due to scarcity of these resources. This situation necessitates comprehensive management and monitoring of all water resources in the country. Water Resources in South Africa has been divided into nineteen (19) water management areas. The Upper Vaal water Management Area is number eight (8) on the list with the major rivers being Wilge, Libenbergsvlei and Vaal. Vaal River is the main source of water to the Gauteng Province, the most populous and economic nerve centre of the country as well as some parts of the North West Province. Water is abstracted from different parts of the river for domestic, industrial, mining, recreational and agricultural purposes. However, other provinces apart from Gauteng Province do not have portable water service providers like Rand Water, thus municipalities carry out their own water purification for domestic use (DWAF, 2006). For example, Dipaleseng Municipality in Balfour, in the Mpumalanga Province abstracts water from the Suikerbosrand and treats it for domestic purposes or portable use.

The Blesbokspruit drains into the Vaal River, downstream of the Vaal Dam. Water pollution in this catchment has witnessed steady increase over time. One of the major sources of pollution of the river is the disposal of untreated or semi-treated sewage effluent into water bodies. This is a common problem throughout South Africa because the feedback from National survey indicated that most of the sewage works are not properly operated and maintained and discharge poor quality effluent into streams and rivers (DWAF, 2006).

Various activities of anthropogenic nature take place in South Africa's river systems. In many parts of the country, quality of the surface waters has deteriorated as a result of industrial and mining effluents, sewage return flows and nutrients from agricultural activities (Hohls *et al*., 2002). The Blesbokspruit is no different from these river quality alterations. This Spruit forms part of the Ekurhuleni municipality in the East Rand and extends to the Lesedi Municipality in Heidelberg. The Blesbokspruit is one of the water resources that are under constant threat in the Upper Vaal Water Management Area.

Mining activities in this area started as far as 1934 with about thirty one (31) mines mining gold in the East Rand (Schoeman and Steyn, 2000). Currently, several activities exist along the river stretch with mining, industrialization, and urbanization being predominant. The main potential anthropogenic activities that may impact on the Blesbokspruit include:

- The Grootvlei mine and its associated mining activities which include dewatering, as well as other mines like Daggafontein and Sub- Nigel gold mines,
- The hazardous waste disposal site that belongs to and managed by Zinco,
- Nutrient loading as a result of sewage discharge to the water resource,
- Previous mining activities which left several mine dumps in the area,
- Agricultural activities which include livestock watering, and
- Urbanization as well as informal settlements.

All these activities may impact negatively on the Blesbokspruit and are the major concerns in this study.

Table 1.1: A description of water use in the Blesbokspruit Catchment

Water Use	Extend of Use
1. Raw Water for Drinking Water Supply.	⇒ Water is abstracted at Suikerbosrand in Balfour and treated to potable use.
2. Recreational Water Quality	⇒ Suikerbosrand Nature Reserve, Marivale Bird Sanctuary Nature Reserve and a few Golf Estates in the Catchment depend on River Water.
3. Fresh Aquatic Life	⇒ Marivale Bird Sanctuary is a RAMSAR site at Blesbokspruit.
4. Agricultural Use	⇒ Very little irrigation occurs around the Catchment. Karan Beef is the largest feedlot that depends on Blesbokspruit.
5. Sewage Treatment Works (STW)	⇒ Seven STW occurs in the Catchment. Ratanda Sewage works, Heidelberg sewage works, Ancor Sewage Works, Tsakane sewage Works, Herbert Bickley Sewage Works, Grundling Sewage Works and Balfour Sewage Works. All these sewage works discharges directly of indirectly into the Blesbokspruit with the exception of Balfour Sewage works that discharges into the Suikerbosrand.
6. Industrial Use and Mining	⇒ Grootvlei Mine discharges into the Blesbokspruit, Old mine dumps, Daggafontein (Closed Mine), Coal Mine (Closed) and Clay mines exist in the Catchment. Zinco industry is also present.

1.2 Problem statements

The constitution of South Africa and the National Water Act guarantee the right of every citizen to clean potable water and gives the people the confidence that “water belongs to us all” and therefore need to be managed and protected. South Africa is a country with diverse cultures and beliefs and proud to have cultural heritage and recreational sites that gives its integrity. In the year 1986, the Blesbokspruit was listed as a Wetland of International Importance (Ramsar Site) under the Ramsar Convention with roughly 20 km total length under protection at the Marivale Bird Sanctuary. The Blesbokspruit is one of the larger wetlands in the Highveld region of Southern Africa lying at an altitude of 1600m (Dini, 1999).

The value of the system lies in its ability to purify industrial and domestic effluent discharged into the Blesbokspruit River from local industries, sewage works and mines, thereby reducing pollutant loads entering the Vaal River. In addition, the Blesbokspruit wetland acts as an important refuge for many water bird species, particularly in the context of the highly industrialized urban environment of the far East Rand where most of the wetland habitats have been lost (Dini, 1999). This represents a valuable aquatic life and if pollution is not controlled and effluent not well managed, the RAMSAR site can be lost forever. There are several activities that may adversely contribute to the degradation of the integrity (pollution) of this Spruit. Among these include Beef Feedlot that supplies meat to a large portion of the country and releases waste effluent into the river, mining and sewage effluent among others. These impactors are located at different stretches of the river with cumulative effect. Consequently, good water quality management practice and skills are required to avoid the potential to cause human diseases and economic degradation.

1.3 Rationale / justification of the research

Surface water serve as an excellent solvent and transport medium for particulates, and as such tend to become contaminated both by natural processes such as erosion and dissolution of salts geologically present in soils, as well as by man-induced processes and wastes such as discharges from mines, industries sewage works etc. The latter are processes that could be said to be natural e.g. contamination from runoff water with geological salts and metals while contamination from mine water, industrial effluents, synthetic chemicals such as pesticide residues could be regarded as anthropogenic.

Land and water developments worldwide have brought many benefits to humans but have also led to a decline in the ecological form and functioning of rivers (King *et al*., 2003). The sewage works in this catchment are not authorised under the National Water Act (NWA) and pose a health risk to the community and the environment at large (www.reserviour.co.za; accessed on 13 Nov 2008). Contaminated water either by chemicals or microbes not only holds the potential to cause adverse affects to human health, but also result in economic loss (Venter *et al.*, 1996). The presence of slimes dams near the water resource pose seepage risks, acid mine drainage and contamination of the water resource. Heavy metals can be toxic to aquatic and animal lives. Radioactive isotopes can be a threat to aquatic ecosystem and human use of water resource (Ashley and Napier, 2005). As much as DWAF does not promote direct consumption of water from the rivers, people especially in rural and informal settlement do not have access to potable water and often resort to rivers as the main supply of water.

1.4 The aims of the research

The main thrust of the research is to:

Conduct an investigation into the integrity (quality status) of the Blesbokspruit as a result of possible contaminations emanating from anthropogenic activities on the water. In order to comprehensively address this, the following investigations would also be carried out:

- To conduct selected physical and chemical analyses (pH, EC, TDS etc) of collected water samples.
- Carry out qualitative and quantitative analysis of selected trace metal (Cd, Cu, As, Fe and Zn) of human and environmental health importance in water samples.
- Perform some microbial evaluation (FC, TC, *E. coli*, and Heterotrophic Plate Count) of water samples.
- Evaluate the results of water quality investigations obtained with acceptable national and international safety limits.
- Compare the results obtained with local and international data from similar studies.
- Make appropriate recommendations to water resource managers, scientists, decision-makers, and the public with respect to usage and management of surface water resource.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Water use in South Africa relates to the consumption of water as well as to the activities that may affect the quality and condition of the Water Resource itself. According to the NWA: 36 of 1998, Waste Water Treatment Plant, Industries, Mines, Agriculture and Recreational facilities are water users and needs to be managed.

In many developing nations, the problem of achieving economic development at the expense of degrading the natural environment has reached alarming proportions (Leung *et al*., 2004). As a result, in the last decade or so, more effective monitoring and management of the natural environment have attracted public concerns and have become a focus of academic research (Nirel and Revaclier, 2003; Leung *et al*., 2004). According to Venter *et al.,* (1996), there is a need to integrate environmental planning into economic planning and allow the former to guide the latter. Unfortunately, rapid economic development and urbanisation has been a series of environmental problems and mostly noticeable in the serious pollution of the river waters (Leung *et al.*, 2004). The need for balanced environmental, ecological and economic perspectives has led to the over-arching concept of sustainability that emphasises decentralized and collaborative decision making for the overall ecosystem (Chen *et al.*, 2002).

Polluted water is a major cause of human disease, misery and health problems. A case of typhoid contamination in the Delmas area, South Africa was reported in September, 2005 that caused a lot of death (www.doh.gov.za; accessed on 10 Feb 2009). Another incident was the year 2008 cholera contamination in Zimbabwe which spread to neighbouring countries like Botswana and South Africa (www.doh.gov.za; accessed on 10 Feb 2009). According to Tempelhoff (2008), a meeting held in South Africa's Gauteng Province in November 20, 2007 ended up in chaos when the findings of independent laboratory revealed evidence of dangerously high levels of faecal pollution in the Vaal River Barrage. Furthermore, about 4 million children die every year as a result of diarrhoea caused by water-borne infections (Inco-CT, 2004). The bacteria most commonly found in polluted water are coliforms excreted by humans due to improperly designed sanitary facilities. These incidents have become part of everyday life in many developing countries. Wall (2006), pointed out that in South Africa, local authorities are increasingly unable to cope

with the constant demand for effective sewage treatment due to the former apartheid regime, and today the Vaal River Barrage is essentially a reservoir of sewage that is constantly diluted with water from the Vaal Dam when DWAF provides water for consumers below the Vaal Dam.

2.2 Pollution and sources of pollution

According to online dictionary, pollution can be defined as the contamination of air, water, or soil by substances that are harmful to living organisms. Pollution can occur naturally, for example through volcanic eruptions, or as the result of human activities such as the spilling of oil or disposal of industrial waste (http://www.thefreedictionary.com/pollution; accessed on 03 June 2009)

The NWA, 36 of 1998 describes pollution as man-made substances or energy that have adverse effects on the living or non-living environment. Pollution can be classified on the basis of source of pollution and resource that is impacted upon (DWAF, 2001). Pollution-causing substances originate mainly from waste materials and can occur in any form such as the gaseous, liquid and solid.

2.2.1 Pollution characterised by source

Pollution occurs mostly as a result of human activities where waste substances are channelled into the environment. These include waste waters from households, industries, mines, transport, recreation, and agriculture. This pollution is related to the concept of urbanisation and has a direct relation to economic growth. Therefore without sustainable development; waste emission, discharge or disposal can lead to irreversible conditions and has the potential to deterioration or damage the environment at large (DWAF, 2001).

2.3 Point and non-point sources of pollution of surface waters

2.3.1 Point source discharges in the catchment

Point source pollution into aquatic system occurs as a result of human activities where wastewater is channelled directly into the receiving water bodies. Discharges from Sewage Treatment Works (STW), effluent from domestic and industrial sources, mining activities among others have been identified as point source discharges into surface waters. An

opportunity for a new Coal Mining activity has been identified in the Catchment at Heidelberg. A description of some of the activities that may impact or influence the water quality of the Blesbokspruit is provided below:

a) Mine water

Mine water discharge by Grootvlei mine is currently being monitored, the quantity of the discharge is estimated at 80-100mL/d I 2000 (Schoeman and Steyn, 2000). The Grootvlei mine is situated east of the town of Springs, part of the Ekurhuleni Metropolitan Municipality in the East Rand and has been operating underground mining activities since 1934 (Schoeman and Steyn, 2000). Tshikalange (1999), reported that Grootvlei mine exploits the Kimberly Reef and the Black Reef for gold at approximately 700 meters underground. One of the major problems the mine experiences is the increasing ingress into the underground mine workings, pumping in this mine commenced in October 1995 (Barradas and Loggenberg, 1996). Grootvlei mine is the only mine in the East Rand that is pumping underground water and helps keep the other mines like Sub-Nigel Mine operational. The pumped water is currently being treated using the Rhodes BioSURE process before it can be released to the Blesbokspruit (Rose *et al*., 2002). Points around the closed mines and mine dumps are also being monitored.

b) Sewage Treatment Work/Plant

Six sewage works exist in this catchment and are all managed by the East Rand Water (ERWAT). All these Waste Care Works (WCW) discharges effluent directly into the Blesbokspruit. The summary of these WCW is outlined below (www.erwat.co.za accessed on 28 April 2009).

Table 2.1: A summary of WCW discharges effluent directly into the Blesbokspruit

Name of WCW	Location	Volume of raw sewage treated	Treatment method employed
Ancor WCW	Springs	28 mega-litres per day of industrial effluent and raw sewage	Conventional biological filtration is employed as the main treatment process.
Carl Grundling WCW	Vorsterkroon, Nigel	2,5 mega-litres per day of industrial effluent and raw sewage	Activated sludge is employed as the main treatment process.
Herbert Bickley WCW	Maraisdrift, Nigel	12 megalitres per day of industrial effluent and raw sewage	Both biological filtration and activated sludge are employed as treatment processes.
Tsakane WCW	Tsakane	12 megalitres per day of raw sewage	Conventional activated sludge is employed as the main treatment process.
Heidelberg WCW	Heidelberg	6 megalitres per day of industrial effluent and raw sewage	Activated sludge is employed as the main treatment process.
Ratanda WCW	Ratanda	2,5 megalitres per day of raw sewage	Conventional activated sludge is employed as the main treatment process

The effluent discharged is currently being monitored and analysed for chemical and microbial quality. Monitoring points has been set upstream and downstream of the discharge.

2.3.2. Non point source discharges in the catchment

Non-point source pollution is that type of pollution where pollutants have no obvious point of entry, for example mine dumps, landfill sites, agricultural area, etc. It occurs as a result of runoff, precipitation, atmospheric deposition, drainage, interflow, seepage, groundwater flow or river course modification (Pegram and Görgens, 2001). Agricultural use and industrial use were identified as non point sources in the Catchment. Sludge from Karan Beef Feedlot and Waste Dams at Zinco have the potential to pollute Water Resources.

2.4 Water Quality Management

The term water quality refers to the microbial, physical, chemical and radiological properties of water (DWAF, 2001). These properties affect both the ecosystem health and the fitness for water use. The South African National Water Act (36 of 1998) recognises that water resources are part of the integrated cycle made up of water ecosystems (rivers, wetlands, lakes, dams, estuaries and groundwater) and the processes of precipitation,

transcription, infiltration and evaporation. The NWA promotes protection of water resources for the current and future generations. The natural water quality change may directly or indirectly be caused by four major categories of human activities:

- Changes in the hydrological cycle which modify the dilution and mixing capacities of water bodies and the hydrological balance. River damming has a great ability to modify water quality through particle settling, increase of water resistance time and evaporation. As a result, reservoir outlet waters are generally very low in suspended sediments, depleted in nutrients due to the trend towards eutrophication and sometimes more saline. Rainfall, evapotranspiration and runoff directly define the flow regime (Tetzlaff *et al*., 2005).

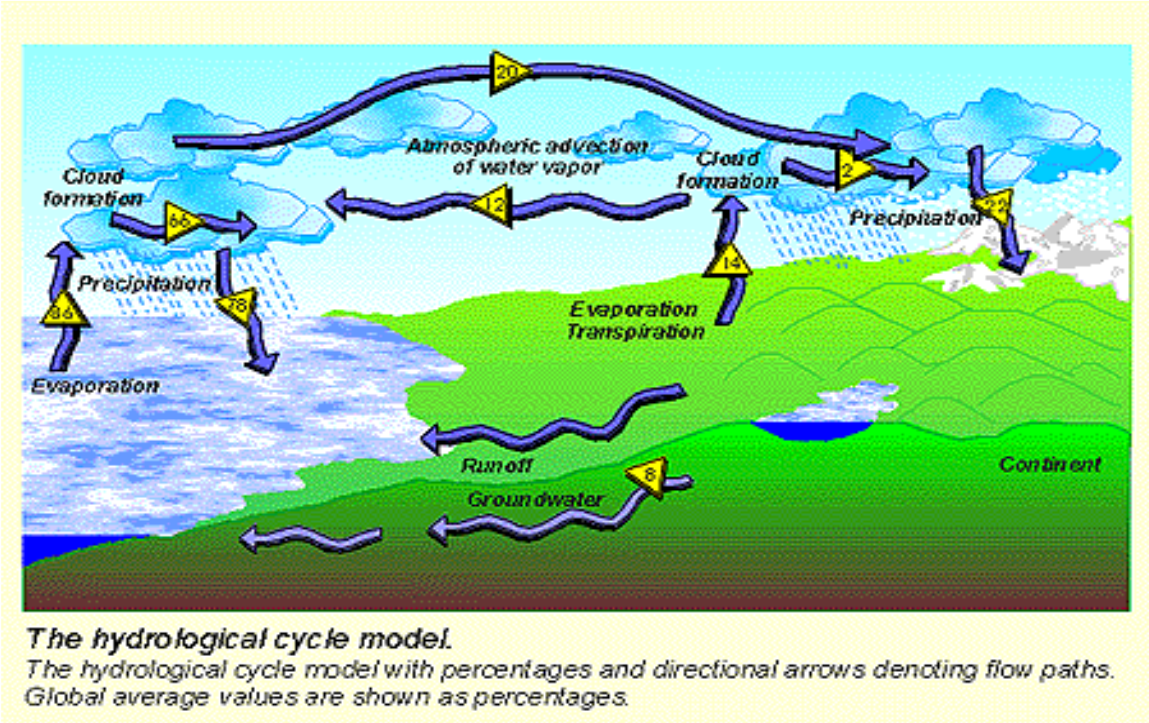

Figure 1: The hydrological cycle (mynasadata.larc.nasa.gov/L13_Bradley.html;accessed on 28 Feb 2008)

- Enhancement or slowing down of natural biogeochemical cycles as weathering, primary production and mechanical erosion. Forest cutting, international fires, road construction, weathering of mine tailings, extensive agricultural ad release of domestic waste can be considered in these categories.
- Direct or indirect dumping of natural substances in water bodies, examples include coal and petroleum burning, metal based industrial activities, etc.
- Direct or indirect release of synthetic substances, both organic and inorganic, examples include radioactive waste, use of plastic substances, etc.

2.5 Types of water users and their associated water quality concerns

Transmission of diseases by polluted water has a long history and remains a problem even today. Contaminated water either by chemicals or microbes not only holds the potential to cause human suffering, but also result in economic loss (Venter *et al.*, 1996). Control of water pollution and management of water quality for human health is therefore both an economic and a social development necessity. Much of the water extracted from the river is returned to the river after various human uses, at which toxic point there is a high risk of it introducing potential toxic species to the river (Aydinalp *et al.*, 2005). Salination is one of the major concerns in water quality. When this wastewater is discharged into the environment without prior treatment, it can cause damage by contaminating soil, surface and ground water (Lefebure and Moletta, 2006). Improvements in wastewater disposal, protection of water resources, and treatment of water supplies has greatly reduced the exposure of humans to waterborne diseases and chemicals in developing countries (Craun, 1986).

2.5.1. Mines

Most of the big mines discharges directly into a water resource or might need to undergo dewatering for safer mining operations as subsurface mining often progresses below the water table. The mining industry is responsible for the major part of waste generation in South Africa, contributing approximately 318 million tonnes or 75% of waste per annum of the total waste production stream (Vermaak *et al.*, 2004). Mining does not only have an impact through large waste tailings but also through seepages from natural watercourses which transport tailings underground for the purpose of backfilling excavations. The average composition of underground drainage waters has been estimated at 51% from natural watercourses, 14% from mine backfill; 34% from service and process sources and the remaining 1% from other unspecified sources (Sengupta, 1993). These water use has an adverse impact on water resource and the types of pollution associated with this water use are normally heavy metals, salinity, and low pH levels. Heavy metals can be toxic to aquatic and animal lives. Radioactive isotopes can be a threat to aquatic ecosystem and human use of water resource (Ashley and Napier, 2005). The major issues of concern from public to the mining industry include:

- Waste management and its long-term storage and disposal
- Health, effects and radiation

- Land-use conflicts and their adverse economic impacts

Coal mining is important for electricity generation however, this activity has a dramatic impact on both surface and ground water. Ground water pollution by coal mines is often due to the removed coal that leaves large underground voids in which ground water can accumulate as well as increased storage capacity and transmissivity due to the strata overlaying the coal being broken or shattered in the case of open cast and high extraction mining. Deterioration of surface water resources have been attributed *inter alia,* operational coal mine where, in the past, poor water management practices have resulted in contamination of some rivers (Vermaak *et al*., 2004).

Acid mine drainage is associated with most mining activities, including rock dumps, low-grade ore, abandoned mines and shafts. Acid Mine Drainage refers to the outflow of acid water from (usually) abandoned mines. It occurs naturally within the environment as part of the rock weathering process but is exacerbated by large scale earth disturbances characteristics of mining and other large construction activities usually within rocks containing an abundance of sulphide minerals (Wildeman *et al*., 1991). Acid mine drainage occur as a result of the oxidation of sulphite minerals, mainly pyrite.

$$4FeS_2 + 15O_2 + 8H_2O \rightarrow 2Fe_2O_3 + 8SO_4^{2-} + 16H^+$$

This reaction result in low pH that has a potential to mobilise heavy metals and transport them to the receiving environment. The solid pyrite, when introduced to oxygen and water, is catalysed to form iron (II) ions, sulphate and hydrogen ions. The hydrogen ions bind to the sulphate ions to produce sulphuric acid and the pH can be as low as 3.6 (Hedin *et al*., 1994).

Before coal is mined, very little of the pyrite is exposed to the conditions necessary to produce acid drainage. A ton of coal containing 1% pyretic sulphur has the potential of producing 33 lb of ferric hydroxide precipitate commonly known as “yellow boy” and over 60 lb of sulphuric acid (Sengupta, 1993). The drainage; if discharged into surface water or ponds constitutes an extensive, expensive and persistent environmental problem. Acid Rock Drainage (ARD) is produced by the exposure of certain sulphite minerals and can be produced from locations in which sulphuric rock has been exposed as a result of mining, construction, or other activities. The sources of ARD from mining operations include:

- Drainage from underground workings
- Runoff from open pit workings
- Waste rock dumps from mining activities
- Mill Tailings
- Ore stockpiles

ARD from underground workings has been known since earlier times because generally occurs as a point discharge of substantial flows of low pH water. Waste rocks produces from mining operations are exposed to precipitation, runoff, and possibly seepage. Waste rocks containing sulphides are potentially large sources of ARD. Acidification and salination of surface water occurs due to the presence of acid and high concentrations of sulphate and metal pollution (Maree *et al.,* 2004). Gold mines also have an impact of surface and ground water in a similar way. Water usually contains high salt levels (TDS in excess of 2500mg/L) (Cowan and Skivington, 1993). The acidity of waters affects metal mobilization in the environment, and also increases the leaching of nutrients from soil resulting in reduced soil fertility, the availability and toxicity of metals. (Meybeck *et al*., 1990)

During mining activities, when most of the valuable minerals have been recovered, the unwanted solids such as silicates, oxides, hydroxides, carbonates and sulphides are dumped in tailings. Historically, tailings were routinely discharged directly into the nearest water resource (Vick, 1990). In some parts of the country, i.e. the Upper Olifants Water Management Area, South Africa, Mpumalanga Province, this is still practised even today and the process is referred to as controlled release. This process allows controlled saline mine-water releases during flood conditions as one of the water quality management tools in the Upper Olifants River catchment, within Witbank Dam catchment (Wates *et al.,* 2002).

Gold mining in South Africa resulted in vast volumes of tailings, which have been deposited in impoundments. Poor management of most of the tailings resulted in the escape of seepages polluting soils and water. Most of these mine dumps are still visible today in the Witwatersrand area, Johannesburg and the East Rand. Similarly, the environmental impacts of tailings and impoundments are related to control and management, either directly as in the case of ground or surface water contamination, or indirectly as in the case of airborne transport of dry tailings. Recently, environmental issues have come to the forecast of tailings design, with special concerns over the quality

of the effluent and seepages from tailings to both ground and surface water (Vick, 1990). This has lead to an increased treatment of toxic tailings effluent prior to discharge and more effort towards total containment. Even though total containment is a challenge to archive, seepage control methods are effective in different ways.

2.5.2. Industries

Industrialization is considered the cornerstone of development strategies due to its significant contribution to the economic growth and human welfare. It has become a yardstick for placing countries in the League of Nations and an index of its political stature (FEPA, 1991). Industrialization, like other human activities that impacts on the environment often result in pollution and degradation. It carries inevitable costs and problems in terms of pollution of the air, water resources and general degradation of the natural environment (Thomas *et al*., 1992). Depending on the size and type of the industry, discharges into the water resource often result in high Chemical Oxygen Demand (COD), low or high pH levels, high nitrates and phosphates. Thus water bodies worldwide have become the primary means of disposal of waste, especially effluents from industries near them. The initial effect of discharged waste is to degrade the physical quality of the water, later the biological degradation becomes evident in terms of number, variety and organisation of the organisms living in water (Gray, 1989). However some industries discharges effluent into the municipal sewage works.

Industrial waste has turned out to be the most common source of water pollution and increases yearly due to the fact that industries are increasing because most countries are getting industrialised (Ogedengbe and Akinbile, 2004). The extend of discharge is such that the receiving water bodies can no longer manage to dilute rivers to give survival as good quality water sources. Water quality does not only get impacted upon by industrial effluent, but is used extensively for manufacturing or for cleaning and washing in industries and more extensively as a cooling agent. In this way industrial processes contaminate clean water with toxic ingredients and causing destruction of the aquatic life (Subrahmanyam and Yadaiah, 2001). A study on the impact of industrial effluent on water quality of a river carried out in Nigeria (Fakayode, 2005) showed that the chemical parameters studied were above the allowed limits and also tended to accumulate downstream. The increasing demand on water arising from fast growth of industries has put pressure on limited water resources and if industrial effluent is not properly treated before discharge, groundwater will be at risk of being polluted. It is generally known that

many people in rural areas still depend to a large extent on groundwater for domestic use (Olayinka, 2004).

Electricity is a basic necessity for the economic development in the country and water is a major source required to generate electricity, especially in a coal-fired power generation plants. Even though power generation industries like Eskom operates on a zero effluent discharge, this industry has an impact on our water resources because most of the water abstracted is not returned or released back to the environment. Abattoir sector serving both red and white meat industries in South Africa was identified by the Water Research Commission as a sector department where water usage and effluent discharge present problems nationwide. This is due to the fact that significant quantities of high quality water is required for processing purposes and discharge of high organic effluents have severe impacts on both the environment and the infrastructure required for sewage treatment (Rose *et al*., 2002). Oil industries generate large volumes of wastewater containing a variety of chemical contaminants. Wastewater is generated in almost every refining operation from primary distillation, to thermal cracking to the cooling water blow down (Pearce and Whyte, 2005). Wastewater from this industry contains elevated levels of phenols, sulphides, ammonia, COD, suspended solids, and iron exchange.

2.5.3. Waste Water Treatment Works (WWTW)

Sewage Treatment refers to the process of removing contaminants from wastewater, both run-off and domestic. It includes physical, chemical and biological process to remove contaminants. In most cases, sewage can be treated close to where it is created in septic tanks or can be collected and transported through a network of pipes and pump stations to a municipal treatment plant. In South Africa, there has been a significant reduction in the amount or content of chemicals discharged into the streams since the enforcement of the NWA: 36, 1998; NEMA: 107, 1998, ECA: 107, 1989, and other environmental legislations. However deterioration with regard to microbial contamination still occurs (DWAF, 2001). Sanitation systems can have an impact on water in a quantitative way through increased water use and in a qualitative way through the passage of organisms and chemicals from the human body to the natural environment (PDG-UCT, 1993).

Population increase has lead to an increase in human impact on the environment (Bilgrami and Kumar, 1998). About 90 % of all the sewage works in South Africa do not comply with their authorisation conditions. The most significant and ubiquitous source of organic matter

of anthropogenic origin in surface waters is human excreta. Waste from domestic activities, other public uses and small trade effluents are usually combined resulting in municipal sewage which after receiving treatment is discharged into watercourses. Large volumes of nitrates, phosphates, organics, COD, *E .coli*, Faecal coliforms etc reaches our rivers on a daily basis mainly due to poor operations or sewage work overload resulting in eutrophication which in turn encourage the overgrowth of weeds, algae and cyanobacteria. Eutrophication is defined as the enrichment of waters with plant nutrients, primarily phosphorus and nitrogen (DWAF, 2001). This may cause an algal bloom, a rapid growth in the population of algae, floating algal and/or macrophyte mats and benthic algal and submerged macrophyte agglomerations (Meybeck *et al*., 1990). Since algae members are unsustainable, they will eventually die and ultimately decomposed by bacteria. This process uses up so much oxygen in the water and these deoxygenating will encourage some of the algae species to produce toxins that contaminate the river system (Rodhe, 1969). However sewage works not only impact on water resources, an impact on soil has also been noted where treated sludge is applied on agricultural land (DWAF, 2001).

2.5.4. Agriculture

This is the most predominant land use in rural catchments of South Africa especially the Free State Province, and is according to FAO the single largest user of freshwater resources (http://www.fao.org/docrep/W2598E/w2598e04.htm; accessed on 04 March 2009). Most of the water used in agricultural activities is recycled back to surface water and/or groundwater which make agriculture both the course and victim of water pollution. It is a course through its discharge of pollutants and sediments to surface and/ or groundwater, through net loss of soil by poor agricultural practices and through salinization and waterlogging of irrigated land. It is a victim through use of wastewater and polluted surface and groundwater which contaminate crops and transmit diseases to consumers and farm workers. Pesticides and fertilizers primarily cause the contamination of water resources. Excessive levels of pesticides have known health effects. Agricultural activities are however classified into three categories, all of which are treated as non-point sources:

2.5.4.1. Livestock grazing

This has got an impact where livestock has a direct access to wetlands and rivers with regard to overgrazing. The type of pollution expected in this regard is contamination of

surface water with pathogens leading to public health problems. Also contamination by metals contained in urine and faeces.

2.5.4.2. Crop lands

In developing countries, food security is of great concern and with the fast increasing population growth; food production has to be expanded to meet the needs of growth population. Irrigation agriculture is currently the largest producer of food worldwide. Washed off of nutrients from fertilizers and pesticides may have a significant impact on the water resources. In addition to the problems of waterlogging, desertification, salinization, erosion, etc., that affect irrigated areas; the problem of downstream degradation of water quality by salts agrochemicals and toxic leachates is a serious environmental problem. Table 2.2 below outlines the impacts of agriculture on water quality.

Table 2.2: Agricultural Impacts on water quality

Agricultural Activity	**Impacts**	
	Surface water	**Groundwater**
Tillage/ ploughing	Sediments carry phosphorus and pesticides adsorbed to sediment particles, siltation of river beds and loss of habitat.	
Fertilizing	Runoff of nutrients, especially phosphorus, leading to eutrophication causing taste and odour in public water supply, excess algae growth leading to deoxygenation of water and fish kill.	Leaching of nitrate to groundwater, excessive levels are a threat to public health.
Manure Spreading	Carried out as a fertilizer activity, spreading on frozen ground result in high levels of contamination of receiving waters by pathogens, metals, phosphorus and nitrogen leading to eutrophication and potential contamination	Contamination of groundwater by nitrogen
Pesticides	Runoff of pesticides leads to contamination of surface water and biota, dysfunction of ecological system in surface waters by loss of top predators due to growth inhibition and reproductive failure, public health impacts from eating contaminated fish. Pesticides are also carried as dust by wind over very long distances	Some pesticides may leach into groundwater causing human health problems from contaminated wells.

	and contaminate aquatic systems.	
Irrigation	Runoff of salts leading to salinization of surface waters, runoff of fertilizers and pesticides to surface waters with ecological damage, bioaccumulation in edible fish species. High levels of trace metals such as selenium can occur with serious ecological damage and potential human health impacts.	Enrichment of ground water with salts and nutrients, especially nitrate.
Clear Cutting	Erosion of land, leading o high levels of turbidity in rivers, siltation of bottom habitat, etc. Disruption and change of hydrologic regime, often with loss of perennial streams, causes public health problems due to loss of potable water.	Disruption of hydrological regime, often with increased surface runoff and decreased groundwater recharge, affects surface water by decreasing flow in dry periods and concentrating nutrients and contaminants in surface water.

Source: (www.fao.org; accessed on 04 Mar 2009)

2.5.4.3. Irrigation

Salination associated with concentrations of return flows is a major water quality impact (Pegram and Görgens, 2001). Run-offs of salts leading to salination of surface water, run-off of fertilizers and pesticides to surface waters with ecological damage, bioaccumulation in edible fish species, etc. Most of the irrigated land use water from the wastewater treatment plants which is partially treated and often run-offs reaches the water resources.

2.5 Factors Impacting on Water Quality not Classified as Water Users

2.6.1. Dense Settlements

Dense Settlement is identified as areas, mostly in homelands, where people have settled in large numbers in locations where there is little economic base and little infrastructure (PDG/UCT, 1993). On a global scale, informal settlements are significant problems especially in the third world countries housing the world's disadvantaged (May *et al*., 1989). The main source of pollution in these areas is the unavailability of sanitation facilities, littering and lack of removal of domestic and other wastes (DWAF, 1999b). Inadequate sanitation or non-functioning sewerage systems both have the effect that excreta are deposited over a large area in such communities. The presence of garbage and lack of vegetation also contribute to pollution. Any runoff reaching a water resource will carry significant pollution loads of microorganisms, organic matter, nitrogen and

phosphates. Historically, grey water has been defined as wastewater that does not contain significant amount of faecal pollution (DWAF, 1999b). Typically, this consist of water discharged from baths, showers and sinks, however when considering grey water from a dense settlement, this may include other pollutants such as sewage, animal and human faeces, motor oil, paraffin and blood and stomach waste from slaughter areas. In these areas, wastewater is full of microorganisms and can introduce water borne diseases like diarrhoeal and cholera (Saff, 1996). The wastewater generated in the informal settlement is not only a concern to human health and well-being of the community, but is a threat to water resources into which this diffuse pollution flows. The optimal operation of Water Pollution Control System during storm flooding could critically depend upon the reliable predictions of changes in sewage and storm water discharges and dissolved oxygen defect at pertinent river reaches (Chen *et al*., 2002).

An ongoing study by the University of Cape Town (UCT) in South Africa has revealed that there is a need for urgent improved grey water management in South Africa's urban and peri-urban settlements, especially the non-sewered areas (PDG/UCT, 1993). Even though many townships have been connected to municipal water supply as part of Government's effort to eradicate backlog to clean water access, more often taps have been placed at public standpipes or inside the yard outside the houses. Water Wheel of July 2005 shows that this practice has resulted in random tossed grey water on the streets which creates a serious hazard to the health of the community as well as the surrounding environment. Improper grey water management can lead to health concerns including mosquito breeding due to ponding of grey water, contamination of drinking water supplies and odours from stagnant water. Children are especially at risk as they play in this dirty water.

2.6.2. Landfills

Waste is defined as any undesirable or superfluous by-product, emission or residue of any process or activity which has been discarded, accumulated or been stored for the purpose of discarding or processing. It may be gaseous, liquid or solid or any combination thereof and may originate from residential, commercial or industrial area. Waste is therefore considered to be a source of pollution and the policies to address the management of the entire waste handling process from generation to the final disposal are available. Even though the policies encourage Integrated Pollution and Waste Management, which entails Waste avoidance, minimisation and prevention, landfills still occurs. The term land filling refers to the deposition of waste on land, whether it be the filling in of excavations or the

creation of a landfill above grade (DWAF, 1994). Land filling therefore represent the most commonly used method for the ultimate disposal of waste that cannot be eliminated by waste minimisation techniques. Even though the need for environmentally acceptable yet cost-effective waste disposals has become a priority in South Africa, most of the municipal landfill sites are not permitted and are badly managed. This therefore allows for all types of waste to be disposed of at these landfill sites illegally. This is the same with Okhla landfill site in Deli, capital city of India, which receives 1300 tonnes per day (TPD) of solid waste, poorly managed and sitting very closely to the river Yamuna (Zafar and Alappat, 2004). Landfill activities have the potential to alter the quality and the quantity of groundwater and surface water in the locality. The significance of the potential varies according to the phase of operation, scale of the operation and the sensitivity of the local water resources. According to Clark (1998), the potential impact of landfill can be summarised in Table 2.3 as follows:

Table 2.3: An overview of potential impacts of landfill on surface and ground waters

Phase of operation	Impact on Surface water	Impact on groundwater
Construction Phase	• Reduction of surface infiltration and an increase in run-off and sediment erosion, resulting from soil compaction by vehicles and loss of vegetation. • Removal, earthworks and elimination of surface depressions, release of oil and hydraulic fluid from vehicles and fuel storage areas. • Release of sediments from vehicle washing	• Reduced infiltration to groundwater as a result of topsoil removal and exposure of lower permeability subsoil. • Contamination of groundwater by leaching of spilled oil and hydraulic fluid from soil.
Operation Phase	• Release of uncontrolled discharges of surface water from the site. • The break-out of leachate from the site.	• Reduction of infiltration of surface water to groundwater by provision of artificial surfaces and an engineered drainage system. • Contamination from leaching of chemical and oil spillages and leachate break-out.
Closure Phase	• Break-out or leakage of leachate. • Increased run-off and sediments erosion due to low permeability cap	• Reduced inputs of water from the surface infiltration due to low permeability ground surface. • Leakage of leachate through the landfill liner

Leachate is an aqueous solution with a high potential, arising when water is permitted to percolate through decomposing waste. Leachate movement is generally through the underlying strata, although it may also seep sideways towards the surface runoff or interflow regimes (DWAF, 2001). Studies done by Zafar and Alappat (2004), showed a

significant leachate increase due to percolation of rainwater that takes all the organic and inorganic content by advection and diffusion transport process from refuse piles. The same study also showed that the colour has become darker after rainfall, a sign of high hazel values of leachate in both landfills of study. The hardness, nitrate, chloride and iron were noted to be high in both landfill leachates.

Leachate quality is primarily dependent upon the balance between acetogenic and methanogenic phases of degradation. Acetogenic leachate is produced during the early stages of landfill and is of high organic strength while methanogenic leachate occurs during later phases of landfill when organic compounds are actively converted to landfill gases, leaving a residue of humic-type material. Landfills do not only impact on water quality but has shown huge ecological impact on the aquatic environment. The major potential sources of ecological impact from landfill activities are:

1. Land-take and excavations, other construction activities and disturbance including site engineering, maintenance and restoration.
2. Noise, dust and windblown litter
3. Accidental Spillage and leakage
4. Landfill leachate and gasses.

2.6.3. Road Tanker Spills

Road tankers contribute highly to water resource pollution. Because tanker spills are greatly affected by climate and geography of the area where spills occur, Department of Water Affairs and Forestry (DWAF) and other government departments cannot control or avoid their pollution therefore need to be managed strictly. Water pollution after a tanker spills, occurs via run-offs into the streams, rivers, dams and via seepage and leaching into the underground water. Deterioration in water quality has serious effects on humans, animals, aquatic lives and sometimes crops and grazing lands. Freshwater animals can be affected either by direct toxic effect, reduction or increase in pH as well as reduction in oxygen (DWAF, 2001).

2.6 Impact Control

Within each water sector or water use, individual polluter's takes responsibility of their impact and implement their individual water quality management plan. There are three main mechanisms to ensure compliance:

2.7.1. Regulatory Management

Government departments like DWAF impose licences that limit use for both abstraction and discharge. This regulates water quality and quantity. Department of environmental Affairs and tourism (DEAT) on the other hand regulates landfills through permits.

2.7.2. Self regulation

Municipalities and industries have their own Environmental Management Plan (EMP) that they need to comply to. Industries again have international standards that they are undertaking or as targets e.g. ISO 14001, for such industries first world environmental requirements apply and provide an incentive. Municipalities also participate in competitions like "cleanest Town", "Most improved Municipality in terms of environmental and water awareness".

2.7.3. Economic Incentives and Penalties

Economic instruments to reward or punish behaviour are recognised on an international level as effective in the management of natural resources with significant environmental dimensions such as forestry, fisheries, land conservation, water quality and river flows (Milne *et al*., 2003). DWAF is currently in the process of developing the Waste Water Discharge Charge System (WDCS), the aim of which is to recover the costs associated with different water treatment and water quality management programmes and to provide incentives for water users returning water back to the water resource to reduce their pollution loads. Many municipalities also impose charges to cover the costs of their own water treatment programmes, Durban and the City of Tshwane municipalities, for example, have introduced further sanitation charges for waste water discharges that exceed the pollution load of normal waste water (Archer, 2008).

CHAPTER 3

RESEARCH DESIGN AND METHODOLOGY

3. 1 Brief introduction

Five (5) water users were identified as the possible main contributors/ polluters in the catchment, namely mines and tailing dams, industries, sewage works, agricultural activities, and some settlements. To establish the water quality impact, water samples were taken at strategic points along the Blesbokspruit (which empties into Vaal River at Vereeniging downstream of the Vaal Dam). From the said impactors, an impact evaluation Table was drawn-up according to the influence identified in the literature review and presented in Table 3.1:

Table 3.1: Impact evaluation of anthropogenic activities along the river

Impactors	Conductivity	pH	Total Dissolved Solids	Total Suspended Solids	Iron (Fe)	Cadmium (Cd)	Copper (Cu)	Zinc (Zn)	Arsenic (As)	Nitrate Nitrites Nitrogen (NO_3-NO_2-N)	Orthophosphate as phosphorus (PO_4^{3-})	Sulphates (SO_4^{2-})	Chemical Oxygen Demand (COD)	Microbial
Mines		X			X	X	X	X	X			X		
Industries		X			X	X	X	X	X					
Agriculture	X		X	X						X			X	X
Sewage Works	X	X	X	X						X	X	X	X	X
Settlements	X	X	X	X						X	X	X	X	X

3.2 Study area

The study site selected is the Blesbokspruit that drains into the Vaal River at Vereeniging, downstream of the Vaal dam and upstream of the barrage. The Vaal dam and the Barrage are Rand Water's abstraction points. Water abstracted at these points is treated for potable use and supplied to the whole of Gauteng as well as some parts of the Free State and North West Provinces. The Water Management Catchment Areas in the country is as shown in Fig 3.1

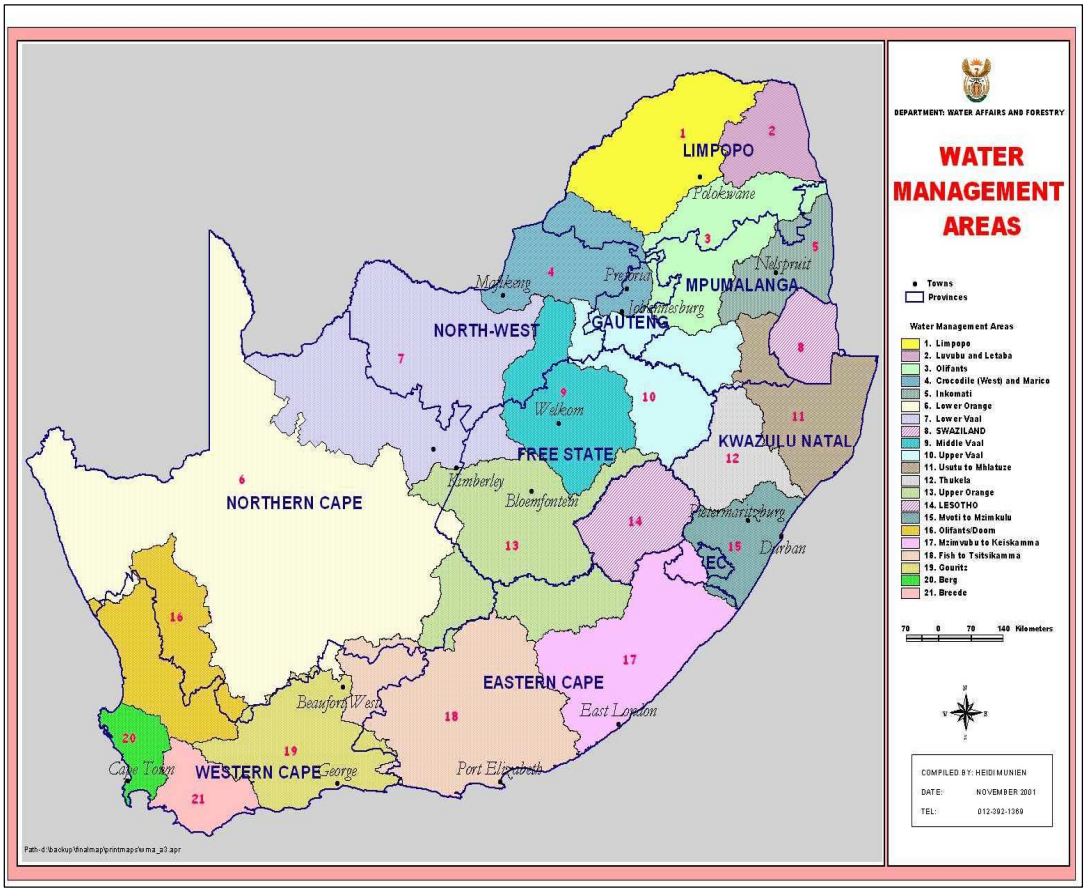

Fig 3.1: Water management (catchment) areas of the Republic of South Africa (Source: www.dwaf.gov.za accessed on 10 June 2009)

3.2 Description and Overview of the Catchment

3.3.1 Location, area and size

The Blesbokspruit runs from Benoni in the East Rand part of the Ekurhuleni Metropolitan Municipality (EMM) to Heidelberg in Lesedi Municipality, Gauteng Province. This study focuses on the Lower Part of the Blesbokspruit, i.e. from Springs to Heidelberg. The study area, Lower Blesbokspruit flows in a southerly direction past Springs where the two branches (Klein Blesbokspruit) join up north east of springs and just north of Grootvlei Mine. Upon entry of the Grootvlei mine water, another ERWAT Water Care Works (WCW), Ancor, discharges effluent further downstream to the Blesbokspruit wetland system. The Blesbokspruit then flows almost due south for about 30 km, after which it flows due west through Nigel, and then through Heidelberg, passing through Heidelberg and Ratanda, and picking up the treated effluent discharge from Tsakane WCW, Grundling WCW, Herbert Bickley WCW, Heidelberg WCW and Ratanda WCW (SRK, 2008). The

Blesbokspruit catchment is highly altered due to urban, industrial and mining development. These developments have significantly affected water quality, flow and water level regime, as well as altering stream morphology. All of these effects have impacts on beneficial water use along the Blesbokspruit itself and Vaal River. As far as its sub-regional context is concerned, the catchment is traversed by three national roads, namely the N17, N12 and the N3, which create certain potentials in terms of future economic development.

3.3.2 Geographical and Climatic Description of the Study Area

Precipitation

The average annual rainfall in Ekurhuleni Metropolitan Municipality is 670 mm recorded over a period of 31 years (Madden, 1987). Hailstorms are uncommon and snow falls occur on rare occasions. The average annual rainfall in Lesedi Municipality is approximately 700mm, occurring mostly from November to March in the form of thunderstorms.

Temperature

In Lesedi Municipality, the average annual surface temperature varies between a minimum of 4°C recorded during June and July and a maximum of 26°C recorded during January, with the lowest and highest recorded as - 8°C and 35°C respectively. In the Ekurhuleni Metropolitan Municipality (EMM), temperature vary from -10^{o}Cin winter to 35^{o}C in summer. (http//www.wetlands.org/RSIS/-COP9 Directory /Directory /1ZA0 04.html)

3.3.3 Topography and Geology

Ekurhuleni Metropolitan Municipality (EMM)

The area underlying EMM has rocks that vary from Swazian to the Mesozoic Eras. The geology of the area is fairly simple and stable with underlying sedimentary rocks of Karoo and Transvaal age overlying formation of gold bearing Witwatersrand (Dini, 1999). The gold bearings reefs of the Witwatersrand Supergroup and Transvaal Sequence sub-outcrop and outcrop along an arc in the East Rand Basin group which stretches from Benoni eastwards towards springs and then southward to Nigel (Barradas and Loggenberg, 1996). Outcrops of the Witwatersrand Reef occur in some areas within the Eastern Basin, at springs against the Transvaal Sediments. The Black Reef Quartzite

Formation and the Malmani dolomites form part of the Transvaal sequence. The Grootvlei Mine is exploiting the Kimberly Reef of the Witwatersrand Supegroup and the Black Reef of the Transvaal Formation for Gold and other precious metals such as silver, osmium, rhodium, ruthenium, iridium and platinum (Digby Wells and Associates, 1996)

Lesedi Local Municipality

The Karoo Sequence consists of a vast accumulation, nearly 8km thick, of shale, sandstone, and mudrock with diamictite and tillite at the bottom and coal about halfway up. These rocks have been extensively intruded by dolerite in the form of dykes and sills. The Karoo sediments are represented in the study area by the Vryheid and Dwyka Formations. The Geology of the area is generally flat positioned sedimentary rocks of Karoo and Transvaal age overlying older formations of Gold bearing Witwatersrand. (Lesedi EMF, 2006)

3.3.4 Land Use and Vegetation

Land Use in the area varies from natural, agricultural, industrial, mining, urban and rural settlements as well as recreational and nature reserves.

i). Natural: Marivale Bird Sanctuary

ii). Agricultural: Irrigated crops such as maize, vegetables, lucerne, lawn grass exist within the catchment and water from the Blesbokspruit is being utilized for irrigation. Animal watering exists in the catchment and also water from the Blesbokspruit is being utilized.

iii). Industrial and mining: The Witwatersrand Basin, made up of East, Central and West Rand Basins in South Africa is famous for its prolific gold, coal and uranium deposits and mining has been going on in the basin since the late 1800 (Handley, 2004). Records of water ingress into underground mines in the East Rand dated back to 1909 (Scott, 1995). Many mines in the vicinity discharge their polluted water into the Blesbokspruit, and the currently the largest and operational mine in springs discharges between 80 and 100Ml/d underground water into the Blesbokspruit (Schoeman and Steyn, 2000). There are also industries in the catchment that dispose their waste into the slimes dams which in turn pollute the Blesbokspruit.

iv). Urban and rural settlements: The towns of Boksburg, Benoni and Brakpan lie in the Northwest, Nigel located on the South, Springs in the East and Heidelberg on the southwest of the Catchment. There is a full sewage reticulation system in these towns and all of them discharge their effluent in the Blesbokspruit. The townships in the area are semi urban and are connected to the sewer system.

v). Recreation and nature reserves: The Marivale Bird Sanctuary, in the southern part of the catchment covers approximately 100 hectares and is about 7.4km long. It attracts mainly birdwatchers and hikers. The Suikerbosrand Nature Reserve forms an enclave into the western edge of the Lesedi Municipal Area. This nature reserve is situated in the Suikerbosrand Hills which contribute substantially to the natural beauty of the area. The nature reserve is well managed and the eco system is fully protected in this area (Lesedi EMF, 2006).

Vegetation

Ekurhuleni Metropolitan Municipality (EMM)

EMM falls within Sac's Grassland Biome (Low and Rebelo, 1996). This grassland biome is one of the most threatened in SA, with 60-80% irreversibly transformed, while only 2% is formally conserved (Bredenkamp, 2002). The biodiversity status of the area includes two grassland vegetation types, according to Low & Rebelo (1996), namely Moist Cool Highveld Grassland (Bredenkamp and van Rooyen 1996b) and Rocky Highveld Grassland (Bredenkamp and van Rooyen 1996a); the former vegetation type covers approximately 55% of the area, while the latter covers 45%. The vegetation has an abundance of grass species and dicotyledonous forbs, while a woody vegetation component occurs as sheltered islands of temperate mountain bushveld within the grassland.

Lesedi Local Municipality (LLM)

Lesedi falls within three vegetation types: (Lesedi EMF, 2006). LLM falls within Moist Clay Highveld Grassland where *Themeda triandra* dominates in primary grassland; 72% of which is transformed and 0,29% conserved, and Rocky Highveld grassland with shallow rocky soil, which is a transitional type between the high inland plateau grassland and the lower inland plateau bushveld. Of the area covered by this type of grassland, 65% is

transformed and 1,38% conserved (Low and Rebelo, 1996). The area is very rich in herbaceous species, both dicotyledons and monocotyledons.

3.4 Samples and sampling sites

3.4.1 Sampling sites

Water samples were collected from the identified nine (9) sampling sites along the Blesbokspruit based on the activities and potential contributors or impactors on the quality of the river water. The sampling points were denoted as BB from 1-9, where BB was acronym of the name of the river "Blesbokspruit" while number 1-9 represent the nine sampling points along the river corresponding to the flow of water from up- to downstream. The quaternary drainage map and the Quaternary drainage topography map showing sampling points are represented in Figures 3.2 and 3.3 respectively.

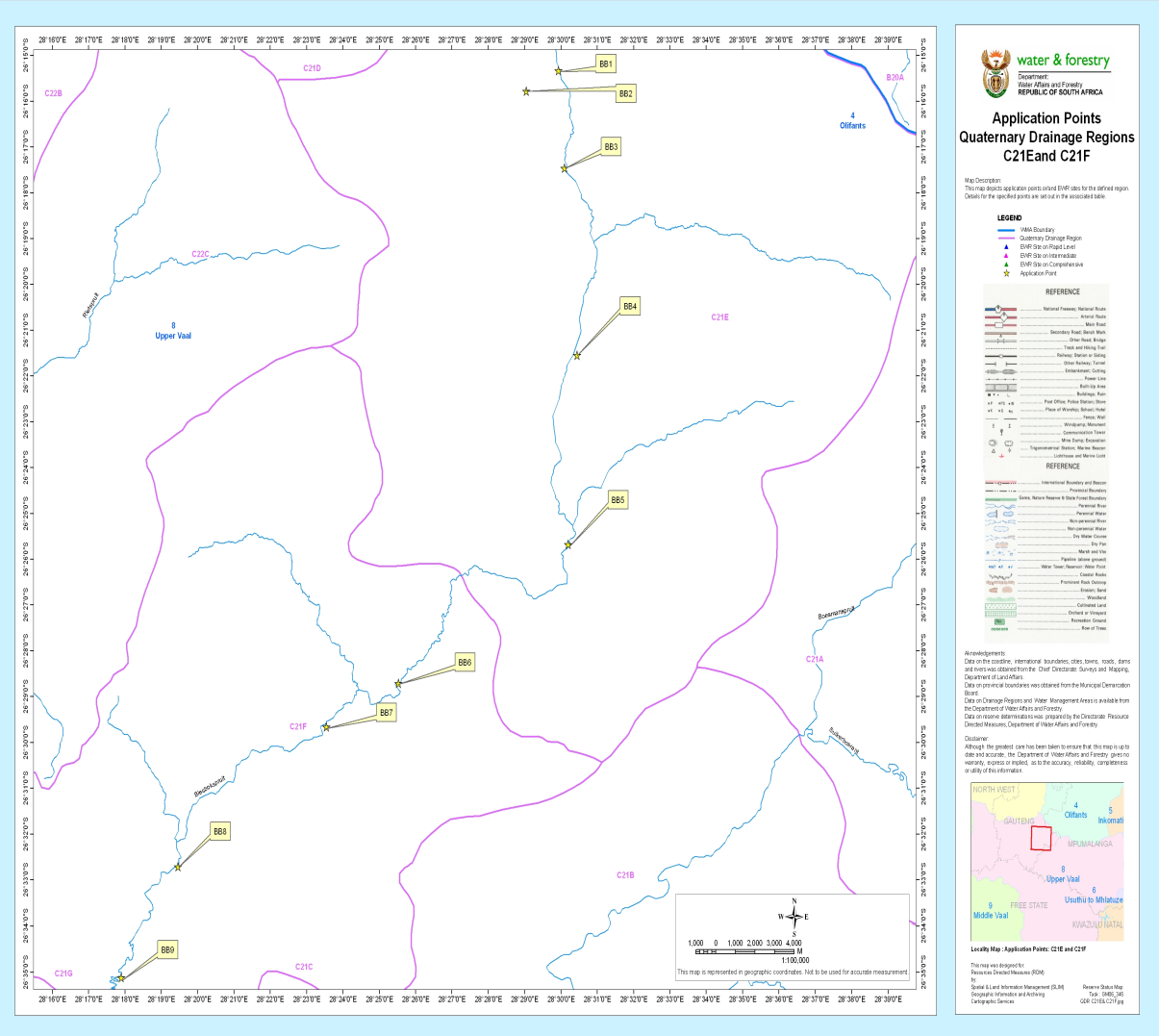

Figure 3.2: A Quaternary Drainage Map indicating the sampling points along the Blesbokspruit (represented as BB 1-9)

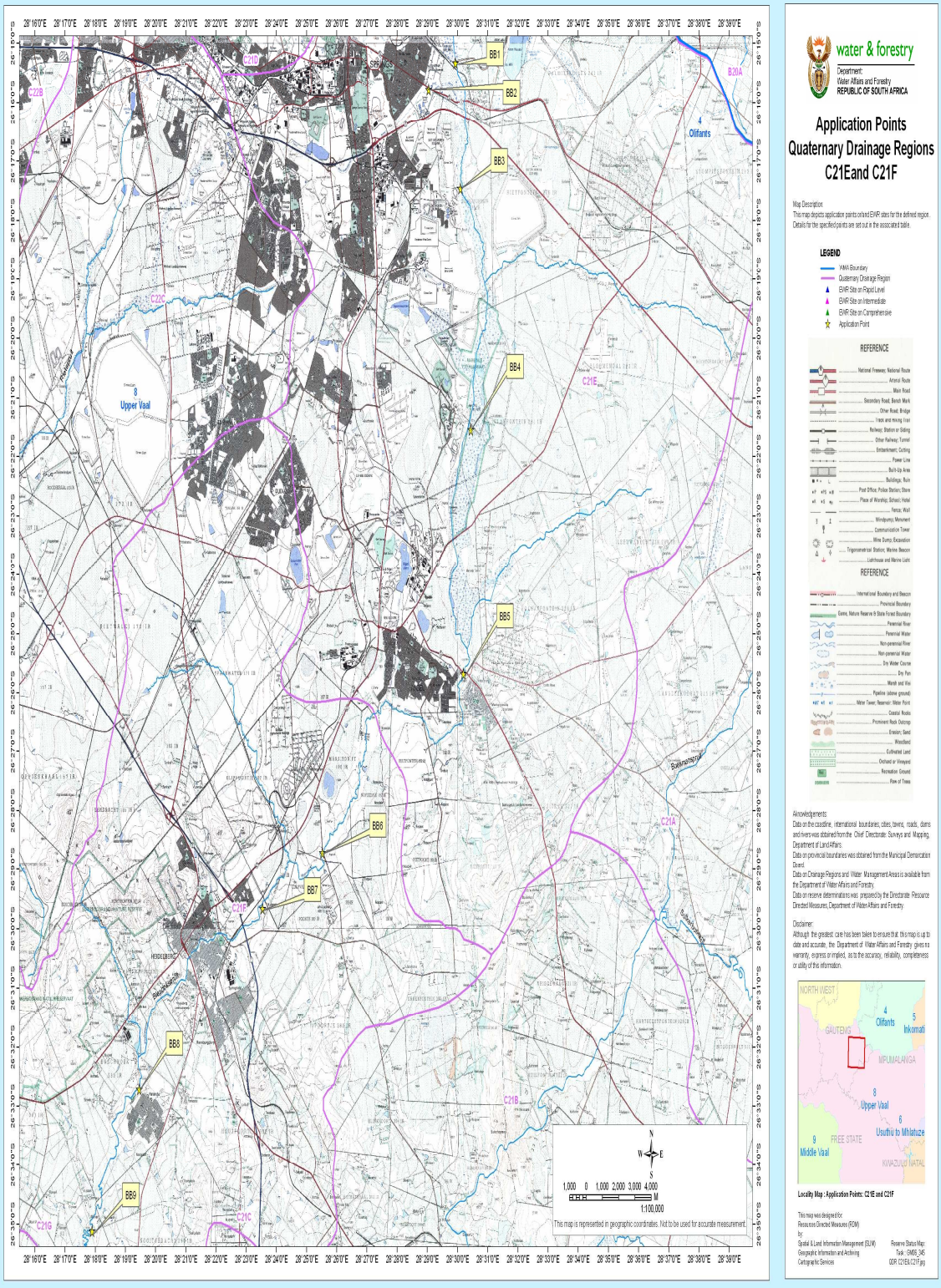

Figure 3.3: A Quaternary Drainage Topography Map indicating the sampling points along the Blesbokspruit (represented as BB 1-9)

The coordinates of the sampling points along the Blesbokspruit were taken using GPS 315 MAGELLIN and are presented in Table 3.2 below:

Table 3.2: Geographic positioning of the sampling sites

Sampling points	Location/position	Co-ordinate	Remark
BB1	Upstream	26° 15' 20" S 28° 29' 56" E	Located downstream of the Grootvlei Mine, where dewatering takes place
BB2		26° 15' 47" S 28° 29' 03" E	Located downstream of Erwat Ancor Sewage Works
BB3		26° 17' 28" S 28° 30' 06" E	Located at Daggafontein Mines (Tailing dams and old mine surrounds this point, there are also pipes indicating river diversion)
BB4		26° 21' 33" S 28° 30' 27" E	Located at Marivale Bird Sanctuary, to the north of this point is another shaft of Grootvlei Mine.
BB5		26° 25' 41.03" S 28° 30' 11.92" E	Located on R51 road to Balfour, also mine dumps around this point.
BB6		26° 28' 43" S 28° 25' 32" E	Located downstream of Erwat Herbert Bickley Sewage Works.
BB7		26° 30' 30" S 28° 25' 32" E	Located downstream of Heidelberg town and its residential areas.
BB8		26^0 32' 43 S 28^0 19' 28 E	Located downstream of Erwat Heidelberg Sewage Works
BB9	Downstream	26^0 35' 07 S 28^0 17' 54 E	Located downstream of Erwat Ratanda Sewage Works

3.5 Water sampling protocol

Two Litre (2L) of surface water samples were collected into clean acid washes plastic containers at about 5 cm below the surface of the water from sampling points. These were kept cool in a cooler box and transported to the laboratory for analyses. Water samples for microbiological investigations were collected in 2L sterile glass bottles and taken immediately to the laboratory for refrigerating. Analyses were carried out within 12h of sample collection according to Clesceri *et al.,* 1998 and RQS 1999. An overview of the physio-chemical parameters investigated is presented in Table 3.3 below

Table 3.3: An overview of the physio-chemical parameters investigated in the study

Determinant	Analytical Technique
Conductivity (mS/m)	Electrode on Radiometer pH meter
Suspended Solids (mg/L)	Gravimetric
Dissolved Solids (mg/L)	Gravimetric
Nitrate and Nitrite Nitrogen (mg/L)	Colorimetric, but with Segmented Flow Analyser
Orthophosphate (mg/LP)	Colorimeter, but with Segmented Flow Analyser
pH	Electrode on Radiometer Conductivity meter
Sulphate (mg/L SO_4)	Colorimetric, but with Segmented Flow Analyser
COD (mg/lO_2)	Coulometric (Potassium dichromate)
Cadmium (ICP)(mg/L Cg)	Perkin Elmer ICP- MS
Copper (ICP)(mg/L Cu)	Perkin Elmer ICP-MS
Iron (ICP)(mg/L Fe)	Perkin Elmer ICP-MS
Zinc(ICP)(mg/LZn)	Perkin Elmer ICPMS
Arsenic (ICP)(mg/L As)	Perkin Elmer ICP- MS
Faecal Coliforms (cfu/100mL)	Membrane Filtration (counts/100mL)
Total Coliforms(cfu/100mL)	Membrane Filtration (counts/100ml)
Heterotrophic Plate Count (cfu/1mL)	Standard plate count – Pour plate method (cfu/mL)
E.coli (cfu/100mL)	Indole Test

3.6 Materials and Methods

3.6.1 Conductivity (EC) in ms/m

Electrical conductivity measurement of water samples were carried out using Radiometer Conductivity Meter Model CDM83. The meter was equipped with electrode-CDC241-9 as well as temperature Sensor–T101. A commercial conductivity standard, 101.5mS/m±0.5% at 25°C (0.05% NaCl) was used to perform the precision calibration of the conductivity meter. The standard is stable for four (4) months Precision Calibration and determination of the temperature coefficient (TC %). The cell constant and TC (%) need to be adjusted monthly using a solution of known conductivity (101.5 mS/m) at a reference temperature of 25°C.

Instruments

1. Radiometer Conductivity Meter Model CDM83
2. SAC 80 sample changer with polyethylene sample cups
3. Conductivity electrode - CDC241-9
4. Temperature Sensor – T101

Reagents

1. Milli-RX water
2. Milli-Q water
3. Sodium Chloride (NaCl), AR grade
4. Renovo–N soaking solution
5. Conductivity standard (Radiometer 0.05%NaCl solution of 101.5mS/m±0.5% at 25°C
6. Ethylenediaminetetra-acetic acid, either the disodium or tetra sodium salt (EDTA) ($C_{10}H_{14}N_2Na_2O_8.H_2O$) or ($C_{10}H_{12}N_2Na_4O_8.2H_2O$)

Preparation of Reagents

All glassware was rinsed with Milli-RX water prior to reagent preparation.

1. Ethylenediaminetetre-acetic acid (EDTA) solution, 1%

Ten grams (10g) of Ethylenediaminetetra-acetic acid, di sodium or tetra sodium salt was dissolved in one litre (1L) Milli-RX water. This solution was stored in a polyethylene container and is stable for three (3) months at room temperature.

Standards

All glassware was rinsed with Milli-Q water prior to standard preparation.

1. Precision calibration standard

A commercial conductivity standard, 101.5mS/m±0.5% at 25°C (0.05%NaCl) was used to perform the precision calibration. The standard is stable for four (4) months

2. Validation Standard

1.0g Sodium chloride dried for one (1) hour at 105°C and left to cool in a desiccator was dissolved in a two (2) litre volumetric flask with Milli-Q water. This validation standard has a conductivity of 101.5mS/m. The solution was stored in the volumetric flask and remains stable for six (6) weeks at room temperature.

Procedures

1. Precision Calibration and determination of the temperature coefficient (TC%). The cell constant and TC (%) need to be adjusted monthly using a solution of known conductivity (101.5mS/m) at a reference temperature.

Determination of EC in water samples

The stirrer was connected to the Conductivity Meter Model CDM83. Samples were poured into clean sample cups and placed in a sample tray. The validation sample standard was

also poured into clean sample cups and supposed to be analysed every 10th and 30th position of the sample tray. Since the samples were less that 10, the second last sample was the validation standard. The electrodes were rinsed with Milli-RX water in between measurements to prevent contamination.

The instrument is connected to the IMS (Instrument Management System) from where sample runs are electronically executed, and to where measurements results are automatically written. Once all the samples have been analysed and results recorded, the stirrer was disconnected and the electrode stored in Milli-RX water overnight.

Quality Control of EC determination of water samples

A validation standard measuring 101.5mS/m was placed in sample position 8. The results are reported in mS/m, to 1 decimal place.

Conversion of results

To convert results expressed in mS/cm or µS/cm to mS/M, the following formula was used:

1 µS/cm = 0.1mS/m (divide the results by 10)

1mS/cm = 100mS/m (multiply the results with 100)

3.6.2 pH measurement of water samples

The pH measurement of water samples were carried out using Radiometer TTT85 Titrator pH Meter filled with a Radiometer GK2401C electrode and a Radiometer T101 Temperature sensor. The pH meter was regularly calibrated using a commercial Radiometer certified buffer solution, pH 4.005±0.010 at 25°C, and pH10.012±0.010 at 25°C were used for calibration. The buffer solutions were stored at room temperature and are stable for two (2) months once the containers have been opened.

Reagents

All reagents used were of analytical grade

1. Milli-RX water
2. Renovo–N soaking solution
3. Potassium Chloride (KCl) for storage
4. Potassium Chloride (KCl)
5. Saturated potassium chloride (KCl-L). This solution was obtained commercially.

6. Ethylenediaminetetra-acetic acid, either the di-sodium or tetra sodium salt (EDTA) ($C_{10}H_{14}N_2Na_2O_8.H_2O$) or ($C_{10}H_{12}N_2Na_4O_8.2H_2O$)
7. Buffer, pH 7 (Merck or BDH).
8. Buffers, pH 4 and pH 10 (Radiometer).

Preparation of Reagents

Storage Solution

- 0.5g potassium chloride was dissolved in 100mL pH 7 buffer
- The solution was stored in a glass container and is stable for one month at room temperature

Ethylenediaminetetra-acetic acid (EDTA) solution, 1%

10g of Ethylenediaminetetra-acetic acid, di-sodium or tetra sodium salt was dissolved in one litre (1L) Milli-RX water. This solution was stored in a polyethylene container and is stable for three (3) months at room temperature.

Standards

Calibration buffer solution

- A commercial Radiometer certified buffer solution, pH4.005±0.010 at 25°C, and pH 10.012±0.010 at 25°C were used for calibration.
- The buffer solutions were stored at room temperature and are stable for two (2) months once the containers have been opened.

Validation buffer solution

- A commercial colourless certified buffer solution, pH 7.00±0.02 at 20°C was used as a validation standard.
- The buffer solution was stored at room temperature and remains stable for two (2) months once the containers have been opened.

Procedures

The stirrer was connected to the Radiometer TTT85 Titrator pH Meter and the speed set between 7 and 8. The stirrer was taken out of the KCl, pH 7 solution. The cap covering the filling hole was removed to check the level of the saturated potassium chloride solution as well as the potassium chloride crystals in the pH electrode. The level of the saturated potassium chloride solution must be 0.5 to 1cm below the filling hole. The layer of the

potassium chloride crystals must be 0.5cm deep and the crystals must be able to move freely in the potassium chloride solution.

Calibration of the instrument

The instrument is calibrated prior to use in accordance with the standard operating instruction contained in the DTS 800 users' handbook.

1. Sample analysis

Before starting the analysis, it is essential to ensure that the instrument is calibrated and that the stirrer is connected correctly to the Radiometer TTT85 Titrator pH Meter. Samples were poured into clean sample cups and placed in a sample tray. The validation standard (pH7.0±0.02) was also poured into clean sample cups and supposed to be analysed every 19th position of the sample tray. Since the samples were less that 10, the second last sample was the pH 7 buffer standard. The pH electrodes were rinsed with Milli-RX water in between measurements to prevent contamination.

The instrument is connected to the IMS (Instrument Management System) from where sample runs are electronically executed, and to where measurements results are automatically written. Once all the samples have been analysed and results recorded, the stirrer was disconnected and the electrode stored in the potassium chloride solution overnight and the filling hole of the pH electrode closed with the cap.

3.6.3 Total Dissolved Solids (TDS) in mg/L

Equipments

1. Evaporating dishes: Dishes of 100mL capacity made of one of the following materials:
- Porcelain, 90-mm diameter
- Desiccator, provided with a desiccant containing a colour indicator of moisture concentration or an instrumental indicator
- Drying oven, for operation at 103°C to 105°C
- Analytical balance, capable of weighing to 0.1mg
- Glass-fibre filter disks without organic binder
- Filtration apparatus

i. Membrane filter funnel

ii. Filtration apparatus with reservoir and coarse (40µm to 60µm) fritted disk as filter support

- Suction flask
- Drying oven, for operation at 180°C±2°C

Procedures and Sample Analysis

Preparation of glass-fibre filter disk: Inset disk with wrinkled side up into filtration apparatus. Apply vacuum and wash disk with three successive 20mL volumes of reagent-grade water. Continue suction to remove all traces of water. Discard washings. Preparation of evaporating dish: Heat clean dish to 108°C±2°C for 1h in an oven. This is stored in a desiccator until needed. Weigh immediately before use.

Selection of filter and sample sizes: Choose sample volume to yield between 205 and 200mg dried residue. If more than 10 min are required to complete filtration, increase filter size or reduce volume.

Sample Analysis:

Stir sample with a magnetic stirrer and pipette a 10mL onto a glass-fibre filter with applied vacuum. Wash with three successive 10mL volumes of reagent –grade water, allowing complete drainage between washings, and continue suction for about three (3) minutes after filtration is complete. Transfer total filtrate (with washings) to a weighed evaporating dish and evaporate to dryness on a steam bath or in a drying oven. Dry evaporated sample for at least 1h in an oven at 108°C±2°C, cool in a desiccator to balance the temperature and weigh. Repeat drying cycle of drying, cooling, desiccating and weighing until a constant weight is obtained or change is less than 4% of previous weight. Analyse samples in duplicates.

Calculation and recording of result

The total dissolved solids were calculated as follows:

$$\text{mg total dissolved solids/L} = \frac{(A - B) \times 100}{\text{Sample volume, mL}}$$

Where:

A = Weight of dried residue + dish, in mg

B = Weight of dish, in mg

3.6.4 Total Suspended Solids (TSS) in mg/L

Equipments

1. Whatman glass microfibre, GF/C 47mm diameter
2. Filtration apparatus and receiving flask
3. Vacuum pump
4. Desiccator containing dried silica gel
5. Clean, dry watch glasses
6. Smooth tipped stainless steel forceps
7. Gradated measuring cylinder, glass
8. Thermometer, calibrated at 104°C
9. Timer
10. Stainless steel tray

Instruments

- Analytical balance, calibrated and capable of weighing to at least 0.0001g.
- Drying oven, thermostatically controlled, capable of maintaining a temperature of 104°C±1°C

Reagents

1. Cellulose powder DS-0
2. Milli-RX water

Procedure and analysis

Prior to analysis the glass microfibre filters were prepared as follows: Filters were soaked in Milli-RX water for 24 hours. The filters were then removed from the water and placed on the filtration apparatus. 200mL of Milli-RX water was filtered through with continued suction for approximately 3 min until the excess water has been drawn off. Filters were transferred to a Stainless steel tray and dried at 104°C±1°C for two (2) hours ±10 minutes and left to cool down. The filters were then stores in a desiccator containing dried silica gel for 1.5 weeks before use.

The samples were analysed within 24 hours of sampling and each sample done in supplicates. A bank was prepared by filtering 1000mL of Milli-RX water. The water from the same container was used for the blank and rinsing. The dried filter from the desiccator

was weighed to the nearest 0.0001g. The pre-weight filter was placed on the filtration apparatus using smooth tipped forceps. The samples were allowed to be at room temperature and sample shaken well. 100mL of the sample filtered at the pressure not exceeding 360mmHg and the filters remained flooded during the filtration process. With the vacuum still on, the sides of the cylinder was washed with Milli-RX water and added to the funnel. The funnel was also washed with Milli-RX water and the filter allowed to suck dry of excess water for 3 minutes. The vacuum was switched off and the filters transferred to the watch glass with smooth tipped forceps and dried in an oven at 104°C±1°C for 2 hours ±10 minutes. After drying the filters were removed and allowed to cool in a desiccator containing dried silica gel for 1 hour and the watch glasses containing filters weighed.

Calculation and recording of results

The total Suspended solids were calculated as follows:

$$\text{Dry mass (mg/L)} = \frac{Wfs - Wf}{V}$$

Where:

Wfs = Mass of filter plus suspended matter, in mg

Wf = Mass of filter prior to filtration, in mg

V = Volume of sample filtered, in L

The results were recorded to the nearest 0.1mg/L

Quality Control

The sample results were accepted if:

1. The duplicate sample results D2 is within ±10% of the average of D1 and D2. The results of D1 and D2 were recorder and the limits calculated as follows:

Average of D1 and D2 X 0.9 = Lower limit

Average of D1 and D2 X 1.1 = Upper limit

The results of the blank is less that the detection limit (DL)

3.6.5 Determination of Heavy Metals in Water Samples

The following equipments, instrument, reagents, standards, procedures and methods were used in the analysis of all the investigated metals i.e. Iron, Arsenic, Cadmium, Copper, and Zinc.

3.6.5.1 Iron (Fe)

Equipments

1. Polyethylene rinse solution bottles, 2L
2. Polyethylene sample vials, 15mL
3. Polyethylene standard vials, 50mL
4. Glass beaker, 100mL
5. Tygon sampling tubes, 0.76mm ID
6. Tygon auto sampler rinsing solution and waste tubes, 1.143mm ID
7. Tygon tube for nebuliser, 3.0mm ID
8. Teflon capillary tube, 0.71mm ID
9. Scott Type spray chamber
10. Demountable axially aligned quartz torch
11. Auto sampler racks
12. Polyethylene waste containers

Instruments

Perkin Elmer dual View Model (optima) 4300DV Inductively coupled plasma Atomic Emission Spectrometer (ICP-AES) equipped with background correction.
Radio frequency generator at 40MHz
UV Detector detecting in the range 167-403nm
Visible Detector, detecting in the range 404-782nm
Compressor equipped with driers for shear gas generation, (pressure 550-825kPa)
Chiller filled with coolant fluid (pressure 310-550kPa)
Argon gas supply, instrument grade gas (5.0), (pressure 275kPa)

Reagents

1. Milli-RX water, resistivity >10M.cm
2. Nitric acid (HNO_3) solution, 1% (v/v)
3. Ethanol
4. Potassium dichromate ($K_2Cr_2O_7$)

5. Sulphuric acid, concentrated

Standards

The following standards were used:

1. Working Calibration standards
2. Control standards
3. Validation standards
4. Spike standards
5. Mn solution, 10mg/L

Blank Samples

Two types of blanks were prepared for the analysis of samples. The calibration blank was used in establishing the analytical curve while the method blank was used to correct for possible contamination resulting from varying amounts of the acids used in the sample preparation.

1. Calibration Blank

A 1% (v/v) HNO_3 solution was used as the calibration blank and placed in position 1 of the auto sampler rack.

2. Method Blank

The method blank was prepared using Milli-RX water as a sample. The method blank was placed in position 10 on the auto sampler rack.

Spiked Samples

A spiked sample was prepared to contain an additional concentration of 1mg/L of each of the elements to be analysed (Cu, Fe, Zn, Cd, As) for the full trace analysis.

Procedure

The room temperature was adjusted to 17°C. The Perkin Elmer Dual View Inductively Coupled Plasma (ICP) was switched on and all the equipments attached checked if they are working properly.

Table 3.4: The instrumental operating conditions used for analyses

Parameter	Settings
Power	1300W at 40MHz
Cooling gas flow rate	15L/min Argon
Auxiliary gas flow rate	0.20L/min Argon
Nebuliser gas flow rate	0.80L/min Argon
Pump flow rate	1.5 mL/min

1% (v/v) HNO_3 was used to rinse between different analyses. The calibration on the instrument was done before the start of the analyses using calibration blank 1% (v/v) HNO_3 as well as the working calibration standards as shown below:

Standard	Auto Sampler Position	Elements
	1	Calibration Blank
1.1	2	Fe and Zn
1.2	3	Fe and Zn
1.3	4	Fe and Zn
1.4	5	Fe and Zn
2.1	6	Cd, and Cr
2.2	7	Cd, and Cr
2.3	8	Cd, and Cr
2.4	9	Cd, and Cr
3.1	10	As and Zn
3.2	11	As and Zn
3.3	12	As and Zn
3.4	13	As and Zn
Control Std	20	All elements

The programme calculates the correlation coefficient value using the linearity calculated intercepts method. The value must be ≥0.99 for each element for the calibration to be accepted, and then the analysis can begin.

Analysis

The following sequence was followed when analysing samples using the ICP-

Auto Sampler Location	Sample
21	Method Blank
	Control Standard (2mg/L)
22	Validation Standard
23	Sample (Sample used to prepare spike)
24	Spiked Sample
25	Sample 1
26	Sample 2
27	Sample 3
28	Sample 4
29	Sample 5
30	Sample 6

	Sample 7
	Control Standard (2mg/L)
	10 Samples, Control Standard, 10 Samples, Control Standard, etc
	The last analysis was the Control Standard

Quality Control and Data Processing

The instrument was calibrated with 1%(v/v) HNO_3 solution as well as the working calibration standards.

1. Control Standard

The standard was done to check if any significant drift has occurred.

2. Method Blank

The results of the method blank were subtracted from each sample to make correction for any possible contamination during sample preparation.

3. Validation Standard

The validation standard was analysed before running the samples and concentration values within the 10% of the actual value was accepted.

4. Spiked sample

The sample (sample used to prepare the spike) and the spiked samples were analysed. The % recovery accepted was between 80% and 120%. The % recovery was calculated as follows:

$$\% \text{ recovery} = \frac{C_{spike} - C_{sample}}{Z} \times 100$$

Where:

C_{spike} = analyte concentration in the spiked sample (mg/L)

C_{sample} = analyte concentration of the sample, (mg/L)

Z = Concentration added to the spiked sample, (mg/L)

Detection Limit (DL)

The DL was determined by running a method blank 10 times and the 2mg/L control standard 10 times. This was done separately for the dissolved and acid extractable metals. The formula used for the determination of the DL is as follows:

$$DL = \frac{3\sigma_{\text{blank intensity}} \text{ X Standard Concentration}}{\text{(Std Intensity-Blank Intensity)}}$$

3.6.6 Nitrate nitrites nitrogen (NO_3-NO_2-N) in mg/L

Equipments

- Phosphate- free membrane filter, 47mm and 0.45µm pore size
- Glass beaker
- Measuring cylinders
- Non-calibrated volumetric flask for the preparation of reagents
- Amber glass containers for storage of reagents
- Open tube cadmium reactor, coated, 30cm
- Copper tube, 2mm ID and ±10cm long
- Calibrated volumetric flask and pipettes for the preparation of standards
- Polyethylene containers for the storage of standards and reagents

Instruments

- TRAACS 800 equipment with a 10mmX0.5mm diameter flow cell and a 520nm filter and Auto sampler with polystyrene sample cups

Reagents

Only AR grade reagents were used

- Milli-RX water and Milli-Q water
- Phosphoric acid (H_3PO_4)
- Sulphanilamide ($C_6H_8N_2O_2S$)
- N-(1-naphthyl) ethylenediamine dihydrochloride ($C_{10}H_7NHCH_2CH_2NH_2.2HCl$)
- Ammonia chloride (NH_4Cl)
- Copper sulphate ($CuSO_4.5H_2O$)
- Brij-35 (30% w/v)
- Potassium nitrite (KNO_3)

- Ammonia Solution (NH_3)
- Hydrochloric acid (HCl), concentrated
- Ethylenediaminetetra-acetic acid, disodium (EDTA) ($C_{10}H_{14}N_2Na_2O_8.H_2O$)
- Ethylenediaminetetra-acetic acid, tetra sodium salt (EDTA) ($C_{10}H_{12}N_2Na_4O_8.2H_2O$)

Preparation of Reagents

All glassware was rinsed with Milli-RX water prior to reagent preparation.

i. Colour Reagent

12.5mL of concentrated phosphoric acid was added to 100mL Milli-RX water and 5g of sulphanilamide and 0.25g N-(1-naphthyl) ethylenediamine dihydrochloride was dissolved into the phosphoric solution and diluted with Milli-RX water. The solution was stored in a closed amber glass bottle at room temperature. The solution is stable for 2 weeks.

ii. Wash Water: Milli-RX water was used as wash water

iii. Ammonium Solution for pH adjustment

10mL concentrated ammonia solution was diluted to 100mL with Milli-RX water and stored in a closed polyethylene container. The solution is stable for three months at room temperature.

iv. Hydrochloric acid solution for pH adjustment, 50%

50mL of concentrated hydrochloric acid was added to 50mL Milli-RX water and stored in a closed polyethylene container. The solution is stable for three months at room temperature.

v. Ammonium Chloride Buffer Solution

30g ammonium chloride and 0.2g EDTA disodium salt were dissolved in 750mL Milli-RX water. 3mL Brij-35 wetting agent wad added and diluted to 1 litre. The pH was adjusted to 6.6 by adding 50% (v/v) hydrochloric acid. The solution was stored in a closed polyethylene container and is stable for one month at room temperature.

vi. <u>Activating copper solution</u>

1.25g of copper sulphate was dissolved in 600mL of Milli-RX water and 3mL Brij-35 added and the solution mixed thoroughly and diluted to 1 litre. The solution was stored in a closed polyethylene container and is stable for three months at room temperature.

vii. Hydrochloric acid, 2mol/vol

165mL concentrated hydrochloric acid (HCl) was diluted in 1 litre Milli-RX water and 0.5mL Brij-35 added. The solution was stored in a closed polyethylene container and is stable for three months at room temperature.

viii. Ethylenediaminetetre-acetic acid (EDTA) solution, 1%

Ten grams (10g) of Ethylenediaminetetra-acetic acid, tetra sodium salt was dissolved in one litre (1L) Milli-RX water. This solution was stored in a polyethylene container and is stable for three (3) months at room temperature.

Standards

All glassware was rinsed with Milli-Q water prior to standard preparation.

1. Nitrate Stock Standard Solution

0.7217g potassium nitrate (dried overnight at 60°C and left in a desiccator to cool) was dissolved in 700mL Milli-Q water and 1mL of a 8.12g/L mercury chloride solution was added to give a final concentration of 6mg Hg (II) and then diluted to 1 litre with Milli-Q water. This solution contains 0.1mg NO_3-N/mL and remains stable for six (6) months when stored in a polyethylene container at < 6°C.

2. Nitrate Calibration Standard Solution

A series of standard solutions was prepared in a 1 litre volumetric flask by quantitative dilution of the stock solution as per table below. The standard solutions was preserved by adding 1mL of a 8.12g/L mercury chloride prior to final dilution to give a final concentration of 6mg/L Hg (II) and stored at < 6°C.

Table: 3.5: Volume of Standard Stock Solution diluted to a 1 litre to prepare the Calibration Standard

Volume of Stock Solution (mL)	NO_3-N (mg/L)
4.0	0.40
10.0	1.00
20.0	2.00
30.0	3.00
40.0	4.00

3. Validation Standard

A commercial potassium nitrate was used to prepare the stock standard.

0.7217g potassium nitrate (dried overnight at 60°C and left in a desiccator to cool) was dissolved in 700mL Milli-Q water and 1mL of a 8.12g/L mercury chloride solution was added to give a final concentration of 6mg Hg (II) and then diluted to 1 litre with Milli-Q water. This solution contains 0.1mg NO_3-N/mL and remains stable for six (6) months when stored in a polyethylene container at < 6°C.

Quantitatively, 7.0mL of the validation stock solution was diluted with Milli-Q water in a 1 litre volumetric flask and preserved with 1mL of a 8.12g/L mercury chloride solution prior to final dilution. The validation standard contains a final concentration of 0.70mg NO_3-N/mL and remains stable for six (6) weeks when stored in a polyethylene container at < 6°C.

Procedure

The sample should be filtered through a pre-washed 0.45µm membrane. All the reagent tubes were thoroughly cleaned before placing in any reagents. The cadmium coil was also rinsed with Milli-RX water before operating the system. The buffer line was put into the Milli-RX water and pumped for 5 minutes. The working buffer line was removed from the Milli-RX water and sequentially a 2mol/L HCL pumped through the buffer line for 2 minutes, activating copper solution for 10 minutes and Milli-RX water for 5 minutes. The working buffer line was placed back into its container and allowed to pump for 10 minutes. A range of diluted samples were prepared and run through the flow system/instrument. Once all the samples have been run through, the flow system was rinsed out by pumping Milli-RX water through the reagent line for 15 minutes.

Quality Control

A 0.70mg/L validation standard was analysed every 5^{th} sample and the results reported as mg/L (NO_3+NO_2) - N.

3.6.7 Orthophosphate as phosphorus (PO_4) in mg/L

Equipments

1. Phosphate- free membrane filter, 47mm and 0.45µm pore size
2. Glass beaker
3. Measuring cylinders
4. Calibrated volumetric flask and pipettes for the preparation of standards
5. Non-calibrated volumetric flask for the preparation of reagents
6. Polyethylene containers for the storage of standards and reagents
7. Amber glass containers for storage of reagents

Instruments

1. TRAACS 800 equipment with a 10mm X 0.5mm diameter flow cell and a 660nm filter
2. Autosampler with polystyrene sample cups

Reagents

Only AR grade reagents were used

1. Milli-RX water
2. Milli-Q water
3. Sulphuric acid (H_2SO_4), concentrated
4. Sodium Chloride (NaCl)
5. Aerosol-22
6. Ammonium molybdate tetrahydrate ($(NH_4)_6$ ($MO_7O_{24}.4H_2O$)
7. Potassium antimony (+) tartrate hemihydrate ($K(SbO)(C_4H_4O_6.½H_2O$)
8. Ascorbic acid ($C_6H_8O_6$)
9. Potassium dihydrogen phosphate ($KH_2.PO_4$)
10. Mercury Chloride ($HgCl_2$)
11. Sodium hydroxide (NaOH)
12. Ethylenediaminetetra-acetic acid, either the disodium or tetrasodium salt (EDTA) ($C_{10}H_{14}N_2Na_2O_8.H_2O$) or ($C_{10}H_{12}N_2Na_4O_8.2H_2O$)

Preparation of Reagents

All glassware was rinsed with Milli-Q water prior to reagent preparation.

i. Wash Water: Milli-Q water was used as wash water.

ii. Sodium hydroxide Solution

5g of sodium hydroxide was dissolved in Milli-Q water and diluted to 500mL and stored in a closed polyethylene container. The solution is stable for three months at room temperature.

iii. Sulphuric Acid Solution

230mL of concentrated sulphuric acid was added to 200mL Milli-Q water and diluted to 500mL. The solution is stable for three months at room temperature and was stored in a closed polyethylene container.

iv. Sodium Chloride Solution

5g of sodium chloride was dissolved in Milli-Q water, diluted to 1 litre and 2mL of Aerosol-22 was added to the solution. The solution remains stable for one month at room temperature and was stored in a closed polyethylene container.

v. Ammonium Molybdate Solution

25g of Ammonium molybdate tetrahydrate was dissolved in 700mL Milli-Q water and diluted to 1 litre. The solution remains stable for three (3) months and was stored in a closed amber glass container at room temperature.

vi. Antimony Potassium Tartrate Solution

0.25g of antimony potassium tartrate was dissolved in 70mL Milli-Q water and diluted to 100mL. This solution is prepared immediately prior to use.

vii. Combined Reagents

275mL of Ammonium molybdate tetrahydrate, 125mL Sulphuric acid solution and 100mL Ammonium molybdate tetrahydrate were combined gradually and thoroughly mixed. The solution remains stable for one (1) month when stored in a closed amber glass container at room temperature.

viii. Ascorbic Acid Solution

6.25g ascorbic acid was dissolved in Milli-Q water and diluted to 250mL. The solution remains stable for one (1) week when stored in a closed amber glass container at <6°C.

ix. Ethylenediaminetetre-acetic acid (EDTA) solution, 1%

Ten grams (10g) of Ethylenediaminetetra-acetic acid, tetra sodium salt was dissolved in one litre (1L) Milli-RX water. This solution was stored in a polyethylene container and is stable for three (3) months at room temperature.

Standards

All glassware was rinsed with Milli-Q water prior to standard preparation.

i. Phosphate Stock Standard Solution

0.0549g potassium dihydrogen phosphate (dried for 1hour at 105°C and left in a desiccator to cool) was dissolved in Milli-Q water and diluted to 1 litre. The solution was preserved by adding 1mL of a 8.12g/L mercury chloride solution to give a final concentration of 6mg Hg (II) prior to final dilution. The stock standard solution contains 0.0125mg PO_4-P/mL and remains stable for six (6) months when stored in a polyethylene container at < 6°C.

ii. Phosphate Calibration Standard Solution

A series of standard solutions was prepared in a 1litre volumetric flask by quantitative dilution of the stock solution as per table below. The standard solutions was preserved by adding 1mL of 8.12g/L mercury chloride prior to final dilution to give a final concentration of 6mg/L Hg (II) and stored at < 6°C.

Table: 3.5: Volume of Standard Stock Solution diluted to a 1 litre to prepare the Calibration Standard

Volume of Stock Solution (mL)	PO_4-P (mg/L)
4.0	0.050
10.0	0.125
20.0	0.250
30.0	0.375
40.0	0.500

i. Validation Standard

A commercial potassium dihydrogen phosphate was used to prepare the stock standard.

0.0549g potassium dihydrogen phosphate (dried for 1hour at 105°C and left in a desiccator to cool) was dissolved in Milli-Q water, preserved by adding 1mL of a 8.12g/L mercury chloride solution to give a final concentration of 6mg Hg (II) and then diluted to 1 litre with Milli-Q water. This solution contains 0.0125mg PO_3-P/mL and remains stable for six (6) months when stored in a polyethylene container at < 6°C.

Quantitatively, 8.0mL of the validation stock solution was diluted with Milli-Q water in a 1 litre volumetric flask and preserved with 1mL of a 8.12g/L mercury chloride solution prior to final dilution. The validation standard contains a final concentration of 0.100mg PO_3-P/L and remains stable for six (6) weeks when stored in a polyethylene container at < 6°C.

Procedure

The sample should be filtered through a pre-washed 0.45 µm membrane. All the reagent tubes were thoroughly cleaned before placing in any reagents. A range of diluted samples were prepared and run through the flow system/instrument. Once all the samples have been run through, the flow system was rinsed with the rinsing solution for 15 minutes followed by Milli-Q water for 10 minutes.

Quality Control

A 0.100 mg/L validation standard was analysed every 5^{th} sample and the results reported as mg/L PO_4-P.

3.6.8 Sulphates (SO_4) in mg/L

Equipments

1. Phosphate- free membrane filter, 47mm and 0.45µm pore size
2. Glass beaker
3. Measuring cylinders
4. Calibrated volumetric flask and pipettes for the preparation of standards
5. Non-calibrated volumetric flask for the preparation of reagents
6. Polyethylene containers for the storage of standards and reagents
7. Amber glass containers for storage of reagents

Instruments

- TRAACS 800 equipment with a 10mm X 0.5mm diameter flow cell and a 660nm filter and auto sampler with polystyrene sample cups

Reagents

Only AR grade reagents were used

1. Milli-RX water
2. Milli-Q water
3. Sulphuric acid (H_2SO_4), concentrated
4. Sodium Chloride (NaCl)
5. Aerosol-22
6. Ammonium molybdate tetrahydrate ($(NH_4)_6$ $(MO_7O_{24}.4H_2O)$
7. Potassium antimony (+) tartrate hemihydrate ($K(SbO)(C_4H_4O_6.½H_2O)$
8. Ascorbic acid ($C_6H_8O_6$)
9. Potassium dihydrogen sulphate ($KH_2.SO_4$)
10. Mercury Chloride ($HgCl_2$)
11. Sodium hydroxide (NaOH)
12. Ethylenediaminetetra-acetic acid, either the disodium or tetrasodium salt (EDTA) ($C_{10}H_{14}N_2Na_2O_8.H_2O$) or ($C_{10}H_{12}N_2Na_4O_8.2H_2O$)

Preparation of Reagents

All glassware was rinsed with Milli-Q water prior to reagent preparation.

i. Wash Water: Milli-Q water was used as wash water.

ii. Sodium hydroxide

5g of sodium hydroxide was dissolved in Milli-Q water and diluted to 500mL and stored in a closed polyethylene container. The solution is stable for three months at room temperature.

iii. Sulphuric Acid Solution

230mL of concentrated sulphuric acid was added to 200mL Milli-Q water and diluted to 500mL. The solution is stable for three months at room temperature and was stored in a closed polyethylene container.

iv. Sodium Chloride Solution

5g of sodium chloride was dissolved in Milli-Q water, diluted to 1 litre and 2mL of Aerosol-22 was added to the solution. The solution remains stable for one month at room temperature and was stored in a closed polyethylene container.

v. Ammonium Molybdate Solution

25g of Ammonium molybdate tetrahydrate was dissolved in 700mL Milli-Q water and diluted to 1 litre. The solution remains stable for three (3) months and was stored in a closed amber glass container at room temperature.

vi. Antimony Potassium Tartrate Solution

0.25g of antimony potassium tartrate was dissolved in 70mL Milli-Q water and diluted to 100mL. This solution is prepared immediately prior to use.

vii. Combined Reagents

275mL of Ammonium molybdate tetrahydrate, 125mL Sulphuric acid solution and 100mL Ammonium molybdate tetrahydrate were combined gradually and thoroughly mixed. The solution remains stable for one (1) month when stored in a closed amber glass container at room temperature.

viii. Ascorbic Acid Solution

6.25g ascorbic acid was dissolved in Milli-Q water and diluted to 250mL. The solution remains stable for one (1) week when stored in a closed amber glass container at <6°C.

ix. Ethylenediaminetetre-acetic acid (EDTA) solution, 1%

Ten grams (10g) of Ethylenediaminetetra-acetic acid, tetra sodium salt was dissolved in one litre (1L) Milli-RX water. This solution was stored in a polyethylene container and is stable for three (3) months at room temperature.

Standards

All glassware was rinsed with Milli-Q water prior to standard preparation.

i. Phosphate Stock Standard Solution

0.0549g potassium dihydrogen sulphate (dried for 1hour at 105°C and left in a desiccator to cool) was dissolved in Milli-Q water and diluted to 1 litre. The solution was preserved by adding 1mL of a 8.12g/L mercury chloride solution to give a final concentration of 6mg Hg (II) prior to final dilution. The stock standard solution contains 0.0125mg SO_4/mL and remains stable for six (6) months when stored in a polyethylene container at < 6°C.

ii. Phosphate Calibration Standard Solution

A series of standard solutions was prepared in a 1 litre volumetric flask by quantitative dilution of the stock solution as per table below. The standard solutions was preserved by adding 1mL of a 8.12g/L mercury chloride prior to final dilution to give a final concentration of 6mg/L Hg (II) and stored at < 6°C.

Table: 3.5: Volume of Standard Stock Solution diluted to a 1 litre to prepare the Calibration Standard

Volume of Stock Solution (mL)	SO_4 (mg/L)
4.0	0.050
10.0	0.125
20.0	0.250
30.0	0.375
40.0	0.500

iii. Validation Standard

A commercial potassium dihydrogen sulphate was used to prepare the stock standard. 0.0549g potassium dihydrogen sulphate (dried for 1 hour at 105°C and left in a desiccator to cool) was dissolved in Milli-Q water, preserved by adding 1mL of a 8.12g/L mercury chloride solution to give a final concentration of 6mg Hg (II) and then diluted to 1 litre with Milli-Q water. This solution contains 0.0125mg PO_3-P/mL and remains stable for six (6) months when stored in a polyethylene container at < 6°C.

Quantitatively, 8.0mL of the validation stock solution was diluted with Milli-Q water in a 1 litre volumetric flask and preserved with 1mL of a 8.12g/L mercury chloride solution prior to final dilution. The validation standard contains a final concentration of 0.100mg SO_4/L and remains stable for six (6) weeks when stored in a polyethylene container at < 6°C.

Procedure and Quality Control

The sample should be filtered through a pre-washed 0.45µm membrane. All the reagent tubes were thoroughly cleaned before placing in any reagents. A range of diluted samples were prepared and run through the flow system/instrument. Once all the samples have been run through, the flow system was rinsed with the rinsing solution for 15 minutes

followed by Milli-Q water for 10 minutes. A 0.100mg/L validation standard was analysed every 5th sample and the results reported as mg/L SO_4.

3.6.9 Chemical Oxygen Demand (COD)

Equipments

1. Matched pair of glass cuvettes with a 1cm path length
2. Digested tubes with mixture and caps with septa (15mL)
3. Propipette
4. Glass beaker, 100mL
5. Vortex mixer
6. Calibrated thermometer
7. Calibrated volumetric flasks, 500mL and 100mL
8. Calibrated balance
9. Calibrated pipettes, 3mL, 4mL, 10mL, 20mL, 30mL, 40mL and 80mL
10. Centrifuge

Instruments

1. Digestion block (148°C±5°C)
2. UV-Visible Spectrophotometer, for use at 445nm

Reagents

- Milli-RX water, resistivity > 10MΩ.cm
- Sulphuric acid (H_2SO_4), solution, 1% (v/v)
- Potassium hydrogen phthalate (KHP), from supplier 1
- Potassium hydrogen phthalate (KHP), from supplier 2 for validation
- Merck COD cell test: Method photometric, 10-150mg/L (part nr. 1.14540.0001).
- Merck COD cell test: Method photometric, 100-1500mg/L (part nr. 1.14541.0001).
- COD certified reference material (CRM) with a concentration of 100mgO_2/L

Calibration Standards

i. Potassium hydrogen phthalate (KHP) calibration stock standard

KHP was dried for 2 hours in an oven at 120°C and allowed to cool in a desiccator. 0.2125g KHP was dissolved in Milli-RX water and diluted to 500mL in a calibrated

volumetric flask. The solution has a theoretical value of 500mg O_2/L and remains stable for 2 months when stored at ≤ 6°C.

ii. Calibration working standard

Calibration working standards were prepared in 100mL calibrated volumetric flask by using the volumes as per the table below, and diluted to 100mL using Milli-RX water. The standards were allowed to reach room temperature before use. The standards were prepared before use.

Table 3.6: Preparation of calibration working Standards.

STANDARDS FOR THE LOW CONCENTRATION CALIBRATION RANGE	
Volume of Calibration Stock Standard	**Concentration of KHP calibration standards (mg O_2/L)**
4mL	20
10mL	50
20mL	100
30mL	150
STANDARDS FOR THE HIGH CONCENTRATION CALIBRATION RANGE	
Volume of Calibration Stock Standard	**Concentration of KHP calibration standards (mg O_2/L)**
10mL	50
20mL	100
40mL	200
80mL	400

Note: The 100mg/L calibration standard is used as the control sample if the low concentration are used and a 200mg/L calibration standard is used as the control sample for the high concentration range.

Validation Standards

i. Potassium hydrogen phthalate (KHP) calibration stock standard

KHP from a different supplier (supplier 2) was dried for 2 hours in an oven at 120°C and allowed to cool in a desiccator. 0.2125g KHP was dissolved in Milli-RX water and diluted to 500mL in a calibrated volumetric flask. The solution has a theoretical value of 500mg O_2/L and remains stable for 2 months when stored at ≤ 6°C.

ii. Validation working standard

100mg/L validation working standard was prepared for the low concentration calibration range by using 20mL of the validation stock standard and diluting it to 100mL with Milli-RX water. 200mg/L validation working standard was prepared for the high concentration calibration range by using 40mL of the validation stock standard and diluting it to 100mL with Milli-RX water.

Blanks

Method blank: For the unpreserved samples, the blank is prepared with Milli-RX water, if the samples are preserved with sulphuric acid, the blank is prepared using 1% (v/v) sulphuric solution.

Calibration blank: Milli-RX water is used to prepare the calibration blank.

Spiked sample

If the low concentration calibration range is done, a sample spiked with 100mg O_2/L must be prepared using one of the samples in the batch, Add 20mL of the 500mg/L calibration stock standard to a calibrated 100mL volumetric flask and make up to the mark with the chosen sample.

If the high concentration calibration range is done, a sample spiked with 200mg O_2/L must be prepared using one of the samples in the batch, Add 40mL of the 500mg/L calibration stock standard to a calibrated 100mL volumetric flask and make up to the mark with the chosen sample.

Procedure

The digestion block was switched on and all samples done in duplicates. The digestion tubes for all the samples in the batch as well as the blank were labelled; method blank, calibration blank, four calibration standards, CRM, validation standard and the spiked sample. All samples and standards were allowed to reach room temperature before starting with the analysis. The precipitate was suspended by using a vortex mixer before use. 3mL of the sample, standard, CRM or blank was allowed to rum down the inside of the tilted reaction cell into the reagent using a calibrated pipette. Turbid samples were shaken before analysis. The cap of the cell was screwed tightly and once all the samples, standard, and blank were added, the vortex mixer was used to vigorously mix the contents of each cell reaction. The samples were immediately placed in the digestion block. The prepared tubes were placed in a pre-heated digestion block and digested at 148°C±5°C for 2 hours. The tubes were removed from the digestion block and left to cool to room temperature before taking a reading. **(Readings were taken within 2 hours)**. The UV-vis spectrophotometer was set to 445nm. After every 10^{th} sample, either 100mg/L or the 200mg/L standard was read. The last sample for each batch must also be a control standard.

The following sequence was followed when analysing samples;

Table 3.7: Sequence followed when analysing for COD

Analysis Sequence	Sample
1	Calibration blank
2	Calibration Standards
3	Method blank
4	Validation Standard
5	Sample (sample used to prepare spike)
6	Spiked sample
7	CRM
8	Sample 1 to 10
9	Control standard (100 mg/L)
10	Sample 11 to 20
11	Control Standard (100 mg/L)
	Etc

Quality Control

The calibration R-squared value must be ≥0.99. The detection limit (DL) is 10mg O_2/L and the value of the blank reading should be less that the DL. The recovery on the spike should be within 15% of the real value. The validation standard must also fall within ±15% range.

Calculations

1. The % recovery was calculated as follows:

$$\% \text{ recovery} = \frac{C_{spike} - C_{sample}}{Z} \times 100$$

Where:

C_{spike} = measured concentration in the spiked sample,(mg/L)

C_{sample} = measured concentration of the sample, (mg/L)

Z = Concentration added to the spiked sample, (mg/L)

2. Detection Limit (DL)

The DL was determined by running a blank and the 100mg O_2/L standard 10 times on absorbency. The DL is calculated at better that 99% confidence level.

$$DL = \frac{3\sigma_{blank\ absorbance} \times \text{Standard Concentration}}{\text{(Std absorbance-Blank absorbance)}}$$

3.6.10 Total Coliforms (TC) in cfu/100mL

Equipments

1) Incubator capable of maintaining a temperature of 35°C±1°C
2) Filtration apparatus, manifold with receiving filter flask
3) Moisture trap between the filter flask and the vacuum pump
4) Sterile membrane filter units (filter base and funnel)
5) Vacuum pump
6) Sterile, white, gridded, 47mm diameter membrane filters, 0.45µm pore size
7) Sterile, disposable, plastic Petri dishes, 50mm, with tight fitting lids (Millipore PD10 047 or equivalent)
8) Smooth tipped forceps
9) Calibrated pipettors, 1mL, 5mL, 9mL, and 10 mL with sterile tips
10) Sterile graduated measuring cylinder, glass, 100mL
11) Autoclave
12) Test Tubes with caps, 20mL
13) Bunsen burner
14) Test tube rags, polypropylene or metal
15) Calibrated analytical balance
16) Biological safety cabinet
17) Inoculating loop
18) Calibrated maximum thermometer (121°C)
19) Calibrated thermometers at the relevant temperatures
20) Vortex Mixer
21) Autoclave tape
22) Min/Max thermometer

Reagents and Media

1) m-Endo Agar LES, Difco
2) Ringer's Dilution Solution
3) Ethanol, 99.8%
4) Disinfectant
5) Biological/ Chemical Indicator for autoclaves

Preparation of Reagents

i. m-Endo Agar LES

The medium is obtained commercially and should be stored in dark areas as it is sensitive to light. The agar plates can be store in the refrigerator for 1 week.

ii. Ringer's Dilution Solution: The solution is obtained commercially

iii. Ethanol:

Prepare 70% (v/v) by adding 700mL ethanol (99.8%) to a 1L volumetric flask and make up to the mark with Milli-RX water. The solution is stable for two months at room temperature when stored in a glass container.

iv. Disinfectant: The solution is obtained commercially

v. Biological/Chemical Indicator: The solution is obtained commercially

Control Cultures

- Positive Control Culture: A commercially certified pure culture of *Enterobacter cloacae* was used as a positive control culture
- Negative control Culture: A commercially certified pure culture of *Staphylococcus aureus* was used as a negative control culture

Analysis

Samples must be kept in a refrigerator below 6°C and analysed within 24hours. The working area and the pipettors to be used were cleaned with 70% (v/v) ethanol before starting wit the analyses. The volume of sample or dilution thereof filtered depend on the source of the sample, below are the used sample volumes and dilutions (Table 3.5.4)

Table 3.8: Suggested sample volumes to be filtered

Sample Source	Sample Volume (concentration)					
	10X	5X	1X	0.1X	0.01X	0.001X
River Water	X	X	X	X	X	
Polluted small river	X	X	X	X	X	X
Chlorinated Sewage	X	X	X	X	X	

Shake the sample vigorously to ensure homogeneous distribution of the bacterial cells. A vortex mixer was used to mix dilutions in the test tubes. The filtration apparatus was assembled, using the flame sterilised forceps, a membrane filter was placed onto the apparatus. The forceps are sterilised by dipping the tips in ethanol, passing them through a flame and allowing the ethanol to burn off. The sample was then filtered through. After filtration and with the vacuum still on, the sides of the filter funnel were rinsed once with a quarter strength ringer's solution, allowing the rinsing to pass through the filter and suck dry the excess water. The vacuum is then switched off, taking care not to damage the

membrane, the filter was removed using flame sterilised forceps and transferred to petri dish containing m-Endo agar.

For each batch of samples, dilutions of the positive and negative control cultures were prepared as follows: Micropipettes were cleaned with ethanol before use. Starting with the negative control culture, 1mL of the negative control culture was added to 9mL of the sterile ringer's solution, and the tubes mixed with a vortex mixer, filtered and the membrane placed on the media.

For the positive control culture, 1mL of the positive control culture was added to 9mL of the sterile ringer's solution, and the tubes mixed with a vortex mixer, filtered and the membrane placed on the media. **Do not rinse the filter funnel**. A sterile membrane filter was placed on the filtration apparatus used above and rinsed with 50mL of ringer's and the membrane placed on the media. This step is repeated three times. All the filters were incubated within 30 minutes of filtration, upside down at 35°C±1°C for 20h to 24h. The working area was cleaned with 70% (v/v) ethanol after analysis.

Counting

After incubation, all plates with typical coliform colonies that are golden-green with metallic sheen were selected, counted and results recorded.

Calculation

The coliform were calculated as follows:

$$\text{Total Coliforms/100 mL} = \frac{\text{No. of colonies X 100}}{\text{Vol of sample filtered (mL)}}$$

Acceptance and Recording of results

The results are accepted if:

The positive control plates produced typical total coliform colonies and the negative control plate produced no growth.

Total Coliform bacteria numbers are quoted as the number of colonies per 100mL

3.6.11 *Escherichia coli (E. coli)* in cfu/100mL

Equipments

1. Incubator equipped with a calibrated thermometer, capable of maintaining a temperature of 44.5°C±1°C
1. Test Tubes with caps, 20mL
2. Bunsen burner
3. Sterile, disposable, plastic Petri dishes, 90 mm
4. Inoculating loop
5. Biological safety cabinet
6. Test tube rags, polypropylene or metal
7. Pipette, graduated
8. Pipette aid
9. Pasteur pipettes, sterile
10. Analytical balance, calibrated
11. Autoclave
12. Fume cupboard
13. Volumetric Flask, 1L
14. Measuring cylinder, 1L
15. Min/Max thermometer
16. Autoclave tape
17. Calibrated thermometers at the relevant temperatures

Reagents and Media

1. Tryptone water
2. Nutrient Agar
3. Kovac's Indole Reagent, commercially obtained
4. Ethanol, 99.8%
5. Biological/ Chemical Indicator for autoclaves

Preparation of Reagents

i. Tryptone water

The water is obtained commercially and 10mL aliquots were dispensed into the test tubes and sterilised according to the manufacturer's instructions

ii. Nutrient Agar

Also obtained commercially and prepared according to the manufacturer's instruction, 90mm agar plates were prepares for streaking.

iii. Ethanol, 99.8%

Prepare 70% (v/v) by adding 700mL ethanol (99.8%) to a 1L volumetric flask and make up to the mark with Milli-RX water. The solution is stable for two months at room temperature when stored in a glass container.

iv. Biological/Chemical Indicator: The solution is obtained commercially

Control Cultures

1. Positive Control Culture

A commercially certified pure culture of *Escherichia coli* was used as a positive control culture

2. Negative control Culture

A commercially certified pure culture of *Enterobacter faecium* was used as a negative control culture

Procedure

All procedures are done in the biological safety cabinet.

The cabinet and the incubator were cleaned with 70% (v/v) ethanol before analyses using a soft paper cloth. Select the m-FC plates that were used for the final faecal coliform count. 10 colonies from the m-FC plates were randomly picked and streaked on the nutrient agar using the streak plate method. The sterile uninoculated agar plates were used as blank controls. The plates were incubated upside down at 44.5°C±1°C for 24 hours. Before continuing with the analyses, ensure that all blanks have no growth. 10mL sterile Tryptone water at room temperature was placed in tubes and inoculated with one purified single colony from each nutrient agar plate. The uninoculated sterile Tryptone water tubes were used as blank controls.

With each batch of samples, aseptically inoculate a tube of sterile Tryptone with the positive control culture using a sterile Pasteur pipette. The same procedure was followed for the negative control culture.

All the tubes were incubated at 44.5°C±1°C for 20h to 24h. Before continuing with the analyses, ensure that all blanks have no growth. 0.5mL of Kovac's reagent was added to each tube using a glass pipette, excluding the blank control tubes. The tubes were shaken

gently and allowed to stand for 10 minutes. The tubes that develop a red layer are positive and denote the presence of indole and confirm the presence of *Escherichia coli.*

Calculation

The *E. coli* count was calculated as follows:

$$\textit{E. coli}/100\text{mL} = \frac{\text{No. of FC colonies X 100}}{\text{Vol of sample filtered (mL)}} \times \frac{\text{No. of positive tubes}}{\text{No. of tubes inoculated}}$$

Acceptance and Recording of results

The results are accepted if:

The blank control plates and tubes produced no growth

The positive control tubes produced a red layer and the negative control tubes gave no change in colour when adding Kovac's reagent.

The results of the duplicate samples must be equal to or less than precision criterion.

Escherichia coli numbers are quoted as the number of colonies per 100mL

3.6.12 Faecal Coliforms, (FC) in cfu/100mL

Equipments

- Incubator capable of maintaining a temperature of 44.5°C±0.5°C
- Filtration apparatus, manifold with receiving filter flask
- Moisture trap between the filter flask and the vacuum pump
- Sterile membrane filter units (filter base and funnel)
- Vacuum pump
- Sterile, white, gridded, 47mm diameter membrane filters, 0.45µm pore size
- Sterile, disposable, plastic Petri dishes, 50mm, with tight fitting lids (Millipore PD10 047 or equivalent)
- Smooth tipped forceps
- Calibrated pipettors, 1mL, 5mL, 9mL, and 10mL with sterile tips
- Sterile graduated measuring cylinder, glass, 100mL
- Autoclave
- Test Tubes with caps, 20mL
- Bunsen burner
- Test tube rags, polypropylene or metal

- Calibrated analytical balance
- Biological safety cabinet
- Inoculating loop
- Calibrated thermometers at the relevant temperatures
- Vortex Mixer
- Autoclave tape
- Min/Max thermometer

Reagents and Media

1. M-FC Agar, Difco
2. Ringer's Dilution Solution
3. Ethanol, 99.8%
4. Disinfectant
5. Biological/ Chemical Indicator for autoclaves

Preparation of Reagents

i. m-FC Agar

The media is obtained commercially and prepared according to the manufacturer's instruction. The agar plates may be stored in the refrigerator for up to two (2) weeks.

ii. Ringer's Dilution Solution: The solution is obtained commercially

iii. Ethanol: Prepare 70% (v/v) by adding 700mL ethanol (99.8%) to a 1L volumetric flask and make up to the mark with Milli-RX water. The solution is stable for two months at room temperature when stored in a glass container.

iv. Disinfectant: The solution is obtained commercially

v. Biological/Chemical Indicator: The solution is obtained commercially

Control Cultures

1. Positive Control Culture

A commercially certified pure culture of *Enterobacter cloacae* was used as a positive control culture

2. Negative control Culture

A commercially certified pure culture of *Enterobacter faecium* was used as a negative control culture

Analysis

Samples must be kept in a refrigerator below 6°C and analysed within 24hours. The working area and the pipettors to be used were cleaned with 70% (v/v) ethanol before starting wit the analyses. The volume of sample or dilution thereof filtered depend on the source of the sample, below are the used sample volumes and dilutions (Table 3.5.4)

Table 3.8: Suggested sample volumes to be filtered

Sample Source	Sample Volume (concentration)					
	10X	5X	1X	0.1X	0.01X	0.001X
River Water	X	X	X	X	X	
Polluted small river	X	X	X	X	X	X
Chlorinated Sewage	X	X	X	X	X	

Shake the sample vigorously to ensure homogeneous distribution of the bacterial cells. A vortex mixer was used to mix dilutions in the test tubes. The filtration apparatus was assembled, using the flame sterilised forceps, and a membrane filter was placed onto the apparatus. The forceps are sterilised by dipping the tips in ethanol, passing them through a flame and allowing the ethanol to burn off. The sample was then filtered through. After filtration and with the vacuum still on, the sides of the filter funnel were rinsed once with a quarter strength ringer's solution, allowing the rinsing to pass through the filter and suck dry the excess water. The vacuum is then switched off, taking care not to damage the membrane, the filter was removed using flame sterilised forceps and transferred to petri dish containing m-FC agar.

For each batch of samples, dilutions of the positive and negative control cultures were prepared as follows:

Micropipettes were cleaned with ethanol before use. Starting with the negative control culture, 1mL of the negative control culture was added to 9mL of the sterile ringer's solution, and the tubes mixed with a vortex mixer, filtered and the membrane placed on the media. For the positive control culture, 1mL of the positive control culture was added to 9mL of the sterile ringer's solution, and the tubes mixed with a vortex mixer, filtered and the membrane placed on the media. **Do not rinse the filter funnel**. A sterile membrane filter was placed on the filtration apparatus used above and rinsed with 50mL of ringer's and the membrane placed on the media. This step is repeated three times. All the filters were incubated within 30 minutes of filtration, upside down at 44.5°C±0.5°C for 20h to 24h. The working area was cleaned with 70% (v/v) ethanol after analysis.

Counting

After incubation, all plates with typical blue colonies (various shades) were selected, counted and results recorded. Small blue colonies were also included in the count, grey colonies were not counted.

Calculation

The faecal coliforms were calculated as follows:

$$\text{Faecal Coliforms/100 mL} = \frac{\text{No. of colonies X 100}}{\text{Vol of sample filtered (mL)}}$$

Acceptance and Recording of results

The results are accepted if:

The positive control plates produced typical faecal coliform colonies and the negative control plate produced no growth.

Total Coliform bacteria numbers are quoted as the number of colonies per 100mL

3.6.13 Heterotrophic Plate in cfu/100mL

Equipments

1) Incubator, capable of maintaining a temperature of 35°C±1°C
2) Sterile 90 mm plastic Petri dishes
3) Calibrated pipettors, 1mL tips
4) Conical flask 1L
5) Volumetric Flask of appropriate size
6) Colony counter with Quebec grid
7) Test Tubes with caps, 20mL
8) Test tube rags, polypropylene or metal
9) Autoclave
10) Bunsen burner
11) Analytical balance, calibrated
12) Biological safety cabinet
13) Vortex mixer
14) Min/Max thermometer
15) Autoclave tape
16) Calibrated thermometers at the relevant temperatures

Reagents and Media

1. Standard plate Count Agar
2. Ringer's Dilution Solution
3. Ethanol, 99.8%
4. Disinfectant
5. Biological/ Chemical Indicator for autoclaves

Preparation of Reagents

1. Standard plate Count Agar

The media is obtained commercially and should be prepared on the day of analyses. Suspend the ingredients in 500mL Milli-Q water in a conical flask and boil to dissolve the constituents. Autoclave the flask containing the media according to the manufacturer's instruction.

2. Ringer's Dilution Solution: The solution is obtained commercially

1. Ethanol

Prepare 70% (v/v) by adding 700mL ethanol (99.8%) to a 1L volumetric flask and make up to the mark with Milli-RX water. The solution is stable for two months at room temperature when stored in a glass container.

2. Disinfectant: The solution is obtained commercially
3. Biological/Chemical Indicator: The solution is obtained commercially

Control Cultures

1. Positive Control Culture

A commercially certified pure culture of *Escherichia coli* was used as a positive control culture

Procedure

Samples must be kept in a refrigerator below 6°C and analysed within 24 hours. The temperature of the agar must be approximately 45°C to 50°C before pouring into the plates containing the sample. All procedures are done in the biological safety cabinet. The cabinet was cleaned with 70% (v/v) ethanol before analyses using a soft paper cloth. Shake the sample vigorously to ensure homogeneous distribution of the bacterial cells. A vortex mixer was used to mix dilutions in the test tubes. The dilutions were prepared as per the table below (Table 3.5.5)

Table 3.9: Mixing dilutions for analyses

Sample Volume	Ringer's diluent volume	Volume pipetted into Petri dish	Original sample volume in petri dish
1mL	-	1mL	1mL
1mL	9mL (= Dilution A)	1mL	0.1mL
1mL of dilution A	9mL (= Dilution B)	1mL	0.01mL
1mL of dilution B	9mL (=Dilution C)	1mL	0.001mL

The samples were analysed in duplicates.

Aseptically 1mL of the sample was pipette into the petri dish and within 30 minutes approximately 18mL sterile molten tempered Standard Plate Count Agar was added. The lid of the petri dish was placed and the dish moved in circular motion to mix the inoculum and the agar. For each batch of samples, the negative control plates were prepared by adding approximately 18mL of sterile plate count agar to petri dishes containing 1mL of sterile ringer's solution, from same batch that was used to prepare the sample dilutions.

For each batch of samples, the positive control plates were prepared by adding approximately 18mL of sterile plate count agar to petri dishes containing 1mL of the positive control cultures. All the plates were left undisturbed until the agar has set. All the plates were then inverted and incubated 30 minutes after pouring, at 35°C±1°C for 44h to 48h. The working area was cleaned with 70% (v/v) ethanol after analysis.

Counting

After incubation, all plates that give a count of between 30 and 300 colonies per plate were selected, counted and results recorded.

Calculation

Calculated the counts by dividing the mean of the two counts by the volume in mL of original sample pipetted into the petri dish to give the results as cfu/mL

Acceptance and Recording of results

The results are accepted if:

The negative control plates produced no growth and the positive control plates produced growth

The Standard Plate Count numbers are quoted in terms of the number of colonies per 1mL of the original sample.

CHAPTER 4

RESULTS

4.1 Results of parameters analysed in collected water samples per sampling period

Results of the analyses of water quality parameters in collected water samples from the Blesbokspruit are as presented in Tables 4.1-4.17 below.

Table 4.1: Results of some physio-chemical and microbial analysis of water samples collected from the Blesbokspruit on 13 March 2008

Sampling points									
Parameters	BB1	BB2	BB3	BB4	BB5	BB6	BB7	BB8	BB9
			Chemical Analysis						
pH	6.8	7.0	7.3	7.6	7.7	7.8	8.0	8.1	8.0
EC (mS/m)	126	113	118	126	125	122	112	105	108
TSS (mg/L)	10	<10	<10	<10	<10	<10	10	26	27
DTS(mg/L)	866	686	796	812	828	788	730	684	720
NO_3-NO_2-N)mg/L N	0.2	0.6	0.1	0.5	0.3	12.3	1.1	0.8	1.8
PO_4(mg/L)	0.6	0.4	0.5	0.7	0.6	0.6	0.6	0.6	0.5
SO_4 (mg/L)	344	168	291	297	300	292	262	220	240
COD (mg/L)	10	<10	<10	<10	<10	<10	27	10	<10
Micro Elements									
Cd (mg/L)	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
Cu (mg/L)	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
Fe (mg/L)	<0.05	0.67	0.09	0.08	0.08	0.12	0.27	0.21	0.31
Zn (mg/L)	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	0.06	<0.06	<0.06
As (mg/L)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Microbial Analysis									
FC (cfu/100mL)	14	720	17	59	8	860	820	260	200
TC (cfu/100mL)	70	5300	140	180	56	9700	6800	7200	420
E.coli (cfu/100mL)	11	600	17	50	7	780	820	200	80
Heterotrophic Plate Count (cfu/1mL)	7000	24000	15900	3800	9000	14700	10400	5400	13800

Table 4.1 shows the results of the analysed parameters in water samples collected from the Blesbokspruit on the 13th of March 2008. The values of the physio-chemical parameters; pH, EC, TSS and TDS varied from 6.8-8.0; 105-126(mS/m); <10-27 and 686-866mg/L respectively. The levels of nitrate, phosphate, sulphate and the COD ranged from 0.2-12.3mg/L N; 0.4-0.7mg/L; 168-344mg/L and <10-27mg/L respectively. The concentration of trace metals in analysed water samples varied from trace <0.07mg/L; trace <0.04mg/L; <0.05–0.31mg/L; trace <0.06mg/L and trace <0.05mg/L respectively for

Cd, Cu, Fe, Zn and As. The Faecal coliform (FC); Total coliform, *E. coli* and HeterotrophicB8 Plate Counts ranged from 8-860 (cfu/100mL); 56-9700 (cfu/100mL); 7-820 (cfu/100mL) and 3800-24000(cfu/1mL) respectively.

Table 4.2: Results of some physio-chemical and microbial analysis of water samples collected from the Blesbokspruit on 21 April 2008

	Sampling points								
	BB1	BB2	BB3	BB4	BB5	BB6	BB7	BB8	BB9
Parameters				**Chemical Analysis**					
pH	7.8	7.5	8.0	8.1	8.1	8.0	7.8	8.2	8.2
EC (mS/m)	154	81	129	128	120	115	106	103	100
TSS (mg/L)	<10	<10	<10	<10	<10	<10	<10	<10	<10
DTS(mg/L)	1026	468	908	800	848	632	774	676	554
NO_3-NO_2-N)mg/L N	<0.1	1.7	<0.1	<0.1	<0.1	0.4	0.7	0.5	0.8
PO_4(mg/L)	0.7	1.0	0.5	0.9	0.9	0.9	0.6	0.4	0.8
SO_4 (mg/L)	425	98	361	342	308	289	261	242	246
COD (mg/L)	16	13	12	<10	<10	<10	<10	51	<10
	Micro Elements								
Cd (mg/L)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Cu (mg/L)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fe (mg/L)	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Zn (mg/L)	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
As (mg/L)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.065
	Microbial Analysis								
FC (cfu/100mL)	24	77	6	2	14	1800	2800	1200	650
TC (cfu/100mL)	34	670	36	15	300	6300	39000	25000	29000
E.coli (cfu/100mL)	22	56	5	2	14	1600	2300	1200	480
Heterotrophic Plate Count (cfu/1mL)	2520	20800	12600	2940	4200	30700	17600	18100	79000

From Table 4.2 above, the values of the physio-chemical parameters; pH, EC, TSS and TDS varied from 7.5-8.2; 81-154(mS/m); constant <10(mg/L) and 468-1026(mg/L) respectively. The levels of nitrate, phosphate, sulphate and the COD ranged from <0.1-1.7mg/L N, 0.4-1.0mg/L, 98-425mg/L, and <10-51mg/L respectively. The concentration of trace metals in analysed water samples varied from trace <0.02mg/L; trace <0.01mg/L, trace <0,01mg/L; trace <0.07mg/L and trace <0.05mg/L respectively for Cd, Cu, Fe, Zn and As. The Faecal coliform (FC); Total coliform, *E. coli* and Heterotrophic Plate Counts ranged from 2-2800(cfu/100mL); 15-39000(cfu/100mL); 2-2300 (cfu/100mL) and 2520-79000 (cfu/100mL) respectively.

Table4.3: Results of some physio-chemical and microbial analysis of water samples collected from the Blesbokspruit on 05 June 2008

	Sampling points								
	BB1	BB2	BB3	BB4	BB5	BB6	BB7	BB8	BB9
Parameters				**Chemical Analysis**					
pH	7.0	7.1	8.1	8.1	8.0	8.3	8.3	8.2	8.7
EC (mS/m)	134	79	145	147	145	141	131	123	127
TSS (mg/L)	<10	14	<10	<10	<10	<10	<10	<10	<10
DTS(mg/L)	746	624	1000	954	1132	960	942	724	888
NO_3-NO_2-N)mg/L N	0.5	1.9	0.2	0.1	0.1	0.5	0.7	0.7	1.0
PO_4(mg/L)	0.1	0.5	0.2	0.2	0.2	0.2	0.3	0.5	0.3
SO_4 (mg/L)	309	78	360	357	357	342	326	295	322
COD (mg/L)	<10	29	<10	<10	<10	<10	11	<10	<10
	Micro Elements								
Cd (mg/L)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Cu (mg/L)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
Fe (mg/L)	0.19	1.37	0.03	0.03	0.01	0.06	0.07	0.10	0.11
Zn (mg/L)	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
As (mg/L)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	Microbial Analysis								
FC (cfu/100mL)	12	14	4	2	63	420	830	320	10
TC (cfu/100mL)	34	22	11	51	20	5400	8600	4100	110
E.coli (cfu/100mL)	11	12	3	2	50	290	820	280	10
Heterotrophic Plate Count (cfu/1mL)	4490	156700	4570	3150	5500	13390	22000	4300	8900

In Table 4.3 above, the values of the physio-chemical parameters; pH, EC, TSS and TDS varied from 7.0-8.7; 79-145mS/m; <10-14mg/L and 624-100mg/L respectively. The levels of nitrate, phosphate, sulphate and the COD ranged from 0.2-1.9mg/L N; 0.2-0.5mg/l; 78-360mg/L and <10-25mg/L respectively. The concentration of trace metals in analysed water samples was constant at trace <0.02mg/L; trace <0.01mg/L; trace <0.07mg/L and trace <0.05 mg/L respectively for Cd, Cu, Zn and As, while the concentrations of Fe varied from trace 0.01-1.37mg/L. The Faecal coliform (FC); Total coliform, *E. coli* and Heterotrophic Plate Counts ranged from 2-830(cfu/100mL); 11-8600(cfu/100mL); 2-820 (cfu/100mL) and 3150-156700(cfu/100mL) respectively.

Table 4.4: Results of some physio-chemical and microbial analysis of water samples collected from the Blesbokspruit on 17July 2008

	Sampling points								
	BB1	BB2	BB3	BB4	BB5	BB6	BB7	BB8	BB9
Parameters	**Chemical Analysis**								
pH	7.5	7.6	7.7	8.3	8.2	8.5	8.4	8.5	8.4
EC (mS/m)	181	79	150	151	149	142	133	133	128
TSS (mg/L)	<10	16	<10	<10	<10	<10	<10	<10	18
DTS(mg/L)	1294	478	1054	1080	1108	1002	918	844	882
NO_3-NO_2-N)mg/L N	0.5	1.0	1.0	0.1	<0.1	0.6	0.8	0.8	1.4
PO_4(mg/L)	0.3	0.4	0.2	0.2	0.2	0.3	0.3	0.3	0.3
SO_4 (mg/L)	500	74	398	390	394	386	350	319	318
COD (mg/L)	<10	81	21	19	32	<10	16	23	27
Micro Elements									
Cd (mg/L)	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
Cu (mg/L)	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
Fe (mg/L)	0.08	1.47	0.11	<0.05	<0.05	<0.05	0.09	0.10	0.13
Zn (mg/L)	<0.06	<0.06	0.50	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
As (mg/L)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Microbial Analysis									
FC(cfu/100 mL)	36	17	0	1	31	10	24	850	840
TC(cfu/100 mL)	53	1700	4	18	33	180	1800	21000	16000
E.coli (cfu/100mL)	34	15	0	1	31	10	19	850	810
Heterotrophic Plate Count (cfu/1mL)	4600	670000	8400	3800	5400	4900	22200	3160	25600

Table 4.4 contained the values of the physio-chemical parameters; pH, EC, TSS and TDS varied from 7.5-8.5; 79-181mS/m; <10-18mg/L and 478-1294mg/L respectively. The levels of nitrate, phosphate, sulphate and the COD ranged from < 0.1-0.8mg/L N; 0.2-0.4mg/L; 74-500mg/L and <10-81mg/L respectively. The concentration of trace metals in analysed water samples was constant at trace <0.07mg/L; trace <0.04mg/L; trace <0.06mg/L and trace <0.05mg/L respectively for Cd, Cu, Zn and As, while the concentrations of Fe varied from trace <0.05-1.47mg/L. The Faecal coliform (FC); Total coliform, *E. coli* and Heterotrophic Plate Counts ranged from 0-850 (cfu/100mL); 4-21000 (cfu/100mL); 0-850 (cfu/100mL) and 3160-670000 (cfu/100mL) respectively

Table 4.5: Results of some physio-chemical and microbial analysis of water samples collected from the Blesbokspruit on 26 August 2008

Sampling points									
	BB1	BB2	BB3	BB4	BB5	BB6	BB7	BB8	BB9
Parameters		**Chemical Analysis**							
pH	7.4	7.6	8.0	8.1	8.1	8.2	8.2	8.2	8.2
EC (mS/m)	163	127	156	161	161	152	132	134	134
TSS (mg/L)	80	15	45	20	28	50	49	34	42
DTS(mg/L)	1150	824	1028	1092	1182	1026	892	880	874
NO_3- NO_2-N) in mg/L N	0.2	2.3	0.1	0.1	0.1	0.7	1.2	1.3	1.4
PO_4(mg/L)	0.8	0.6	0.5	0.3	0.3	0.3	0.5	0.5	0.5
SO_4 (mg/L)	410	222	380	440	370	320	357	345	333
COD (mg/L)	<10	<10	<10	<10	<10	<10	<10	<10	<10
Micro Elements									
Cd (mg/L)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Cu (mg/L)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fe (mg/L)	0.02	0.40	0.05	<0.01	<0.01	0.02	0.20	0.18	0.10
Zn (mg/L)	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
As (mg/L)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Microbial Analysis									
FC(cfu/100mL)	22	590	15	22	28	48	78	680	840
TC(cfu/100mL)	25	4500	18	28	30	1400	250	9700	7100
E.coli (cfu/100mL)	20	560	14	20	23	39	78	640	840
Heterotrophic Plate Count (cfu/1mL)	1820	36400	3250	930	1980	1410	3900	6600	6000

Table 4.5 revealed the values of the physio-chemical parameters; pH, EC, TSS and TDS varied from 7.4-8.2; 127-163mS/m; 15-80mg/L and 824-1182mg/L respectively. The levels of nitrate, phosphate, sulphate and the COD ranged from 0.1-2.3mg/L N; 0.3-0.8mg/L; 222-440mg/L and a constant <10mg/L respectively. The concentration of trace metals in analysed water samples was constant at trace <0.02mg/L; trace <0.01mg/L; trace <0.07mg/L and trace <0.05mg/L respectively for Cd, Cu, Zn and As, while the concentrations of Fe varied from trace <0.01-0.18mg/L. The Faecal coliform (FC); Total coliform, *E. coli* and Heterotrophic Plate Counts ranged from 15-840 (cfu/100mL); 18-9700 (cfu/100mL); 14-840 (cfu/100mL) and 930-36400 (cfu/100mL) respectively

Table 4.6: Results of some physio-chemical and microbial analysis of water samples collected from the Blesbokspruit on 14 October 2008

Sampling points									
	BB1	BB2	BB3	BB4	BB5	BB6	BB7	BB8	BB9
Parameters	**Chemical Analysis**								
pH	7.8	7.6	7.6	7.9	7.9	8.1	8.4	8.4	8.3
EC (mS/m)	182	123	171	179	181	156	140	138	140
TSS (mg/L)	14	14	40	<10	11	<10	15	11	10
DTS(mg/L)	1308	760	1174	1260	1220	1070	962	906	970
NO_3- NO_2-N) in mg/L N	0.3	0.2	0.2	0.1	0.2	1.8	1.8	1.3	1.2
PO_4(mg/L)	2.3	3.6	1.3	0.6	0.4	0.4	0.6	0.5	0.5
SO_4 (mg/L)	520	132	360	400	600	373	322	300	304
COD (mg/L)	<10	47	58	<10	16	15	16	25	23
Micro Elements									
Cd (mg/L)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Cu (mg/L)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fe (mg/L)	0.05	1.38	0.39	0.10	0.70	0.10	0.21	0.12	0.10
Zn (mg/L)	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
As (mg/L)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Microbial Analysis									
FC (cfu/100mL)	11	10700	36	22	51	76	80	65	210
TC (cfu/100mL)	50	230000	70	24	120	250	270	2000	400
E.coli (cfu/100mL)	10	10700	36	19	51	76	80	65	200
Heterotrophic Plate Count (cfu/1mL)	13500	830000	9500	3500	4500	6000	5600	149000	23300

In Table 4.6 above, the values of the physio-chemical parameters; pH, EC, TSS and TDS varied from 7.6-8.4; 123-182mS/m; 11-40mg/L and 906-1308mg/L respectively. The levels of nitrate, phosphate, sulphate and the COD ranged from 0.1-1.8mg/L N; 0.4-3.6mg/L; 132-600mg/L and <10-58mg/L respectively. The concentration of trace metals in analysed water samples was constant at trace <0.02mg/L; trace <0.01mg/L; trace <0.07mg/L and trace <0.05mg/L respectively for Cd, Cu, Zn and As, while the concentrations of Fe varied from trace 0.05-1.38mg/L. The Faecal coliform (FC); Total coliform, *E. coli* and Heterotrophic Plate Counts ranged from 11-10700 (cfu/100mL); 24-230000 (cfu/100mL); 10-10700 (cfu/100mL) and 5600-830000 (cfu/100mL) respectively.

Table 4.7: Results of some physio-chemical and microbial analysis of water samples collected from the Blesbokspruit on 20 November 2008

	BB1	BB2	BB3	BB4	BB5	BB6	BB7	BB8	BB9
Parameters	**Chemical Analysis**								
pH	7.9	7.6	7.9	8.1	8.2	8.2	8.0	8.1	8.1
EC (mS/m)	100	52	99	116	122	118	110	118	117
TSS (mg/L)	< 10	11	95	< 10	22	60	112	100	147
DTS(mg/L)	740	336	718	820	890	916	822	908	740
NO_3- NO_2-N) in mg/L N	0.2	0.2	0.2	0.2	0.1	0.2	1.4	1.6	1.8
PO_4(mg/L)	1.4	1.2	1.4	1.8	1.9	1.2	1.4	1.6	1.3
SO_4 (mg/L)	198	84	224	272	305	306	282	303	295
COD (mg/L)	31	40	32	26	26	30	42	36	44
Micro Elements									
Cd (mg/L)	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07
Cu (mg/L)	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04
Fe (mg/L)	0.13	0.41	0.10	0.05	0.06	0.31	0.39	0.91	0.49
Zn (mg/L)	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06	0.06	< 0.06	0.08	0.06
As (mg/L)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Microbial Analysis									
FC (cfu/100mL)	44	340000	340	16	280	2900	3600	9700	12500
TC (cfu/100mL)	470	3200000	5000	56	620	18000	12000	66000	92000
E.coli (cfu/100mL)	44	340000	340	16	280	2900	3600	9700	12500
Heterotrophic Plate Count (cfu/1mL)	17100	1430000	25700	14000	41200	83000	68000	89000	105000

Values of the physio-chemical parameters: pH, EC, TSS and TDS varied from 7.6-8.2; 52-118mS/m; <10-147mg/L and 336-916mg/L respectively in Table 4.7. The levels of nitrate, phosphate, sulphate and the COD ranged from 0.1-1.8mg/L N; 1.2-1.9mg/L; 84-306mg/L and 26-44mg/L respectively. The concentration of trace metals in analysed water samples was constant at trace <0.07mg/L; trace <0.04mg/L; trace <0.06mg/L and trace <0.05mg/L respectively for Cd, Cu, Zn and As, while the concentrations of Fe varied from trace 0.05-0.49mg/L. The Faecal coliform (FC); Total coliform, *E. coli* and Heterotrophic Plate Counts ranged from 16-340000(cfu/100mL); 56-3200000 (cfu/100mL); 16-340000(cfu/100mL) and 14000-1430000(cfu/100mL) respectively

Table 4.8: Results of some physio-chemical and microbial analysis of water samples collected from the Blesbokspruit on 13 January 2009

	BB1	BB2	BB3	BB4	BB5	BB6	BB7	BB8	BB9
Chemical Analysis									
pH	6.8	7.1	7.3	7.6	8.0	8.0	7.9	7.8	7.6
EC (mS/m)	133	90	131	133	131	129	116	106	106
TSS (mg/L)	10	13	<10	<10	44	20	32	43	48
DTS(mg/L)	880	546	940	944	954	906	784	750	744
NO_3- NO_2-N) in mg/L N	0.1	0.1	0.2	<0.1	0.3	0.1	0.3	0.2	1.8
PO_4(mg/L)	0.1	0.1	0.1	0.2	0.1	0.1	0.1	<0.1	0.8
SO_4 (mg/L)	330	93	328	327	318	302	222	254	31
COD (mg/L)	29	48	22	30	35	34	29	29	35
Micro Elements									
Cd (mg/L)	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
Cu (mg/L)	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
Fe (mg/L)	0.09	0.41	0.16	<0.05	0.12	0.09	0.18	0.31	0.26
Zn (mg/L)	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
As (mg/L)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Microbial Analysis									
FC (cfu/100mL)	2	50	5	3	19	62	51	0	240
TC (cfu/100mL)	7	160	120	13	120	410	350	28	2200
E.coli (cfu/100mL)	1	50	5	3	17	62	50	0	210
Heterotrophic Plate Count (cfu/1mL)	7100	14300	1600	2460	4700	8700	32000	213800	32800

In Table 4.8 above, the values of the physio-chemical parameters; pH, EC, TSS and TDS varied from 7.1-8.0; 90-133mS/m; <10-48mg/L and 546-944mg/L respectively. The levels of nitrate, phosphate, sulphate and the COD ranged from <0.1-0.8mg/L N; <0.1-0.8mg/L; 31-328mg/L and 22-48mg/L respectively. The concentration of trace metals in analysed water samples was constant at trace <0.07mg/L; trace <0.04mg/L; trace < 0.06mg/L and trace <0.05mg/L respectively for Cd, Cu, Zn and As, while the concentrations of Fe varied from trace <0.05-0.41mg/L. The Faecal coliform (FC); Total coliform, *E. coli* and Heterotrophic Plate Counts ranged from 0-240 (cfu/100mL); 7-2200 (cfu/100mL); 0-210 (cfu/100mL) and 2460-213800 (cfu/100mL) respectively

4.2 The mean results of analysed parameters per sampling point

Table 4.9: Mean results of analysed parameters in water samples at sampling point (BB1) across the sampling period

Determinant	13-03-2008	21-04-08	05-06-08	17-07-08	26-08-08	14-10-08	20-11-08	13-01-09	Mean	Standard Deviation
EC (mS/m)	126	154	134	181	163	182	100	133	146.63	26.7
TSS (mg/L)	10	10	10	10	80	14	10	10	19.25	23.0
DTS(mg/L)	866	1026	746	1294	1150	1308	740	880	1001.3	214.7
NO_3-NO_2-N) mg/L N	0.2	0.1	0.5	0.5	0.2	0.3	0.2	0.1	0.2625	0.15
PO_4(mg/L)	0.6	0.7	0.1	0.3	0.8	2.3	1.4	0.1	0.7875	0.70
pH	6.8	7.8	7	7.5	7.4	7.8	7.9	6.8	7.375	0.43
SO_4 (mg/L)	344	425	309	500	410	520	198	330	379.5	99.3
COD (mg/L)	10	16	10	10	10	10	31	29	15.75	8.47
Cd (mg/L)	0.07	0.02	0.02	0.07	0.02	0.02	0.07	0.07	0.045	0.03
Cu (mg/L)	0.04	0.01	0.01	0.04	0.01	0.01	0.04	0.04	0.025	0.02
Fe (mg/L)	0.05	0.01	0.19	0.08	0.02	0.05	0.13	0.09	0.0775	0.06
Zn (mg/L)	0.06	0.07	0.07	0.06	0.07	0.07	0.06	0.06	0.065	0.005
As (mg/L)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	6.94E-18
FC (cfu/100mL)	14	24	12	36	22	11	44	2	20.625	13.0
TC (cfu/100mL)	70	34	34	53	25	50	470	7	92.875	143.7
E.coli (cfu/100mL)	11	22	11	34	20	10	44	1	19.125	13.2
Heterotrophic Plate Count (cfu/1mL)	7000	2520	4490	4600	1820	13500	17100	7100	7266.3	5033.2

Table 4.9 above, showed the mean values and the standard deviations of the physio-chemical parameters; nitrates, phosphate, sulphate; COD; trace metals and microbial results over eight (08) months period in analysed water samples.

Table 4.10: Mean results of analysed parameters in water samples at sampling point (BB2) across the sampling period

Determinant	13-03-08	21-04-08	5-06-08	17-07-08	26-08-08	14-10-08	20-11-08	13-01-09	Mean	Std Dev.
EC (mS/m)	113	81	79	79	127	123	52	90	93	24.20
TSS (mg/L)	10	10	14	16	15	14	11	13	12.875	2.14
DTS(mg/L)	686	468	624	478	824	760	336	546	590.25	153.3
NO_3- NO_2-N) in mg/L N	0.6	1.7	1.9	1	2.3	0.2	0.2	0.1	1	0.801
PO_4(mg/L)	0.4	1	0.5	0.4	0.6	3.6	1.2	0.1	0.975	1.04
pH	7	7.5	7.1	7.6	7.6	7.6	7.6	7.1	7.3875	0.25
SO_4 (mg/L)	168	98	78	74	222	132	84	93	118.63	49.0
COD (mg/L)	10	13	29	81	10	47	40	48	34.75	22.3
Cd (mg/L)	0.07	0.02	0.02	0.07	0.02	0.02	0.07	0.07	0.045	0.03
Cu (mg/L)	0.04	0.01	0.01	0.04	0.01	0.01	0.04	0.04	0.025	0.02
Fe (mg/L)	0.68	0.03	1.37	1.47	0.4	1.38	0.1	0.41	0.73	0.56
Zn (mg/L)	0.06	0.07	0.07	0.06	0.07	0.07	0.06	0.06	0.065	0.01
As (mg/L)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	7.0E-18
FC (cfu/100mL)	720	77	14	17	590	10700	340000	50	44021	111922.1
TC (cfu/100mL)	5300	670	22	1700	4500	230000	3200000	160	430294	1049507.5
E.coli (cfu/100mL)	600	56	12	15	560	10700	340000	50	43999	111930.7
Heterotrophic Plate Count (cfu/1mL)	24000	20800	156700	6.7E04	3.6E02	8.3E04	1.43E04	1.43E02	397775	492949.5

Table 4.10 above, show the mean values and the standard deviations of the physio-chemical parameters; nitrates, phosphate, sulphate; COD; trace metals and microbial results over eight (08) months period in analysed water samples.

Table 4.11: Mean results of analysed parameters in water samples at sampling point (BB3) across the sampling period

Determinant	13-03-08	21-04-08	5-06-08	17-07-08	26-08-08	14-10-08	20-11-08	13-01-09	Mean	Standard Deviation
EC (mS/m)	118	129	145	150	156	171	99	131	137.38	21.4
TSS (mg/L)	10	10	10	10	45	40	95	10	28.75	28.6
DTS(mg/L)	796	908	1000	1054	1028	1174	718	940	952.25	137
NO_3- NO_2-N)mg/L N	0.1	0.1	0.2	1	0.1	0.2	0.2	0.2	0.2625	0.28
PO_4(mg/L)	0.5	0.5	0.2	0.2	0.5	1.3	1.4	0.1	0.5875	0.46
pH	7.3	8	8.1	7.7	8	7.6	7.9	7.3	7.7375	0.29
SO_4 (mg/L)	291	361	360	398	380	360	224	328	337.75	52.6
COD (mg/L)	10	12	10	21	10	58	32	22	21.875	15.5
Cd (mg/L)	0.07	0.02	0.02	0.07	0.02	0.02	0.07	0.07	0.045	0.03
Cu (mg/L)	0.04	0.01	0.01	0.04	0.01	0.01	0.04	0.04	0.025	0.015
Fe (mg/L)	0.09	0.01	0.03	0.11	0.05	0.39	0.1	0.16	0.1175	0.11
Zn (mg/L)	0.06	0.07	0.07	0.5	0.07	0.07	0.06	0.06	0.12	0.14
As (mg/L)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	6.9E-18
FC (cfu/100mL)	17	6	4	0	15	36	340	5	52.875	109.0
TC (cfu/100mL)	140	36	11	4	18	70	5000	120	674.88	1635.4
E.coli (cfu/100mL)	17	5	3	0	14	36	340	5	52.5	109.2
Heterotrophic Plate Count (cfu/1mL)	15900	12600	4570	8400	3250	9500	25700	1600	10190	7384.0

Table 4.11 above, show the mean values and the standard deviations of the physio-chemical parameters; nitrates, phosphate, sulphate; COD; trace metals and microbial results over eight (08) months period in analysed water samples.

Table 4.12: Mean results of analysed parameters in water samples at sampling point (BB4) across the sampling period

Determinant	13-03-08	21-04-08	5-06-08	17-07-08	26-08-08	14-10-08	20-11-08	13-01-09	Mean	Std Dev
EC (mS/m)	126	128	147	151	161	179	116	133	142.63	19.5
TSS (mg/L)	10	10	10	10	20	10	10	10	11.25	3.30
DTS(mg/L)	812	800	954	1080	1092	1260	820	944	970.25	153.5
NO_3- NO_2-N)mg/L N	0.5	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1625	0.13
PO_4(mg/L)	0.7	0.9	0.2	0.2	0.3	0.6	1.8	0.2	0.6125	0.51
pH	7.6	8.1	8.1	8.3	8.1	7.9	8.1	7.6	7.975	0.23
SO_4 (mg/L)	297	342	357	390	440	400	272	327	353.13	52.0
COD (mg/L)	10	10	10	19	10	10	26	30	15.625	7.78
Cd (mg/L)	0.07	0.02	0.02	0.07	0.02	0.02	0.07	0.07	0.045	0.03
Cu (mg/L)	0.04	0.01	0.01	0.04	0.01	0.01	0.04	0.04	0.025	0.02
Fe (mg/L)	0.08	0.01	0.03	0.05	0.01	0,10	0.05	0.05	0.04	0.02
Zn (mg/L)	0.06	0.07	0.07	0.06	0.07	0.07	0.06	0.06	0.065	0.01
As (mg/L)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	7.0E-18
FC (cfu/100mL)	59	2	2	1	22	22	16	3	15.875	18.4
TC (cfu/100mL)	180	15	51	18	28	24	56	13	48.125	52.1
E.coli (cfu/100mL)	50	2	2	1	20	19	16	3	14.125	15.6
Heterotrophic Plate Count (cfu/1mL)	3800	2940	3150	3800	930	3500	14000	2460	4322.5	3760.7

Table 4.12 above, show the mean values and the standard deviations of the physio-chemical parameters; nitrates, phosphate, sulphate; COD; trace metals and microbial results over eight (08) months period in analysed water samples.

Table 4.13: Mean results of analysed parameters in water samples at sampling point (BB5) across the sampling period

Determinant	13-03-08	21-04-08	5-06-08	17-07-08	26-08-08	14-10-08	20-11-08	13-01-09	Mean	Std Dev
EC (mS/m)	125	120	145	149	161	181	122	131	141.75	20.1
TSS (mg/L)	10	10	10	10	28	11	22	44	18.125	11.7
DTS(mg/L)	828	848	1132	1108	1182	1220	890	954	1020.3	147.6
NO_3-NO_2-N)mg/L N	0.3	0.1	0.1	0.1	0.1	0.2	0.1	0.3	0.1625	0.09
PO_4(mg/L)	0.6	0.9	0.2	0.2	0.3	0.4	1.9	0.1	0.575	0.56
pH	7.7	8.1	8	8.2	8.1	7.9	8.2	8	8.025	0.16
SO_4 (mg/L)	300	308	357	394	370	600	305	318	369	93.1
COD (mg/L)	10	10	10	32	10	16	26	35	18.625	10.0
Cd (mg/L)	0.07	0.02	0.02	0.07	0.02	0.02	0.07	0.07	0.045	0.03
Cu (mg/L)	0.04	0.01	0.01	0.04	0.01	0.01	0.04	0.04	0.025	0.02
Fe (mg/L)	0.08	0.01	0.01	0.05	0.01	0.7	0.06	0.12	0.13	0.22
Zn (mg/L)	0.06	0.07	0.07	0.06	0.07	0.07	0.06	0.06	0.065	0.01
As (mg/L)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	7.E-18
FC (cfu/100mL)	8	14	63	31	28	51	280	19	61.75	84.3
TC (cfu/100mL)	56	300	20	33	30	120	620	120	162.38	192.9
E.coli (cfu/100mL)	7	14	50	31	23	51	280	17	59.125	84.8
Heterotrophic Plate Count (cfu/1mL)	9000	4200	5500	5400	1980	4500	41200	4700	9560	12096.4

Table 4.13 above, show the mean values and the standard deviations of the physio-chemical parameters; nitrates, phosphate, sulphate; COD; trace metals and microbial results over eight (08) months period in analysed water samples.

Table 4.14: Mean results of analysed parameters in water samples at sampling point (BB6) across the sampling period

Determinant	13-03-08	21-04-08	5-06-08	17-07-08	26-08-08	14-10-08	20-11-08	13-01-09	Mean	Std Dev
EC (mS/m)	122	115	141	142	152	156	118	129	134.38	14.6
TSS (mg/L)	10	10	10	10	50	10	60	20	22.5	19.2
DTS(mg/L)	788	632	960	1002	1026	1070	916	906	912.5	133.3
NO_3-NO_2-N)mg/L N	12.3	0.4	0.5	0.6	0.7	1.8	0.2	0.1	2.075	3.89
PO_4(mg/L)	0.6	0.9	0.2	0.3	0.3	0.4	1.2	0.1	0.5	0.35
pH	7.8	8	8.3	8.5	8.2	8.1	8.2	8	8.1375	0.12
SO_4 (mg/L)	292	289	342	386	320	373	306	302	326.25	34.6
COD (mg/L)	10	10	10	10	10	15	30	34	16.125	9.36
Cd (mg/L)	0.07	0.02	0.02	0.07	0.02	0.02	0.07	0.07	0.045	0.03
Cu (mg/L)	0.04	0.01	0.01	0.04	0.01	0.01	0.04	0.04	0.025	0.02
Fe (mg/L)	0.12	0.01	0.06	0.05	0.02	0.1	0.31	0.09	0.095	0.09
Zn (mg/L)	0.06	0.07	0.07	0.06	0.07	0.07	0.06	0.06	0.065	0.005
As (mg/L)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	6.9E-18
FC (cfu/100mL)	860	1800	420	10	48	76	2900	62	772	988.0
TC (cfu/100mL)	9700	6300	5400	180	1400	250	18000	410	5205	5835.5
E.coli (cfu/100mL)	780	1600	290	10	39	76	2900	62	719.63	970.2
Heterotrophic Plate Count (cfu/1mL)	14700	30700	13390	4900	1410	6000	83000	8700	20350	25128.6

Table 4.14 above, show the mean values and the standard deviations of the physio-chemical parameters; nitrates, phosphate, sulphate; COD; trace metals and microbial results over eight (08) months period in analysed water samples.

Table 4.15: Mean results of analysed parameters in water samples at sampling point (BB7) across the sampling period

Determinant	13-03-08	21-04-08	5-06-08	17-07-08	26-08-08	14-10-08	20-11-08	13-01-09	Mean	Std Dev
EC (mS/m)	112	106	131	133	132	140	110	116	122.5	12.0
TSS (mg/L)	10	10	10	10	49	15	112	32	31	33.4
DTS(mg/L)	730	774	942	918	892	962	822	784	853	81.1
NO_3- NO_2-N)mg/L N	1.1	0.7	0.7	0.8	1.2	1.8	1.4	0.3	1	0.44
PO_4(mg/L)	0.6	0.6	0.3	0.3	0.5	0.6	1.4	0.1	0.55	0.36
pH	8	7.8	8.3	8.4	8.2	8.4	8	7.9	8.125	0.24
SO_4 (mg/L)	262	261	326	350	357	322	282	222	297.75	45.1
COD (mg/L)	27	10	11	16	10	16	42	29	20.125	10.8
Cd (mg/L)	0.07	0.02	0.02	0.07	0.02	0.02	0.07	0.07	0.045	0.04
Cu (mg/L)	0.04	0.01	0.01	0.04	0.01	0.01	0.04	0.04	0.025	0.02
Fe (mg/L)	0.27	0.01	0.07	0.09	0.2	0.21	0.39	0.18	0.1775	0.11
Zn (mg/L)	0.06	0.07	0.07	0.06	0.07	0.07	0.06	0.06	0.065	0.005
As (mg/L)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	6.9E-18
FC (cfu/100mL)	820	2800	830	24	78	80	3600	51	1035.4	1303.9
TC (cfu/100mL)	6800	39000	8600	1800	250	270	12000	350	8633.8	12208.4
E.coli (cfu/100mL)	820	2300	820	19	78	80	3600	50	970.88	1228.3
Heterotrophic Plate Count (cfu/1mL)	10400	17600	22000	22200	3900	5600	68000	32000	22713	19243.4

Table 4.15 above, show the mean values and the standard deviations of the physio-chemical parameters; nitrates, phosphate, sulphate; COD; trace metals and microbial results over eight (08) months period in analysed water samples.

Table 4.16: Mean results of analysed parameters in water samples at sampling point (BB8) across the sampling period

Determinant	13-03-08	21-04-08	5-06-08	17-07-08	26-08-08	14-10-08	20-11-08	13-01-09	Mean	Std Dev
EC (mS/m)	105	103	123	133	134	138	118	106	120	13.3
TSS (mg/L)	26	10	10	10	34	11	100	43	30.5	28.9
DTS(mg/L)	684	676	724	844	880	906	908	750	796.5	92.4
NO_3-NO_2-N)mg/L N	0.8	0.5	0.7	0.8	1.3	1.3		0.2	0.8	0.37
PO_4(mg/L)	0.6	0.4	0.5	0.3	0.5	0.5	1.6	0.1	0.5625	0.42
pH	8.1	8.2	8.2	8.5	8.2	8.4	8.1	7.8	8.1875	0.19
SO_4 (mg/L)	220	242	295	319	345	300	303	254	284.75	39.4
COD (mg/L)	10	51	10	23	10	25	36	29	24.25	13.6
Cd (mg/L)	0.07	0.02	0.02	0.07	0.02	0.02	0.07	0.07	0.045	0.03
Cu (mg/L)	0.04	0.01	0.01	0.04	0.01	0.12	0.04	0.04	0.0388	0.03
Fe (mg/L)	0.21	0.01	0.1	0.1	0.18	0.21	0.91	0.31	0.2538	0.26
Zn (mg/L)	0.06	0.07	0.07	0.06	0.07	0.07	0.08	0.06	0.0675	0.01
As (mg/L)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	6.9E-18
FC (cfu/100mL)	260	1200	320	850	680	65	9700	0	1634.4	3072.5
TC (cfu/100mL)	7200	25000	4100	21000	9700	2000	66000	28	16879	20339.8
E.coli (cfu/100mL)	200	1200	280	850	640	65	9700	0	1616.9	3079.6
Heterotrophic Plate Count (cfu/1mL)	5400	18100	4300	3160	6600	149000	89000	213800E	39366	53054.1

Table 4.16 above, show the mean values and the standard deviations of the physio-chemical parameters; nitrates, phosphate, sulphate; COD; trace metals and microbial results over eight (08) months period in analysed water samples.

Table 4.17: Mean results of analysed parameters in water samples at sampling point (BB9) across the sampling period

Determinant	13-03-08	21-04-08	5-06-08	17-07-08	26-08-08	14-10-08	20-11-08	13-01-09	Mean	Std Dev
EC (mS/m)	108	100	127	128	134	140	117	106	120	13.5
TSS (mg/L)	27	10	10	18	42	10	147	48	39	43.1
DTS(mg/L)	720	554	888	882	874	970	740	744	796.5	123.7
NO_3- NO_2-N)mg/L N	1.8	0.8	1	1.4	1.4	1.2	1.8	1.8	1.4	0.36
PO_4(mg/L)	0.5	0.8	0.3	0.3	0.5	0.5	1.3	0.8	0.625	0.31
Ph	8	8.2	8.7	8.4	8.2	8.3	8.1	7.6	8.1875	0.30
SO_4 (mg/L)	240	246	322	318	333	304	295	31	261.13	92.7
COD (mg/L)	10	10	10	27	10	23	44	35	21.125	12.5
Cd (mg/L)	0.07	0.02	0.02	0.07	0.02	0.02	0.07	0.07	0.045	0.03
Cu (mg/L)	0.04	0.01	0.01	0.04	0.01	0.12	0.04	0.04	0.0388	0.03
Fe (mg/L)	0.31	0.01	0.11	0.13	0.1	0.1	0.49	0.26	0.1888	0.14
Zn (mg/L)	0.06	0.07	0.07	0.06	0.07	0.07	0.06	0.06	0.065	0.01
As (mg/L)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	6.9E-18
FC (cfu/100mL)	200	650	10	840	840	210	12500	240	1936.3	4003.6
TC (cfu/100mL)	420	29000	110	16000	7100	400	92000	2200	18404	29383.3
E.coli (cfu/100mL)	80	480	10	810	840	200	12500	210	1891.3	4020.5
Heterotrophic Plate Count (cfu/1mL)	13800	79000	8900	25600	6000	23300	105000	32800	36800	33563.0

Table 4.17 above, show the mean values and the standard deviations of the physio-chemical parameters; nitrates, phosphate, sulphate; COD; trace metals and microbial results over eight (08) months period in analysed water samples.

4.3 Overall mean values and graphical representations of analysed parameters in water samples.

These are presented in a series of Tables (Table 4.18- 4.34) and Graphs (Figure 4.1-4.17) as shown below:

1. pH

Table 4.18: Overall mean values of pH of water samples across sampling periods (BB1-9)

Sampling points	pH values
BB1	7.4
BB2	7.4
BB3	7.7
BB4	8
BB5	8
BB6	8.1
BB7	8.1
BB8	8.2
BB9	8.2

Table 4.18 showed the mean pH values of water samples collected and analysed across periods. The mean values ranged from 7.4–8.8. These values could be said to be within most of the standard value ranges for drinking water (6.0-9.0). At this pH levels no significant effects on health due to toxicity of dissolved metal ions and protonated species, or on taste are expected. Very slight effects on taste may be noticed on occasion. The acceptable pH required for irrigation purposes range from 6.5-8.4, thus the results from this study are suitable for irrigation. The soil pH within this range does not present major problems with either unavailability of plant nutrients or toxic levels of element. Direct contact with crop foliage by either high or low pH waters causes foliar damage, which can, depending on the severity and timing of the damage, result in a decreased yield or damage to fruit or other marketable products (DWAF 1996b).

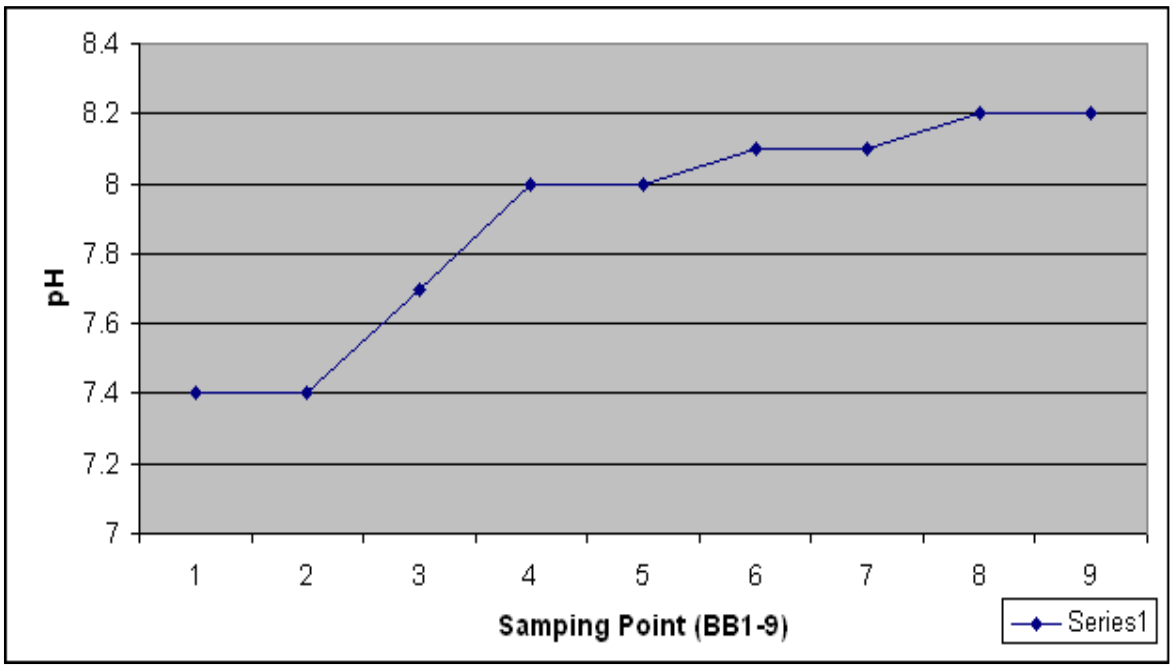

Figure 4.1: Variations in mean pH values of water samples across sampling points

Figure 4.1 showed the variation pattern of water pH values across the sampled sites. From the graph above it could be seen that the pH values was initially steady at 7.4 between water samples at BB1 and BB2 from where it increases steadily up to 8.2 and then stabilised at this value.

2. Electrical Conductivity (EC)

The results presented in Table 4.19 below are the mean values of EC for water samples across periods. The results are represented in the table below and their variations in the graph below.

Table 4.19: Overall mean results of EC of water samples across sampling periods (BB1-9)

Sampling points	EC (ms/m)
BB1	146.6
BB2	93.0
BB3	137.3
BB4	142.6
BB5	141.8
BB6	134.4
BB7	122.5
BB8	120
BB9	120

From the Table, the mean EC values of water samples across periods ranged from 93.0-146.6. EC is not a variable of concern in irrigation and was also not included in both the South African Guidelines for Irrigation. Electrical Conductivity is an indicator of the potential problems in plant growth associated with increasing quantities of salt. The final effect of using irrigation water with varying levels of salt is dependent upon the soil's ability to percolate water. As far as domestic use is concerned, EC has a direct relation with TDS, no health effects are associated with EC of <45mS/m and TDS of 300mg/L (DWAF, 1996a). If EC increases with direct relation to TDS, then no health effects will be observed.

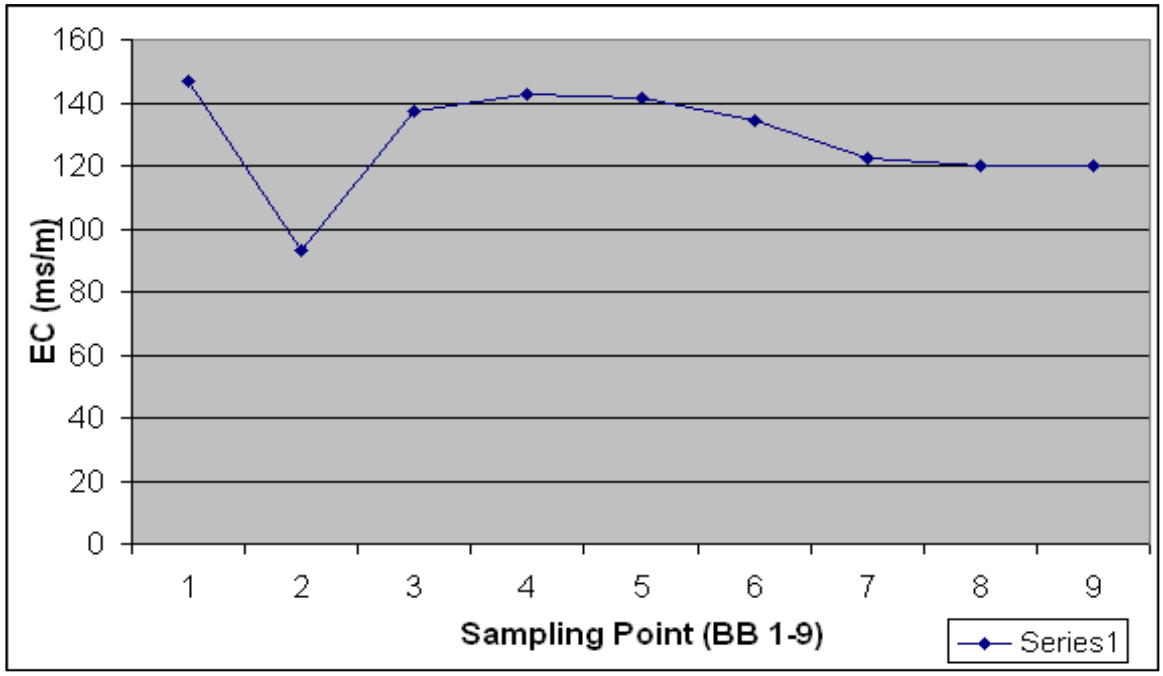

Figure 4.2: Variations in mean EC values of water samples across sampling points

From Figure 4.2, there was a decline in EC from BB1 to BB2 where it increased marginally from BB3 to BB7 and later became steady at BB8 and BB9.

3. Total Suspended Solids (TSS)

The results below are the mean values of TSS for water samples across periods. The results are represented in the Table 4.20 below and their variations graphically presented in Figure 4.3 below.

Table 4.20: Result of the overall mean values of TSS of water samples across sampling periods (BB1-9)

Sampling points	TSS (mg/L)
BB1	19.25
BB2	12.88
BB3	28.75
BB4	11.25
BB5	18.13
BB6	22.5
BB7	31
BB8	30.5
BB9	39

From the Table 4.20 above, the mean TSS values of water samples across periods ranged from 11.25– 39.0 and could be said to be within most of the standard value ranges for drinking water (>10mg/L). Water with a turbidity of >10mg/L is associated with severe aesthetic effects (appearance, taste and odour). Water with high turbidity has the ability to carry associated risk of disease due to infectious disease agents and chemicals adsorbed onto particulate matter. A chance of disease transmission at epidemic level exists at high turbidity. The requirement for irrigation purposes is in the range of (50-100mg/L). Suspended solids are mostly comprised of particulate matter of inorganic origin with no inherent toxic effect for plants or soil.

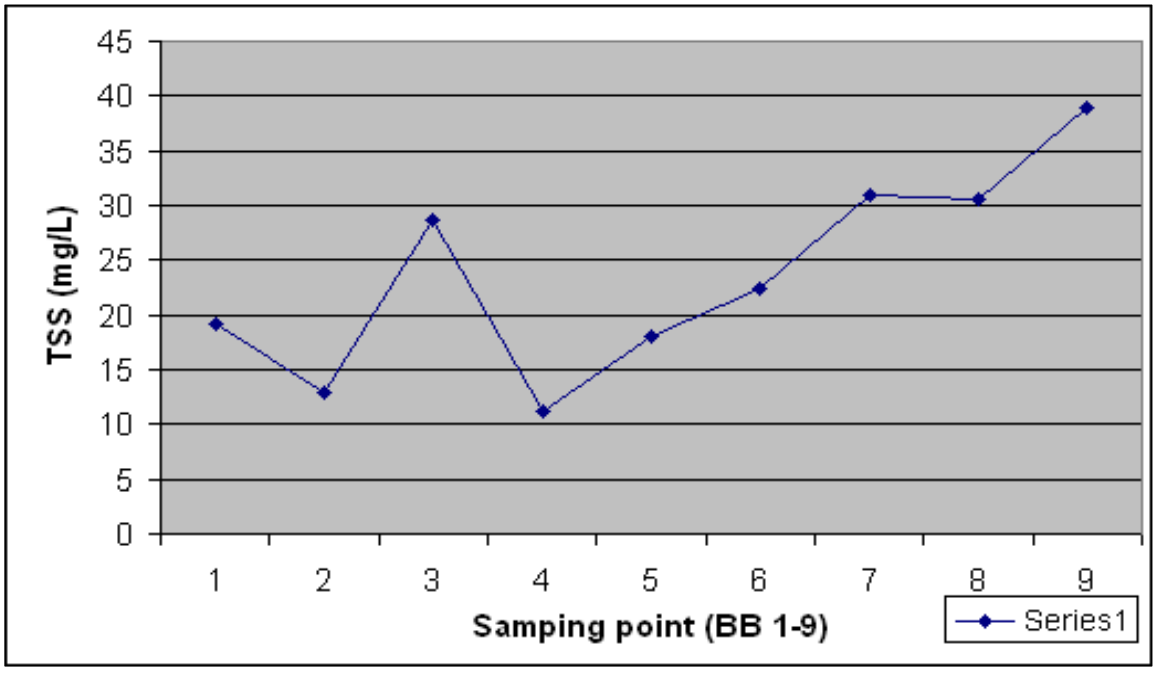

Figure 4.3: Variations in mean TSS values of water samples across sampling points

Figure 4.3 showed the variation pattern of water TSS values across the sampled sites. From the graph above it could be seen that TSS values are fluctuating from BB1-BB4,

and from BB4 a linear increase was observed till BB7, constant to BB8 and then increases to 39.0 at BB9.

4. Total Dissolved Solids (TDS)

The results below are the mean values of TDS for water samples across periods. The results are represented in the Table 4.21 below and their variations graphically presented in Figure 4.4 below.

Table 4.21: Overall mean results of the TDS of water samples across sampling periods (BB1-9)

Sampling points	TDS (mg/L)
BB1	1001.25
BB2	590.25
BB3	952.25
BB4	970.25
BB5	1020.25
BB6	912.5
BB7	853
BB8	796.5
BB9	796.5

The mean values ranged from 590.25–1020.25mg/L and are extremely outside the standard values for irrigation purposes (40-90mg/L in sensitive crops and 90-270mg/L in salt tolerant crops). Irrigation with water containing salt introduces salt into the soil profile. When no or little leaching of salt takes place from the soil profile, salt accumulates and a saline soil is formed. Since crops are sensitive to soil salinity, yield is reduced if grown on salt affected soils.

Having discussed EC and that direct increase relation between the two cannot result in health effects, The above results assessed against EC using the Water Quality Guidelines for Domestic Use as a guideline, the water in the Lower Blesbokspruit cannot cause any health defects because the TDS ranging from 450-1000mS/m is associated with EC of between 70-150, hence no health effects associated with this combination.

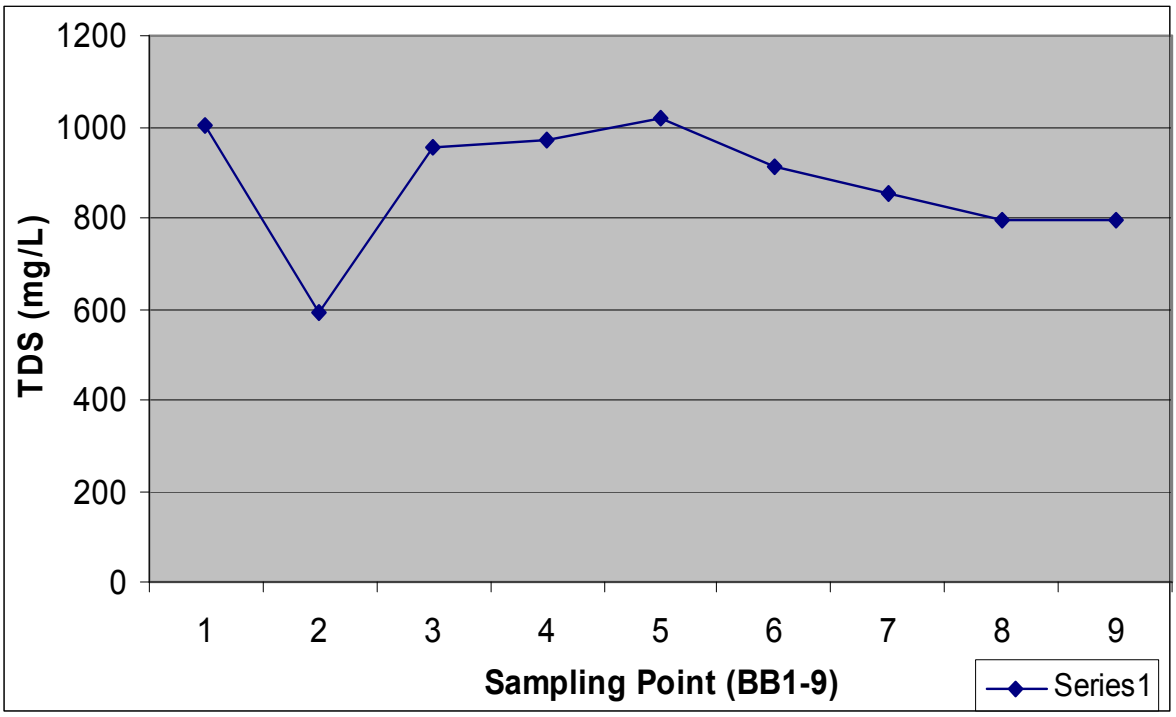

Figure 4.4: Variations in mean Total Dissolved Solids (TDS) values of water samples across sampling points

Figure 4.4 showed the variation pattern of water TDS values across the sampled sites. From the graph above it could be seen that TDS values shows the opposite of TSS above, the results start by fluctuating in a decreasing manner from BB1-BB2, from there it increases linearly from BB3 to BB5 and decreases again from BB5 to BB9.

5. Nitrate-Nitrite-Nitrogen

The results below are the mean values of Nitrate-Nitrite-Nitrogen for water samples across periods. The results are represented in the Table 4.22 below and their graphical variations in the Figure 4.5 below.

Table 4.22: Result of the overall mean values of (NO_3-NO_2-N) in mg/L of water samples across sampling periods (BB1-9)

Sampling points	(NO_3-NO_2-N) in mg/L
BB1	0.26
BB2	1
BB3	0.26
BB4	0.16
BB5	0.16
BB6	2.01
BB7	1
BB8	0.8
BB9	1.4

The mean values of nitrate in analysed water samples varied from 0.16-2.01mg/L and are set to be within the SA standard values for irrigation purposes (5.0-30mg/L). Nitrogen is one of the essential macro plant nutrients and its presence in irrigation water is mostly viewed as beneficial. However, high concentrations may stimulate excessive vegetative growth and cause lodging, delayed crop maturity and poor quality (as is the case when too much nitrogenous fertilizer is applied).

Nitrates values between 0-6mg/L have not adverse health effects (DWAF, 2006a). Still on this characteristic, water in the lower Blesbokspruit may not have any health effects associated with this variable.

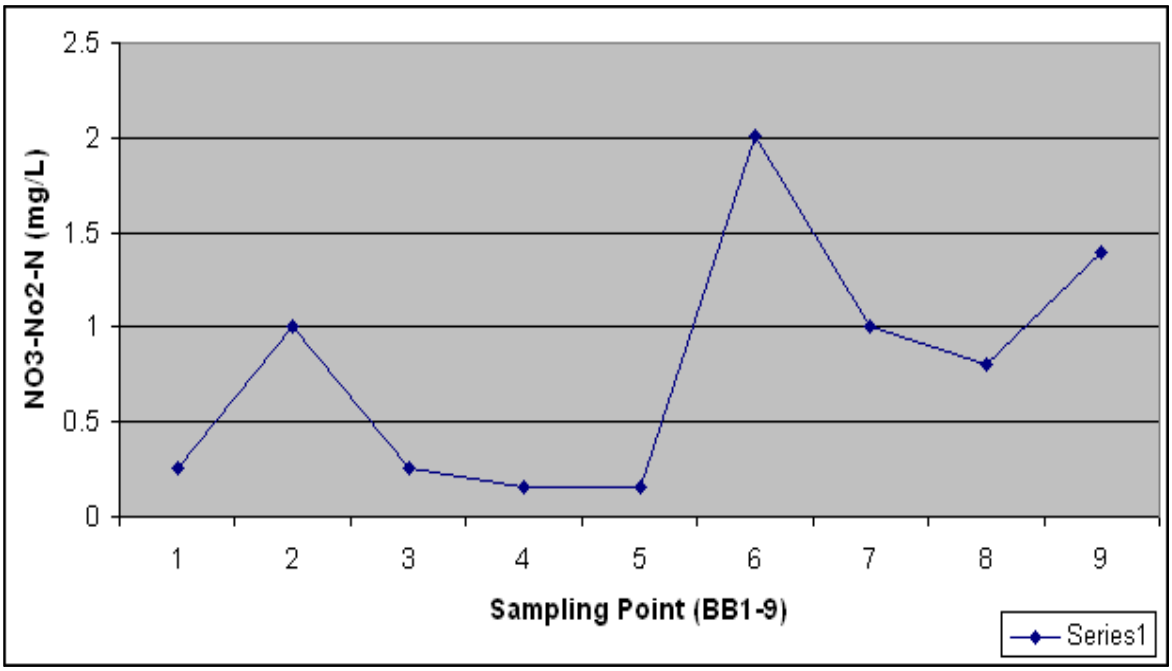

Figure 4.5: Variations in mean Nitrate-Nitrite-Nitrogen (NO_3-NO_2-N) values of water samples across sampling points

From Figure 4.5, there was no distinct pattern in the variation in the nitrate values across the sampled sites. However, there was a sharp increase in value from BB5 to BB6. This sharp increase might be as a result of the seepages from irrigated lawn located close to sampling point 5.

6. Phosphate

The results below are the mean values of Orthophosphates (PO_4^{3-} mg/L) for water samples across periods. The results are represented in the Table 4.23 and their graphical variations in Figure 4.6 below.

Table 4.23: Overall mean results of PO_4^{3-} mg/L of water samples across sampling periods (BB1-9)

Sampling points	PO_4^{3-} (mg/L)
BB1	0.79
BB2	0.96
BB3	0.59
BB4	0.61
BB5	0.58
BB6	0.50
BB7	0.55
BB8	0.56
BB9	0.63

The mean values of phosphates in analysed water samples varied from 0.50-0.96mg/L, phosphates analyses have not been included in the SA standard values for irrigation, but research has shown that continuous medium- to long-term application of nitrogen- and phosphate-rich irrigation water from integrated aquaculture-agriculture systems appeared to have some negative effects on soil conditions (Prinsloo, *et al.*, 2000). The SA guideline for P in water systems that will reduce the likelihood of algal and other plant growth is 5mg/l (DWAF, 1996b). The results obtained in this study are within the acceptable levels to reduce algal bloom.

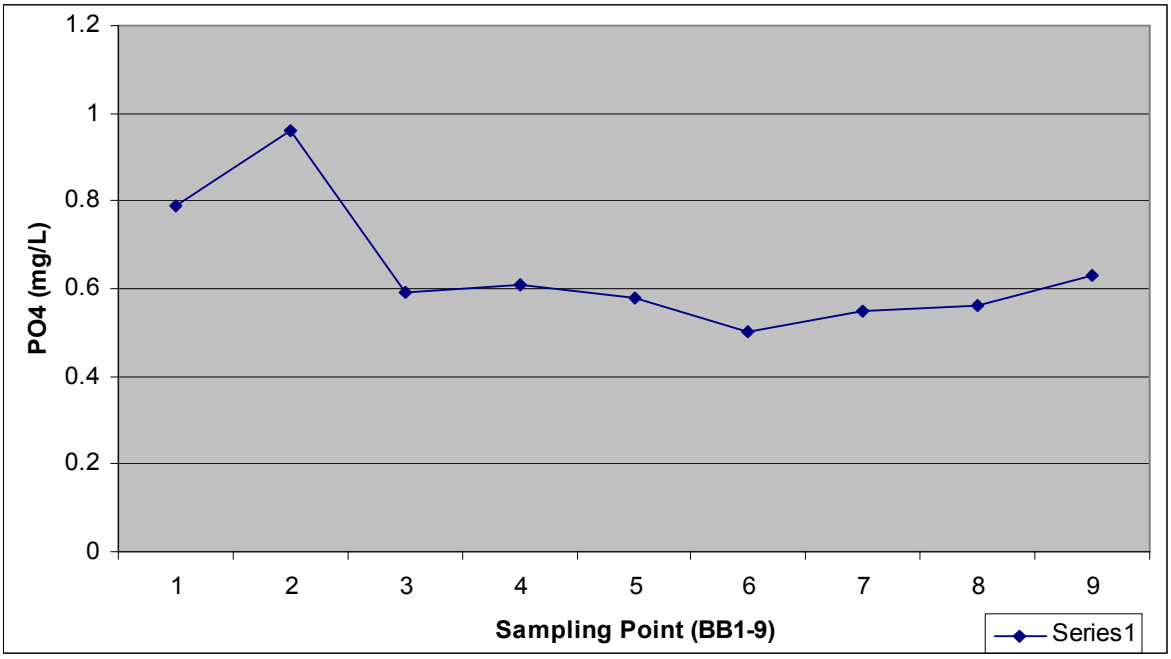

Figure 4.6: Variations in mean Orthophosphates (PO_4^{3-}) values of water samples across sampling points

From Figure 4.6, there is a decrease in PO_4 values from BB2 to BB6, followed by a slight increase from BB6 to BB9. This increase can be associated with sewage works discharges from these points.

7. Sulphate

The results below are the mean values of Sulphates (SO_4^{2-}) in mg/L for water samples across periods. The results are represented in the Table 4.24 and their graphical variations in Figure 4.7 below.

Table 4.24: Result of the overall mean values of SO_4^{2-} (mg/L) of water samples across sampling periods (BB1-9)

Sampling points	SO_4^{2-} (mg/L)
BB1	379.5
BB2	118.63
BB3	337.75
BB4	353.13
BB5	369
BB6	326.25
BB7	297.75
BB8	284.75
BB9	261.13

The mean values of sulphates in analysed water samples varied from 118.63-379.5mg/L. Sulphates analyses are also not included in the SA Guidelines for irrigation, however sulphates have been know to enhance crop growth. With regard to drinking water the acceptable sulphates concentration with no adverse health effects is 0-200mg/L, only one sampling point BB2 is within the acceptable standard, Most of the results lies in the range of 200-400mg/L, and here a tendency to develop diarrhea in sensitive and some non-adapted individuals exists, as well as a slight taste could be noticeable.

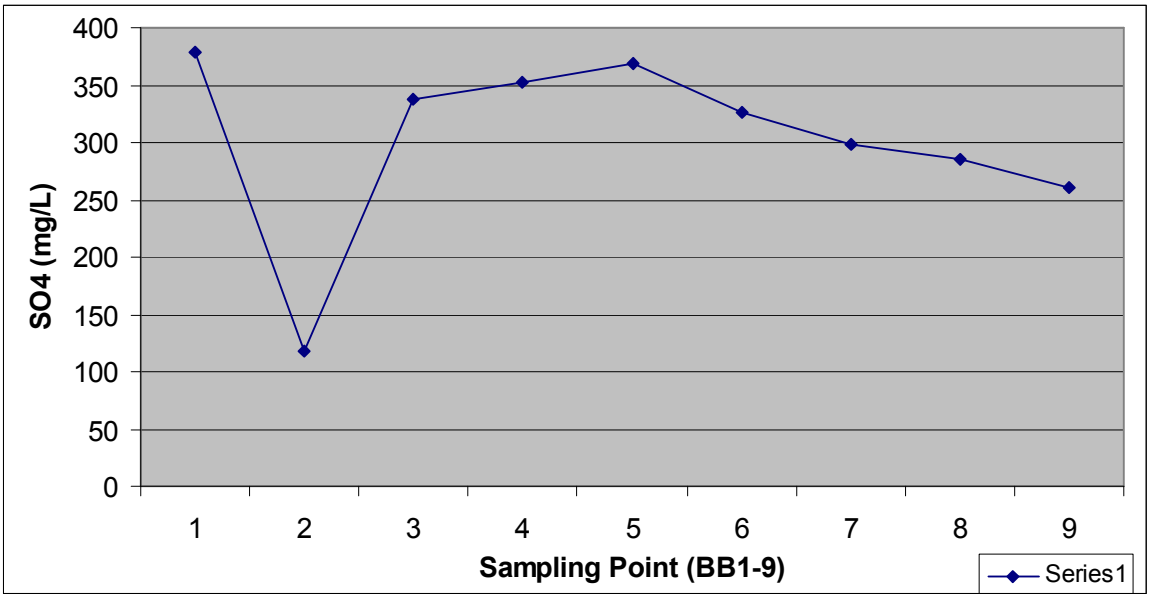

Figure 4.7: Variations in mean Sulphates (SO_4) values of water samples across sampling points

From Figure 4.7, there was no distinct pattern in the variation in the nitrate values across the sampled sites. However, there was a sharp increase in value from BB5 to BB6. A sharp decrease is observed as the river flows away from the mining areas i.e. close to sampling point 5.

8. COD

The results below are the mean values of Chemical Oxygen Demand (COD) in mg/L for water samples across periods. The results are represented in the Table 4.25 and also depicted graphically in Figure 4.8

Table 4.25: Result of the overall mean values of COD (mg/L) of water samples across sampling periods (BB1-9)

Sampling points	COD (mg/L)
BB1	15.75
BB2	34.75
BB3	21.88
BB4	15.63
BB5	18.63
BB6	16.13
BB7	20.13
BB8	24.25
BB9	21.13

The mean values of sulphates in analysed water samples varied from 118.63-379.5mg/L. COD is not a variable of concern for irrigation and hence is not included in the SA Guidelines for irrigation, however according to DWAF 1999, Government Notice 1191, the acceptable COD levels for irrigation with waste water is 400mg/L per 500m3, hence the above results can be set to be within the acceptable levels for irrigation.

Though there are no COD guidelines in the SA Water Quality Guidelines (DWAF 1996d), the oxygen-free water in the Blesbokspruit, would have negative effects on the freshwater quality as well as cause harm to the aquatic life in the river with potentially dire consequences on the aquatic biota.

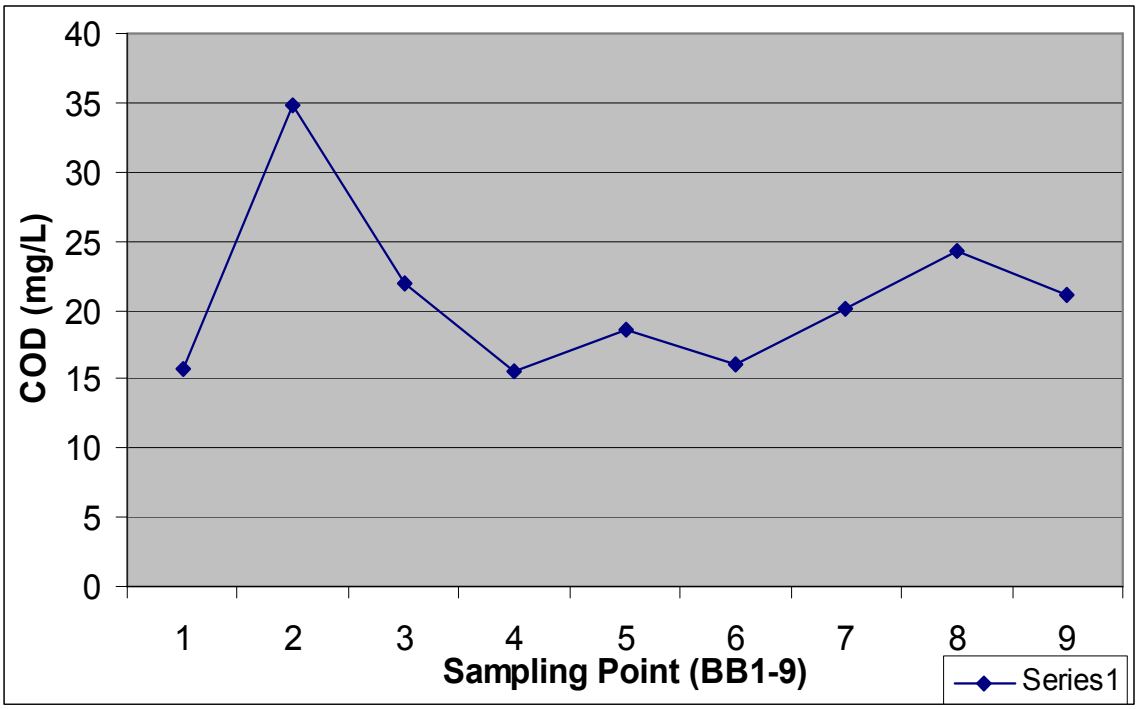

Figure 4.8: Variations in mean Chemical Oxygen Demand (COD) values of water samples across sampling points

From Figure 4.8, there was no distinct pattern in the variation in the COD values across the sampled sites. However, there is an increase in value on points downstream of the sewage works, a sharp increase from BB6 to BB9. Another sharp increase is noted at BB2; also this point is downstream of the sewage works.

9 Trace metals

9.1 Cadmium (Cd)

The results below are the mean values of Cadmium (Cd) in mg/L for water samples across periods. The results are represented in the Table 4.26 and the graphical variation in Figure 4.9 respectively.

Table 4.2: Result of the overall mean values of Cd (mg/L) of water samples across sampling periods (BB1-9)

Sampling points	Cd (mg/L)
BB1	0.045
BB2	0.045
BB3	0.045
BB4	0.045
BB5	0.045
BB6	0.045
BB7	0.045
BB8	0.045
BB9	0.045

The mean value of Cd in analysed water samples from BB1-BB9 were 0.045. There was no variation in all the values. This might be due to the detection limit of the instrument used for this metal and not necessarily the actual value of this metal in all samples across the sites. However, this value is higher than the recommended value of 0.005 mg/l for drinking water (DWAF, 2006a), consuming this water is dangerous as acute or irreversible effects of Cd associated with kidney failure exist.

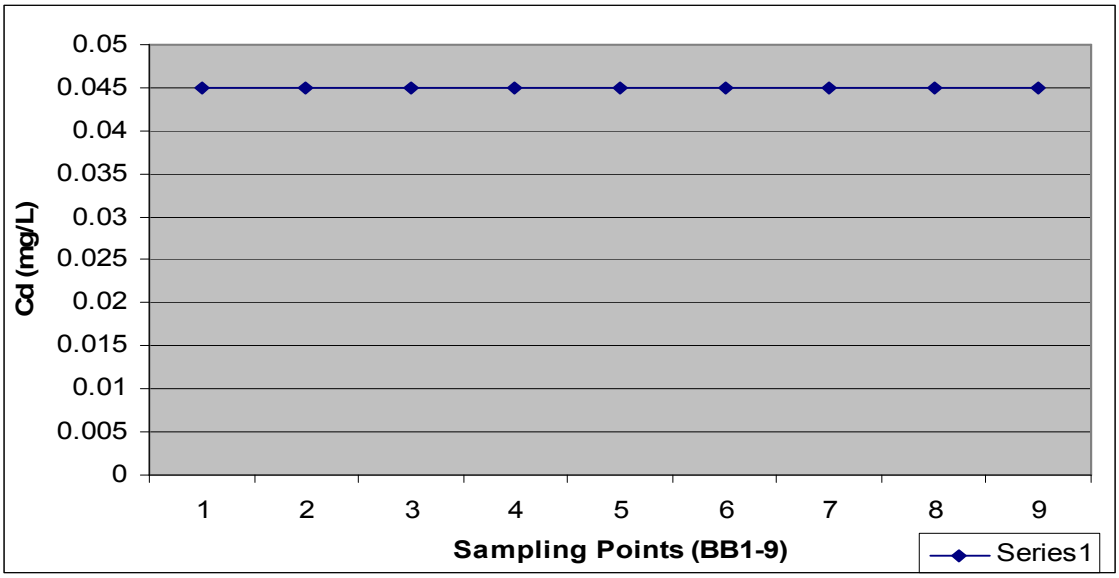

Figure 4.9: Variations in mean Cadmium (Cd) values of water samples across sampling points

Figure 4.9 showed the variation in mean Cd values across sampled points. Cd values are constant throughout the sampling period.

9.2 Copper (Cu)

The results below are the mean values of Copper (Cu) in mg/L for water samples across periods. The results are represented in the table below and their variations in the graph below.

Table 4.27: Result of the overall mean values of Cu (mg/L) of water samples across sampling periods (BB1-9)

Sampling points	Cu (mg/L)
BB1	0.039
BB2	0.025
BB3	0.025
BB4	0.025
BB5	0.025
BB6	0.025
BB7	0.025
BB8	0.039
BB9	0.039

The mean values of Cu in analysed water samples varied from 0.025-0.039mg/L. These values are set to be within the standard values for irrigation (0.2-5.0mg/L) As with water consumption these values are also acceptable. No health effects are visible with Cu values of 0-1.0mg/L

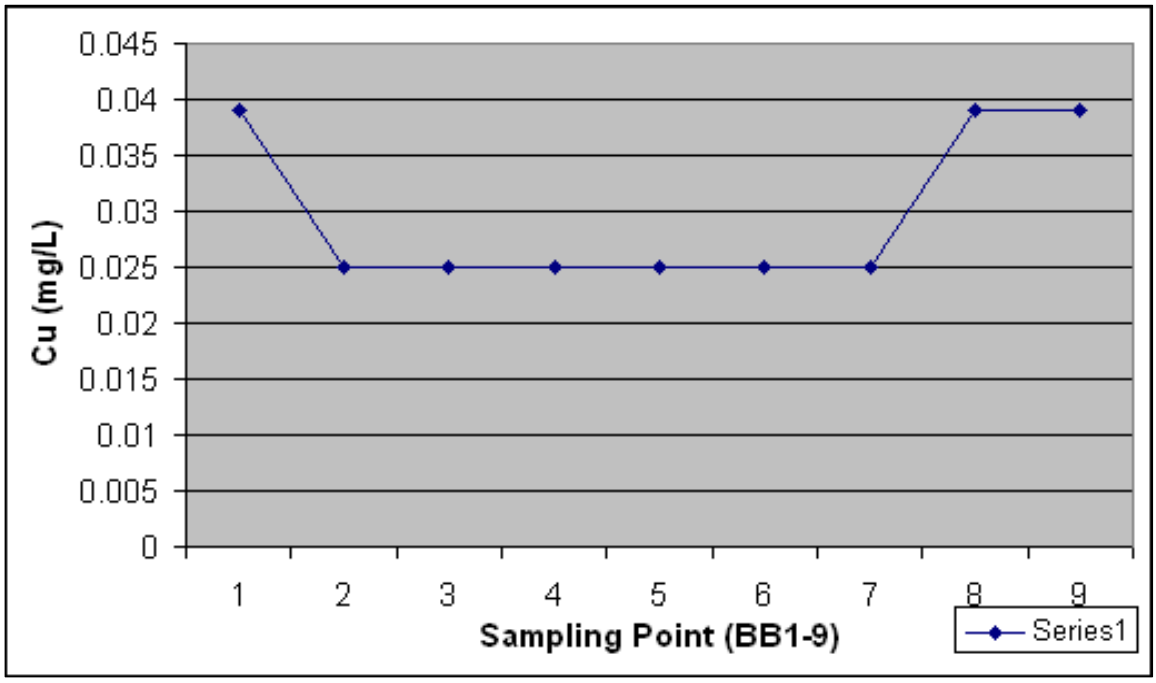

Figure 4.10: Variations in mean Copper (Cu) values of water samples across sampling points

In Figure 4.10, Cu values started high at 0.039mg/L and decreased sharply at BB2, these decreased was then maintained and Cu was constant at 0.025mg/L across a number of sampling points. It then increased back to 0.039mg/L from BB8 and remained constant at this value.

9.3 Iron (Fe)

The results below are the mean values of Iron (Fe) in mg/L for water samples across periods. The results are represented in the Table 4.28 and the variations in Figure 4.11 as shown below.

Table 4.28: Result of the overall mean values of Fe (mg/L) of water samples across sampling periods (BB1-9)

Sampling points	Fe (mg/L)
BB1	0.078
BB2	0.73
BB3	0.12
BB4	0.04
BB5	0.13
BB6	0.1
BB7	0.18
BB8	0.25
BB9	0.19

The mean values of Fe in analysed water samples varied from 0.04-0.73mg/L. These values are set to be within the standard values for irrigation (5.0-30.0mg/L). No health effects are visible for Fe values ranging from 0-0.1mg/L, in this study only two points i.e. BB1 and BB4 are within the acceptable standards for domestic use, the rest may result in slight effects on taste and marginal other aesthetic effects. No health effects; the water is generally well tolerated for Fe values of 0.1-0.3mg/L

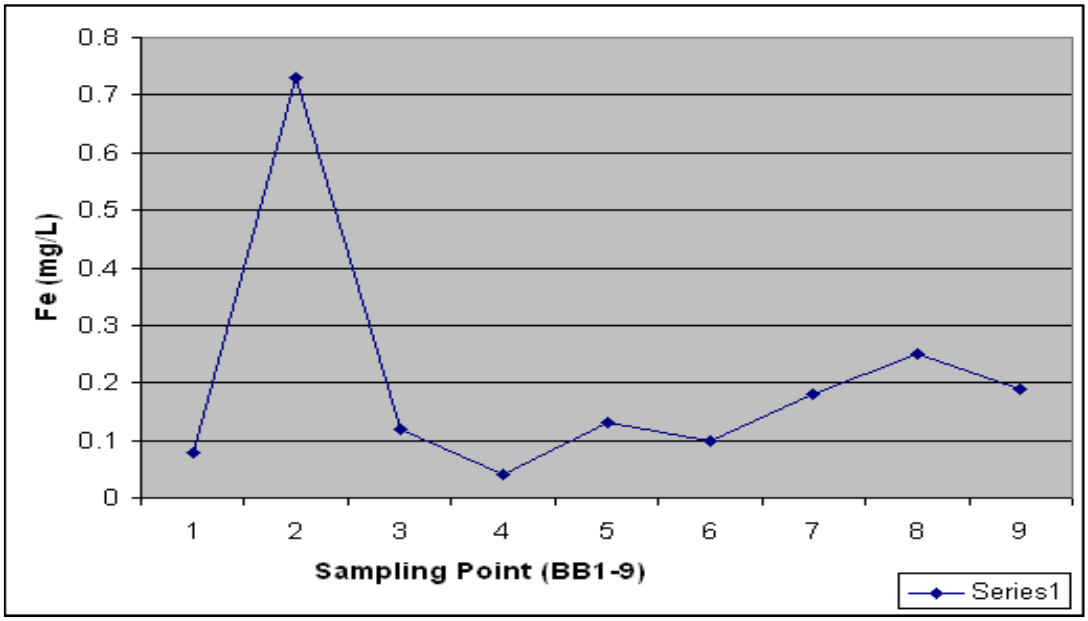

Figure 4.11: Variations in mean Iron (Fe) values of water samples across sampling points

In Figure 4.11, Fe values started at a very low 0.078 and increased sharply at BB2, it then decrease with no distinct pattern. From BB6, the values increased sharply and went down again at BB9

9.4 Zinc (Zn)

The results below are the mean values of Zinc (Zn) in mg/L for water samples across periods. The results are represented in the table below and their variations in the graph below.

Table 4.29: Result of the overall mean values of Zn (mg/L) of water samples across sampling periods (BB1-9)

Sampling points	Zn (mg/L)
BB1	0.065
BB2	0.065
BB3	0.12
BB4	0.065
BB5	0.065
BB6	0.065
BB7	0.065
BB8	0.068
BB9	0.065

The mean values of Zn in analysed water samples varied from 0.065 -0.12mg/L. These values are set to be within the standard values for irrigation (1.0-5.0 mg/L). No health effect associated with domestic use of water in this stream for the variable Zn with the acceptable levels of (0-3.0 mg/L)

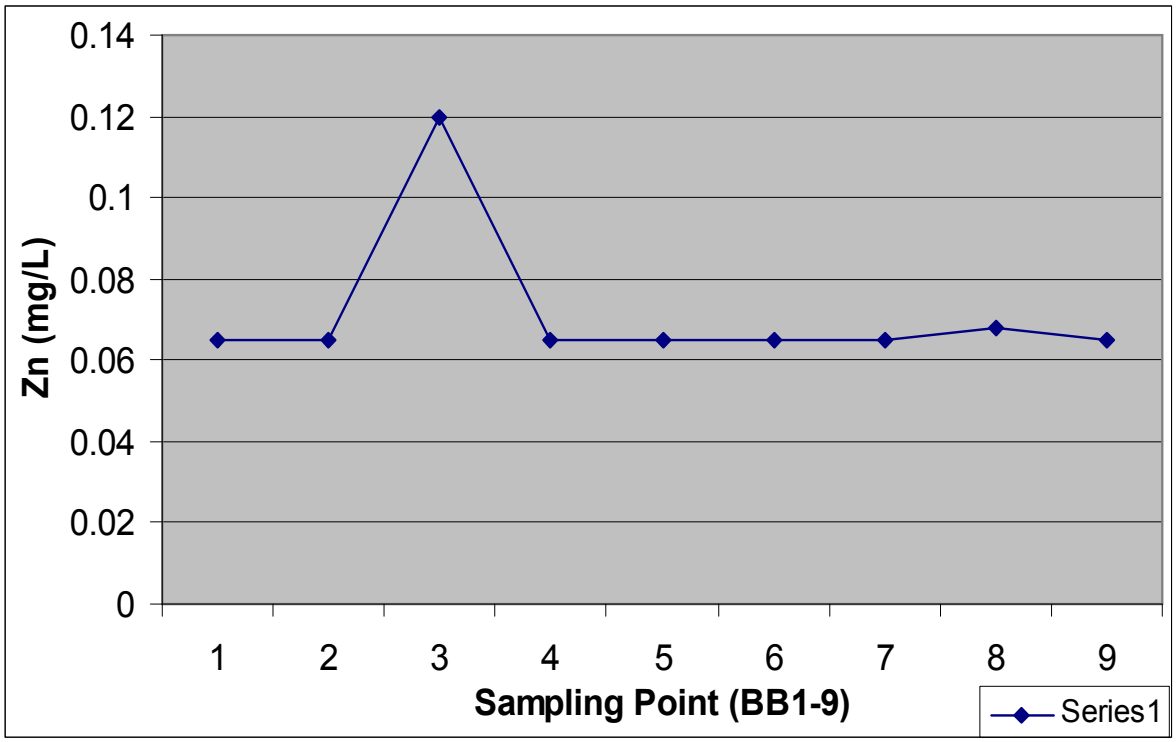

Figure 4.12: Variations in mean Zinc (Zn) values of water samples across sampling points

In Figure 4.12, the Zn pattern is generally constant, a sharp increase is observed at BB3 and the cause is unknown.

9.5 Arsenic (As)

The results below represent the mean values of in Arsenic (As) mg/L for water samples across periods, their variations are also represented in the graph below.

Table 4.30: Overall mean values of As (mg/L) of water samples across sampling periods (BB1-9)

Sampling points	As (mg/L)
BB1	0.05
BB2	0.05
BB3	0.05
BB4	0.05
BB5	0.05
BB6	0.05
BB7	0.05
BB8	0.05
BB9	0.05

Table 4.30 showed the mean As values of water samples collected and analysed across periods. The mean values were constant at 0.05mg/L. These values could be said to be within most of the standard value ranges for drinking water (0-10mg/L), Arsenic when

consumed is slowly excreted from the body, hence it can easily accumulate. Poisoning can be both chronic and acute. Requirements for irrigation purposes is 0.1-2.0, (these is the maximum concentration acceptable for fine textured, neutral to alkaline soils.), However, the sensitivity of the crop to arsenic depend on the crop type, while nutrient solutions containing arsenic can induce toxicity.

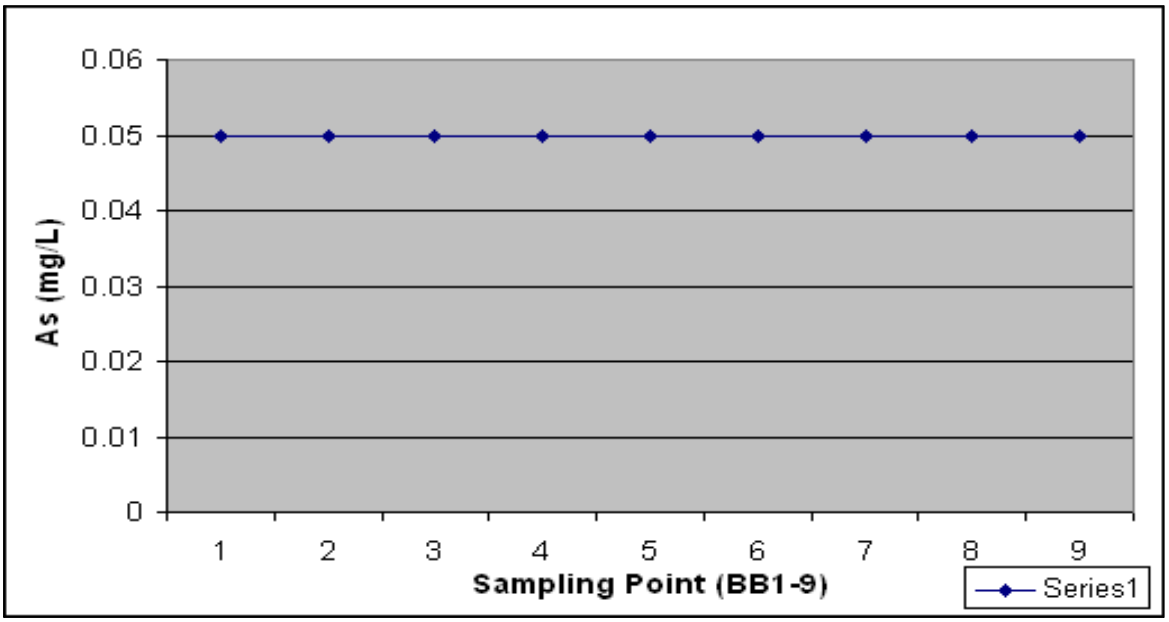

Figure 4.13: Variations in mean Arsenic (As) values of water samples across sampling points

Figure 4.13 showed the variation pattern of water As values across the sampled sites. From the graph above it could be seen that the As values were constant at 0.05mg/L.

10. Faecal Coliforms (FC) in cfu/100mL

The results below are the mean values of Faecal Coliforms (FC) in cfu/100mL for water samples across periods. The results are represented in the Table 4.31 and the graphical representation in Figure 4.14 below.

Table 4.31: Result of the overall mean values of FC (cfu/100mL) of water samples across sampling periods (BB1-9)

Sampling points	FC (cfu/100mL)
BB1	20.63
BB2	44021
BB3	52.88
BB4	15.88
BB5	61.75
BB6	772
BB7	1035.36
BB8	16878,5
BB9	1936.25

Table 4.31 above show the mean FC values of water samples collected and analysed across periods. The mean values range from 20.63 cfu/100mL to 44021 cfu/100mL. There are values that are within the SA guidelines for irrigation (1-1000cfu/100mL), however all the points downstream of the sewage works are outside these guidelines and this indicate poor sanitation facilities in the catchment. Likelihood of contamination from vegetables and other crops eaten raw and of milk from cows grazing on pastures will result in the transmission of human pathogens.

The only ideal FC consumption is 0cfu/100mL; in this study values are alarming. According to DWAF 2006a, coliforms >20cfu/100mL pose a significant and increasing risk of infectious disease transmission. As Faecal Coliform levels increases, the amount of water ingested that is required to cause infection decreases.

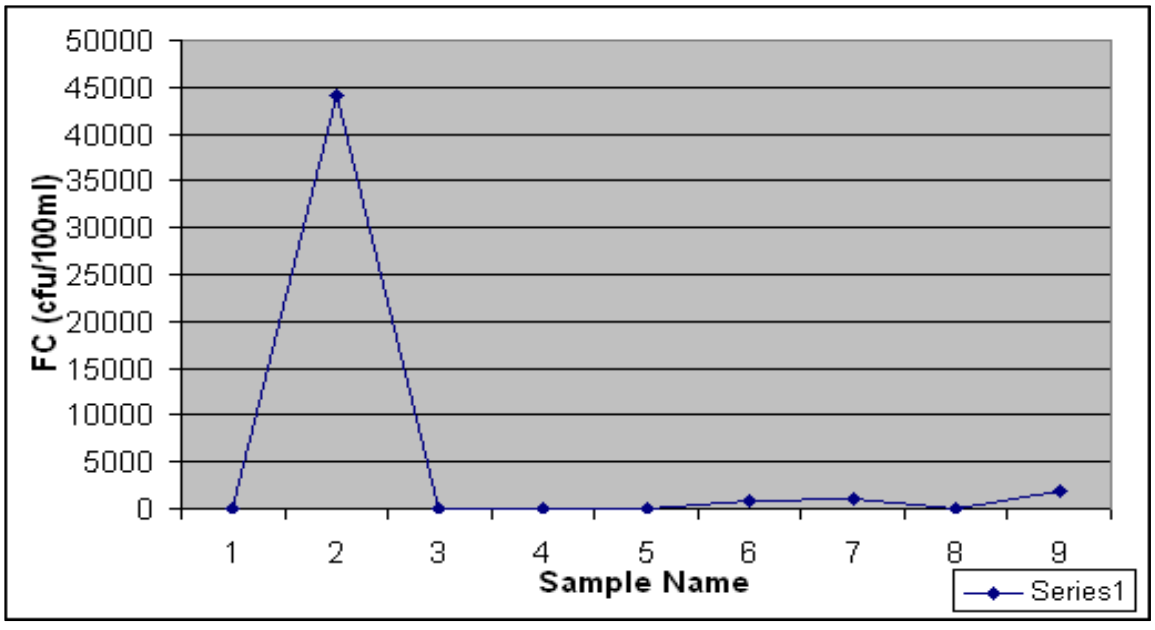

Figure 4.14: Variations in mean Faecal Coliforms (FC) values of water samples across sampling points

Figure 4.14 showed the variation pattern of water FC values across the sampled sites. From the graph above it could be seen that there is no distinct characteristics of FC hence some of the values seem to be very low. The problem lies with sewage works where the results peaks instantly and goes as high as 44021cfu/100mL from 20.63cfu/100mL. In general there is an increase in FC values from BB6 as all the points from BB6 are downstream of sewage works.

11. Total Coliforms (TC) in cfu/100mL

The results below are the mean values of in Total Coliforms (TC) cfu/100mL for water samples across periods. The results are represented in the Table 4.32 and the variation pattern as shown in Figure 4.15 below.

Table 4.32: Overall mean results of TC (cfu/100mL) of water samples across sampling periods (BB1-9)

Sampling points	TC (cfu/100mL)
BB1	92.88
BB2	430294
BB3	674.89
BB4	48.13
BB5	162.38
BB6	5205
BB7	8633.75
BB8	16878.5
BB9	18403.75

Table 4.32 above show the mean TC values of water samples collected and analysed across periods. The mean values range from 92.88 cfu/100ml to 430294 cfu/100mL. This variable is not included in the SA guidelines for irrigation because some of the coliforms recorded here may be from animal faeces whereby animals graze near to the water resource.

Negligible risk of microbial infection is expected in waters containing FC contamination of 0-5cfu/100mL. In this study, the values are way above this level indicative of poor treatment, post-treatment contamination or definite growth in the water distribution system. Significant and increasing risk of infectious disease transmission is highly expected from consuming this water.

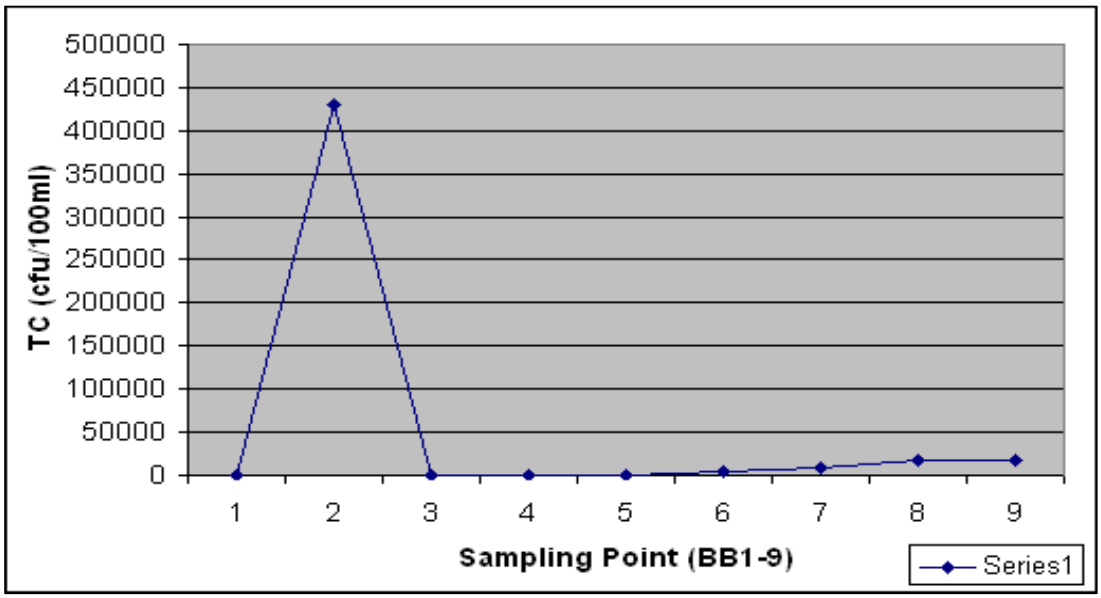

Figure 4.15: Variations in mean Total Coliforms (TC) values of water samples across sampling points

Figure 4.15 showed the variation pattern of water TC values across the sampled sites. From the graph above it could be seen that there is no distinct characteristics of TC hence some of the values seem to be very low. As much as some of the FC maybe as a result of animals grazing in the area, there is a direct relationship with points downstream of sewage works and high FC. From the graph above it could be seen that there is no distinct characteristics of TC hence some of the values. The values peaks instantly at sewage works downstream points and goes as high as 430294 cfu/100mL. In general there is an increase in TC values from BB6.

12. *Escherichia Coli (E. coli)* in cfu/100mL

The results below are the mean values of *Escherichia Coli (E. coli)* in cfu/100mL for water samples across periods. The results are represented in the Table 4.33 and graphical variation pattern in Figure 4.16 below.

Table 4.23: Result of the overall mean values of *E. coli* (cfu/100mL) of water samples across sampling periods (BB1-9)

Sampling points	*E. coli* (cfu/100mL)
BB1	19.13
BB2	43999.125
BB3	52.5
BB4	14.13
BB5	59.13
BB6	719.63
BB7	970.86
BB8	1616.86
BB9	1891.25

Table 4.33 above show the mean *E. coli* values of water samples collected and analysed across periods. *E. coli* is an indicator micro organism of human excreta contamination. The mean values range from 19.13 cfu/100mL to 43999.125 cfu/100mL. This variable is also not included in the SA guidelines for irrigation however as with FC, crops irrigated with water contaminated with *E.coli* pose a serious health risk. *E.coli* was not included in the SA Water Quality Guidelines for Domestic Use; hence the WHO standards shall be utilized to discuss this point. *E. coli* must not be detectable in any 100mL sample. This statement confirms that the water in the Blesbokspruit must not be utilized for domestic purposes.

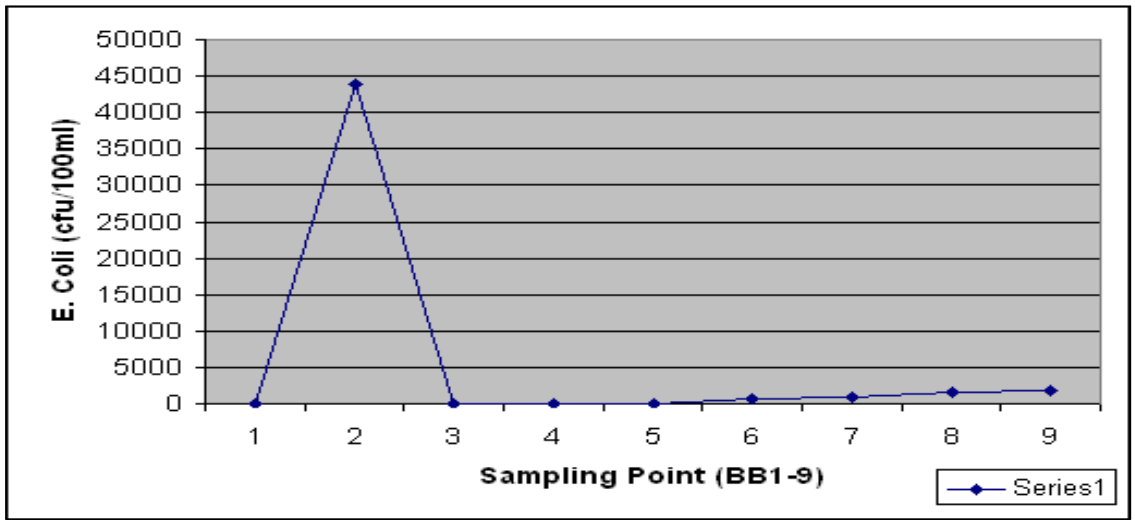

Figure 4.16: Variations in mean *Escherichia coli (E. coli)* values of water samples across sampling points

Figure 4.16 showed the variation pattern of water *E. coli* values across the sampled sites. From the graph above it could be seen that the pattern is similar to the microbes already discussed above. *E. coli* is an indicator micro organism of human excreta contamination; hence high values at points downstream of sewage works (BB2, BB6, BB8, and BB9) are not surprising. A disturbing analysis is the presence of these

microbes throughout the stream, indicating that more often human excreta find their ways to water resources.

13. Heterotrophic Plate Count in (cfu/100mL)

The results below are the mean values of in Heterotrophic Plate Count in cfu/100mL for water samples across periods. The results are represented in the Table 4.34 and the variation pattern graphically presented in Figure 4.17 below.

Table 4.34: Overall mean values of Heterotrophic Plate Count in (cfu/100mL) of water samples across sampling periods (BB1-9)

Sampling points	Heterotrophic Plate Count (cfu/1mL)
BB1	7266.25
BB2	397775
BB3	10190
BB4	4322.5
BB5	9560
BB6	20350
BB7	22712.5
BB8	39365.71
BB9	36800

Table 4.34 above show the mean Heterotrophic plate count values of water samples collected and analysed across periods. Similar to FC, heterotrophic plate count is not a variable of concern because it just indicate the presence micro organisms, does not necessarily differentiate between pathogens and other coliforms. The mean values range from 10190 cfu/100mL to 397775 cfu/100mL. This variable is also not included in the SA guidelines for irrigation because some of the coliforms recorded here may be not be pathogens.

The presence of heterotrophic plate count from 100cfu/100mL is an indicative of poor treatment, post-treatment contamination or definite after-growth in the water distribution system. Increased risk of infectious disease transmission occurs. Pollution of water can give rise to conditions conducive to bacterial growth, such as high nutrient concentrations and high turbidity and can result in a substantial increase of these naturally-occurring organisms.

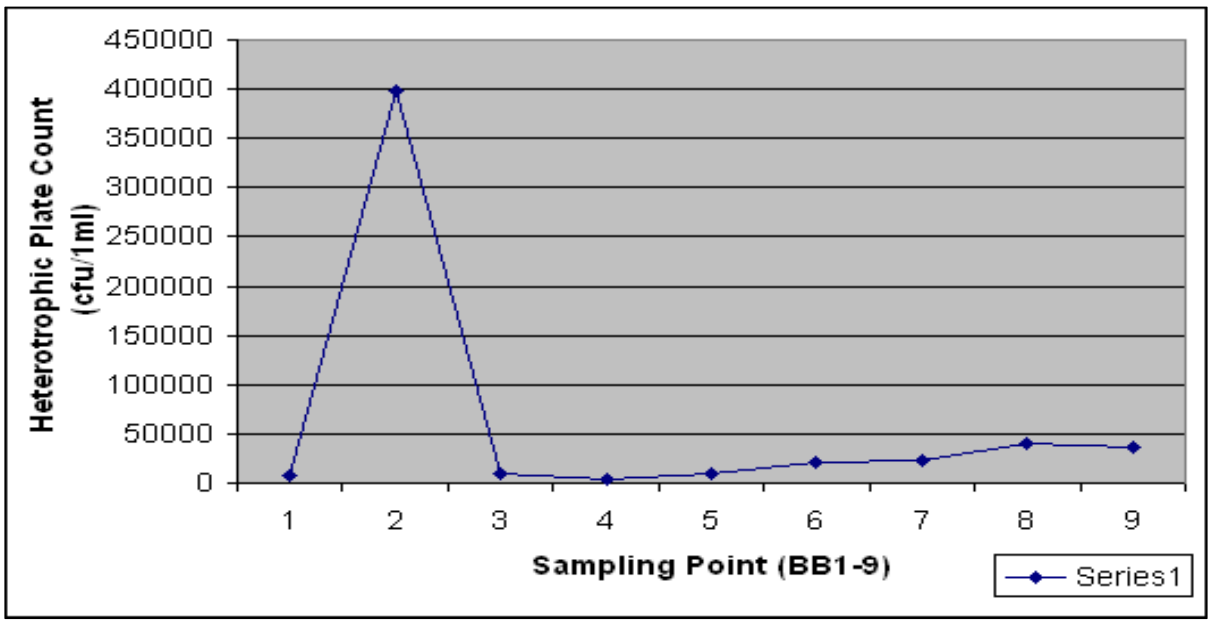

Figure 4.17: Variations in mean Heterotrophic Plate Count values of water samples across sampling points

Figure 4.17 showed the variation pattern of water Heterotrophic plate count values across the sampled sites. From the graph above, it could be seen that there is no distinct characteristics of Heterotrophic plate count hence some of the values seem to be very low. The values peaks instantly at BB2 which is downstream of the sewage works and the value goes as high as 430294 cfu/100mL. In general there is an increase in TC values from BB6.

4.4 The seasonal variation of analysed parameters per sampling point

The results below show the seasonal variations of parameters per sampling point. These are presented in a series of Tables (Table 4.35- 4.51) and Graphs (Figure 4.18-4.34) where the months of June/July are the dry/winter seasons and the months of November/January are the wet/summer seasons as shown below:

The results below are the seasonal variations of pH for water samples across the wet and dry periods. The results are represented in the Table 4.35 and also depicted graphically in Figure 4.18

Table 4.35: The seasonal variation of pH per sampling point

pH				
Sampling point	**Dry/winter seasons**		**Wet/summer seasons**	
	5-Jun-08	**17-Jul-08**	**20-Nov-08**	**13-Jan-09**
BB1	7.0	7.5	7.9	6.8
BB2	7.1	7.6	7.6	7.1
BB3	8.1	7.7	7.9	7.3
BB4	8.1	8.3	8.1	7.6
BB5	8.0	8.2	8.2	8.0
BB6	8.3	8.5	8.2	8.0
BB7	8.3	8.4	8.0	7.9
BB8	8.2	8.5	8.1	7.8
BB9	8.7	8.4	8.1	7.6

From Table 4.35 above, the pH values for the winter season varied from 7.0 to 8.7 in June and from 7.5 to 8.5 in July while for the summer season the pH values varied from 7.6 to 8.2 in November and from 6.8 to 8.0 in January.

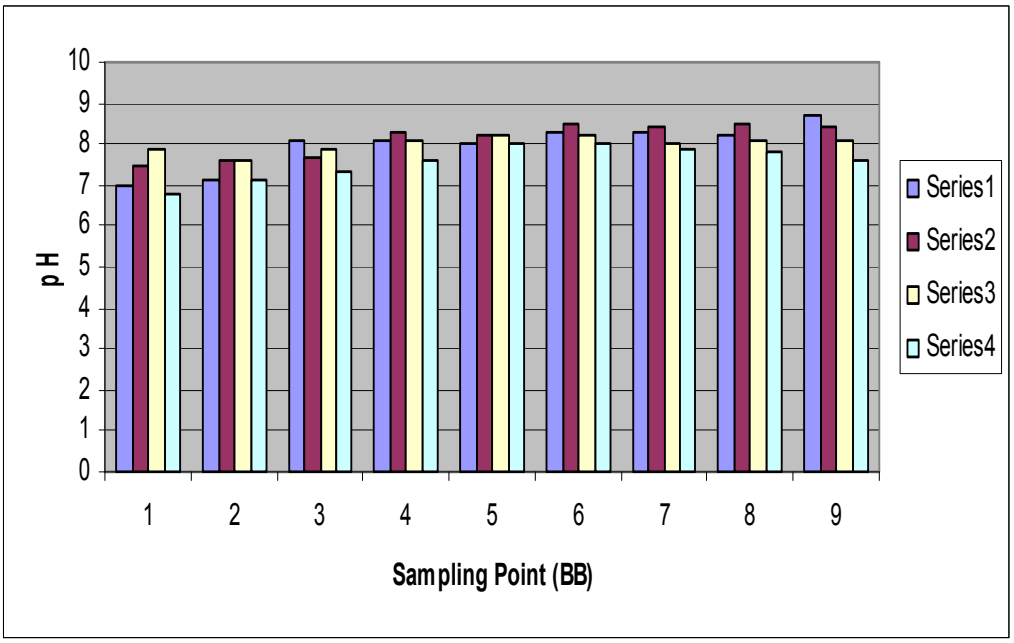

Figure 4.18: Seasonal variations of pH per sampling points

From Figure 4.18, it is evident that the pH of the water samples was within the recommended safety limit by (DWAF, 1996a). The pH values obtained during the rainy also seemed not to have any effect on the ph range. pH value would have been expected to shift towards alkalinity during this period. However, the points from BB6 showed a linear increase which could be associated with the fact that during winter

seasons, the sewage works treat more water mainly coming from the storm water channels, so this increase is not surprising and is acceptable.

Table 4.36: The seasonal variation of EC per sampling point

EC (mS/m)				
Sampling point	**Wet/winter seasons**		**Dry/ summer seasons**	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	134	181	100	133
BB2	79	79	52	90
BB3	145	150	99	131
BB4	147	151	116	133
BB5	145	149	122	131
BB6	141	142	118	129
BB7	131	133	110	116
BB8	123	133	118	106
BB9	127	128	117	106

From Table 4.36 above, the EC values for the winter season varied from 79 to 147 mS/m in June and from 79 to 181 mS/m in July while for the summer season the EC values varied from 52 to 122 mS/m in November and from 90 to 133 mS/m in January.

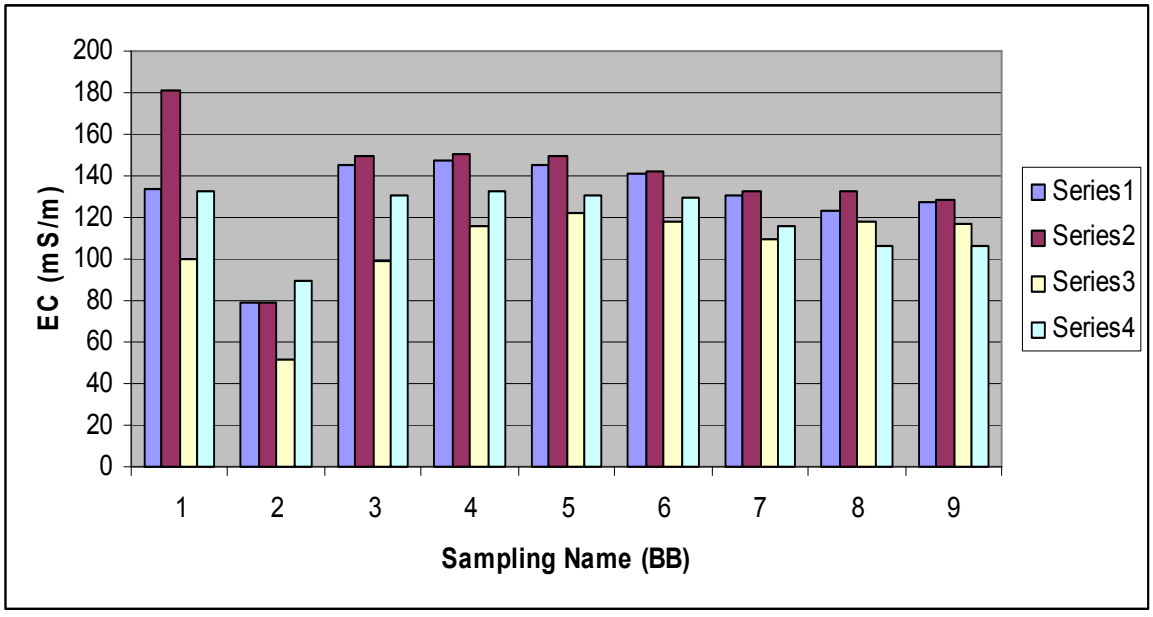

Figure 4.19: Seasonal variations of EC per sampling points

From Figure 4.19, it is evident that the water in the Blesbokspruit had high levels of EC in the winter season than in the summer season across the resource. It can also be seen that the EC levels in series 3 were generally low as opposed to the series 4 of January,

this is an indication that generally the rainfall in November are higher than the rainfalls in January. From this graph it is clear that this variable is affected by rainfalls.

Table 4.37: The seasonal variation of TSS per sampling point

TSS (mg/L)				
Sampling point	Dry/winter season		Wet/summer season	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	10	10	10	10
BB2	14	16	11	13
BB3	10	10	95	10
BB4	10	10	10	10
BB5	10	10	22	44
BB6	10	10	60	20
BB7	10	10	112	32
BB8	10	10	100	43
BB9	10	18	147	48

From Table 4.37 above, the TSS values for the winter season varied from 70 to 14 mg/L in June and from 10 to 18 mg/L in July while for the summer season the TSS values varied from 10 to 147 mg/L in November and from 10 to 48 mg/L in January.

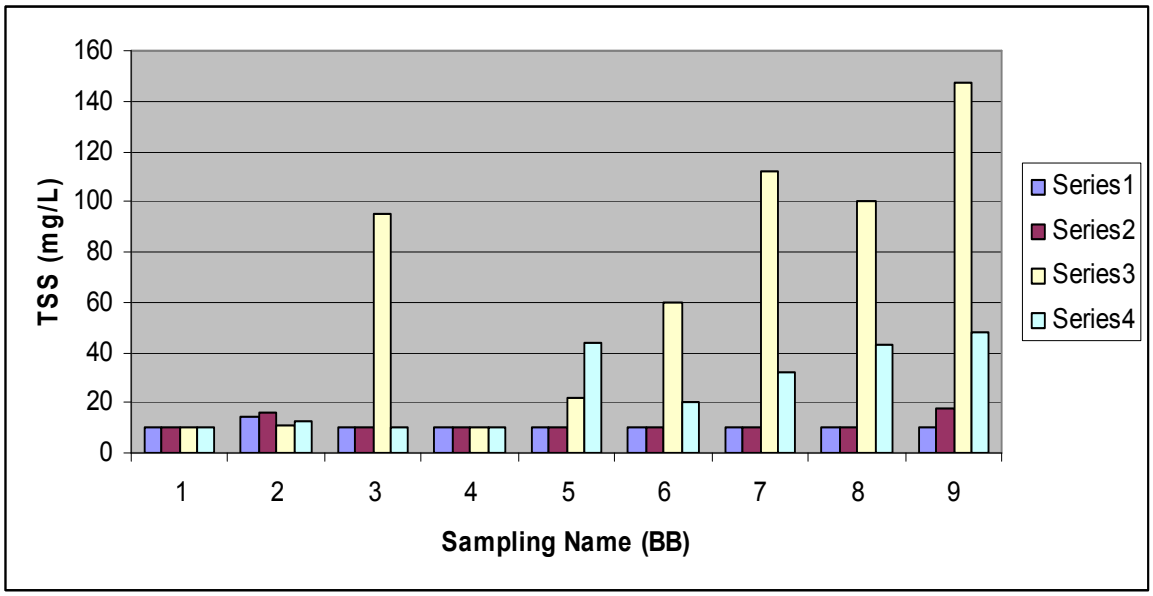

Figure 4.20: Seasonal variations of TSS per sampling points

From Figure 4.20, it is evident that the water in the Blesbokspruit had high levels of TSS in the month of November which is associated with high rainfalls. These results could be

as a result of soil erosion whereby solids are introduced into the river due to high rainfalls. Also from the graph above, from point BB5 there has been a linear increase in TSS levels during the wet/summer seasons, hence these findings confirms that heavy rainfalls has an influence on the amount of TSS in the water source.

Table 4.38: The seasonal variation of TDS per sampling point

TDS (mg/L)				
Sampling point	**Dry/winter seasons**		**Wet/summer seasons**	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	746	1294	740	880
BB2	624	478	336	546
BB3	1000	1054	718	940
BB4	954	1080	820	944
BB5	1132	1108	890	954
BB6	960	1002	916	906
BB7	942	918	822	784
BB8	724	844	908	750
BB9	888	822	740	744

From Table 4.38 above, the TDS values for the winter season varied from 746 to 1000 mg/L in June and from 478 to 1294 mg/L in July while for the summer season the TDS values varied from 336 to 1916 mg/L in November and from 546 to 954 mg/L in January.

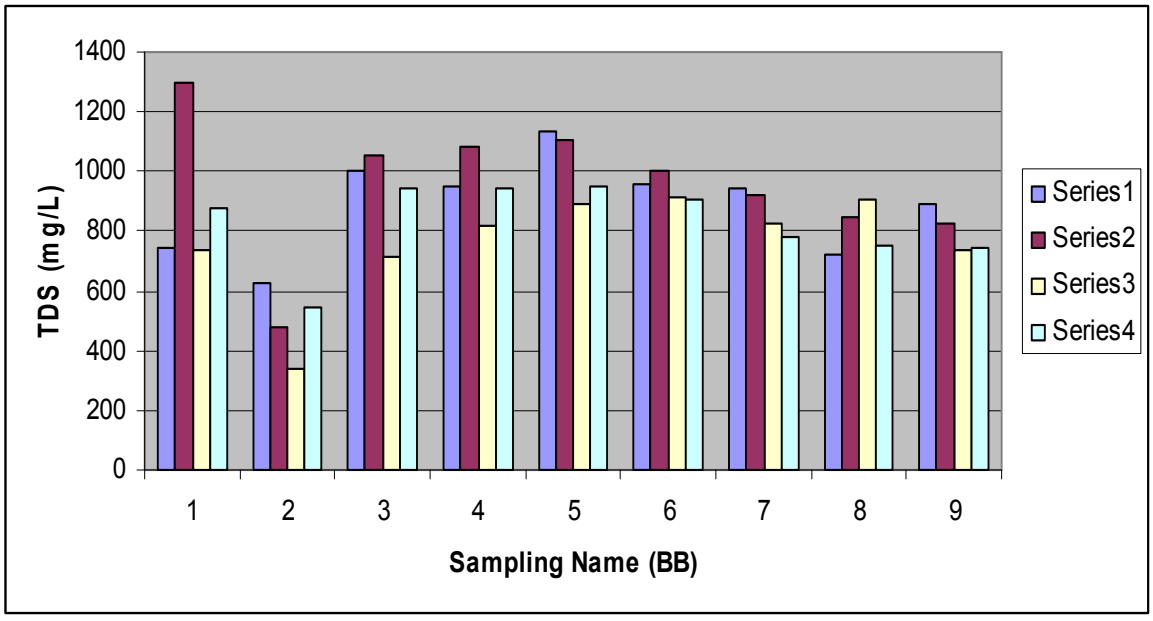

Figure 4.21: Seasonal variations of TDS per sampling points

From Figure 4.21, the water in the Blesbokspruit had high levels of TDS in the dry/winter months and also in the month of January. From the overall results, it can be concluded that during the summer season, rain does not necessarily dissolve solids into the water resource but rather introduce solids by erosion as seen in figure 4.20 above.

Table 4.39: The seasonal variation of NO_3-NO_2-N (mg/L) N per sampling point

NO_3-NO_2-N (mg/L N)				
Sampling point	Dry/winter seasons		Wet/summer seasons	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	0.5	0.5	0.2	0.1
BB2	1.9	1	0.2	0.1
BB3	0.2	1	0.2	0.2
BB4	0.1	0.1	0.2	0.1
BB5	0.1	0.1	0.1	0.3
BB6	0.5	0.6	0.2	0.1
BB7	0.7	0.8	1.4	0.3
BB8	0.7	0.8	1.6	0.2
BB9	1	1.4	1.8	1.8

From Table 4.39 above, the NO_3-NO_2-N values for the winter season varied from 0.1 to 1.9 in June and from 0.1 to 1.4 in July while for the summer season the NO_3-NO_2-N values varied from 0.1 to 1.8 both the months of November and January.

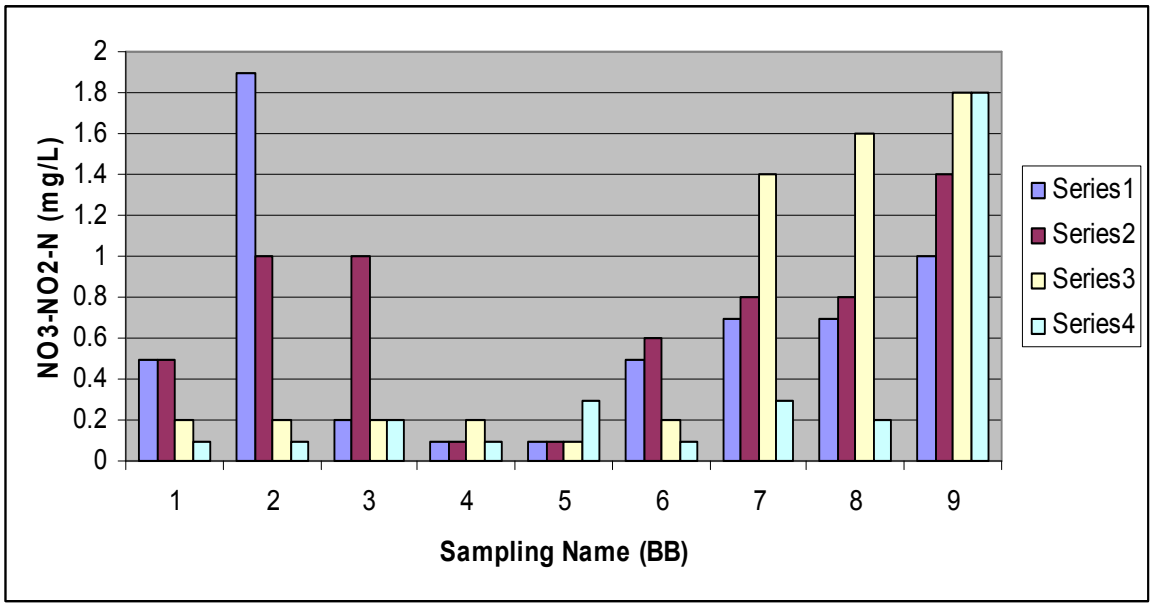

Figure 4.22: Seasonal variations of NO_3-NO_2-N per sampling points

From Figure 4.22, the levels of NO_3-NO_2-N were high for the dry/winter months at point BB1 to BB3 and from BB4 a linear increase in the levels of NO_3-NO_2-N was observed in the month of November and only at BB9 an increase in levels of NO_3-NO_2-N was observed in January. From the overall results, it can be concluded that during the dry/winter seasons, NO_3-NO_2-N is high.

Table 4.40: The seasonal variation of PO_4 (mg/L) per sampling point

PO_4(mg/L)				
Sampling point	**Dry/winter seasons**		**Wet/summer seasons**	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	0.1	0.3	1.4	0.1
BB2	0.5	0.4	1.2	0.1
BB3	0.2	0.2	1.4	0.1
BB4	0.2	0.2	1.8	0.2
BB5	0.2	0.2	1.9	0.1
BB6	0.2	0.3	1.2	0.1
BB7	0.3	0.3	1.4	0.1
BB8	0.5	0.3	1.6	0.1
BB9	0.3	0.3	1.3	0.8

From Table 4.40 above, the PO_4 values for the winter season varied from 0.1 to 0.5 mg/L in June and from 0.2 to 0.4 mg/L in July while for the summer season the PO_4 values varied from 1.2 to 1.9 mg/L in November and from 0.1 to 0.8 mg/L in January.

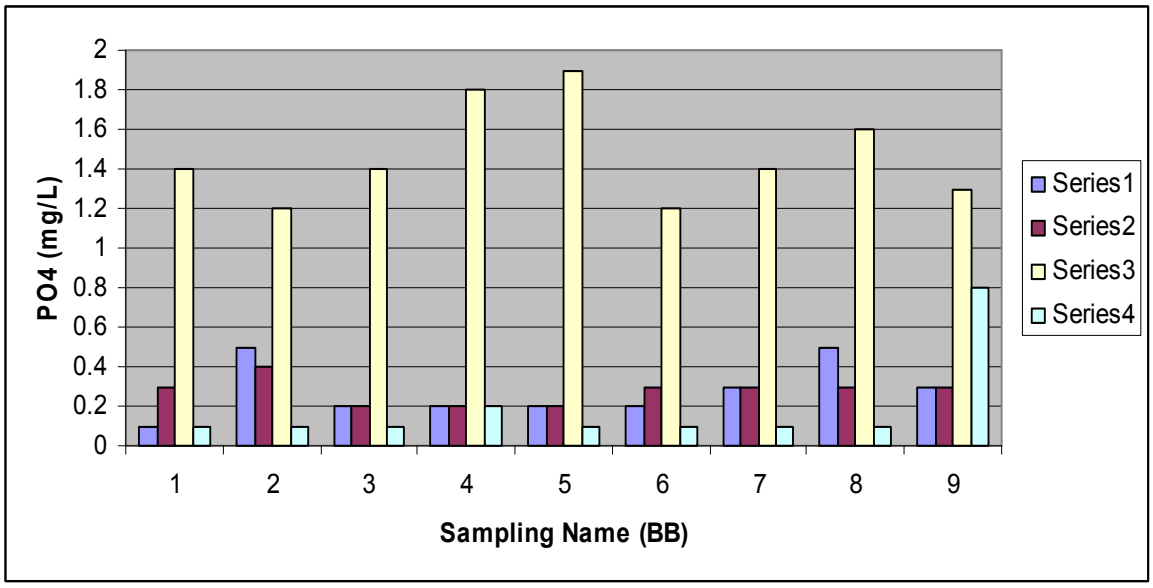

Figure 4.23: Seasonal variations of PO_4 per sampling points

From Figure 4.23, the levels of PO_4 were generally high in November. However in terms of seasonal changes, the results indicate that PO_4 does not depend on seasonal changes because at BB2, PO_4 was high in June, November and July while at BB9, PO_4 was high in November, January, June/July.

Table 4.41: The seasonal variation of SO_4(mg/L)per sampling point

SO_4 (mg/L)				
Sampling point	Dry/winter seasons		Wet/summer seasons	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	309	500	198	330
BB2	78	74	84	93
BB3	360	398	224	328
BB4	357	390	272	327
BB5	357	394	305	318
BB6	342	386	306	302
BB7	326	350	282	222
BB8	295	319	303	254
BB9	322	318	295	310

From Table 4.41 above, the SO_4 values for the winter season varied from 78 to 357 mg/L in June and from 74 to 500 mg/L in July while for the summer season the SO_4 values varied from 84 to 306 mg/L in November and from 93 to 328 mg/L in January.

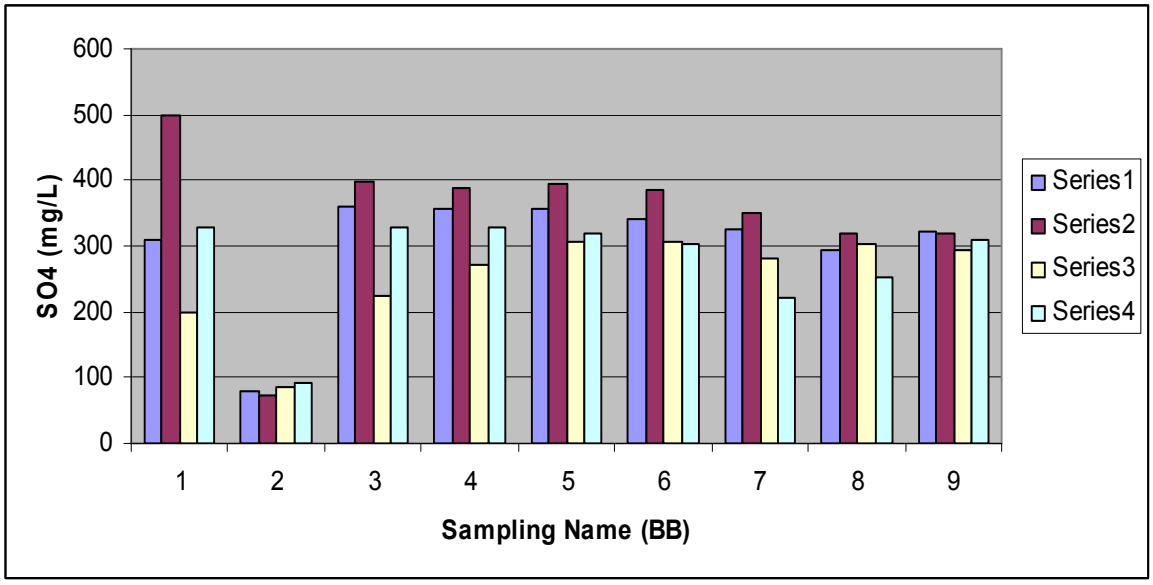

Figure 4.24: Seasonal variations of SO_4 per sampling points

From Figure 4.24, the levels of SO_4 were generally high in dry/winter seasons and lower in the wet/summer seasons except for BB2 where the opposite was observed.

Table 4.42: The seasonal variation of COD(mg/L)per sampling point

COD (mg/L)				
Sampling point	Dry/winter seasons		**Wet/summer seasons**	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	10	10	31	29
BB2	29	81	40	48
BB3	10	21	32	22
BB4	10	19	26	30
BB5	10	32	26	35
BB6	10	10	30	34
BB7	11	16	42	29
BB8	10	23	36	29
BB9	10	27	44	35

From Table 4.42 above, the COD levels for the winter season varied from 10 to 29 mg/L in June and from 10 to 81 mg/L in July while for the summer season the COD levels varied from 31 to 44 mg/L in November and from 22 to 48 mg/L in January.

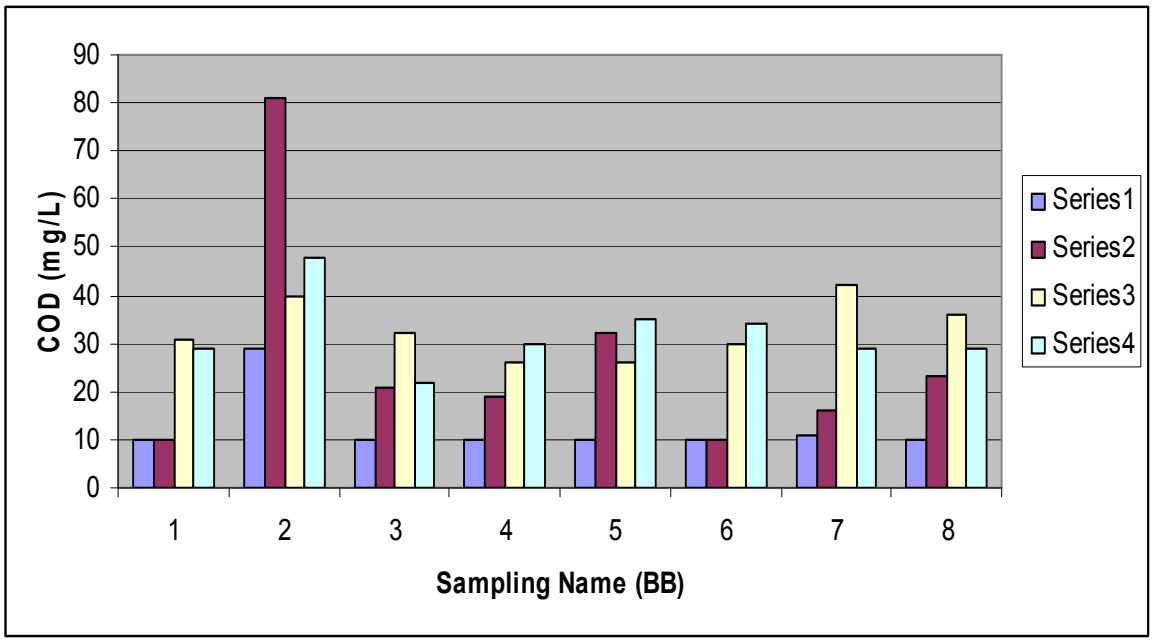

Figure 4.25: Seasonal variations of COD per sampling points

From Figure 4.25, the levels of COD were generally high in the wet/summer seasons with picking up exceptions at BB2 and BB5. From these results, it can be concluded that COD levels are dependent on the rainy seasons.

Table 4.43: The seasonal variation of Cd(mg/L)per sampling point

Cd (mg/L)				
Sampling point	Dry/winter seasons		Wet/summer seasons	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	0.02	0.07	0.07	0.07
BB2	0.02	0.07	0.07	0.07
BB3	0.02	0.07	0.07	0.07
BB 4	0.02	0.07	0.07	0.07
BB5	0.02	0.07	0.07	0.07
BB6	0.02	0.07	0.07	0.07
BB7	0.02	0.07	0.07	0.07
BB8	0.02	0.07	0.07	0.07
BB9	0.02	0.07	0.07	0.07

From Table 4.43 above, the Cd levels for the winter season was constant at 0.02 mg/L in June and 0.07 mg/L in July while for the summer season the Cd levels were constant at 0.07 mg/L for both the months of November and January.

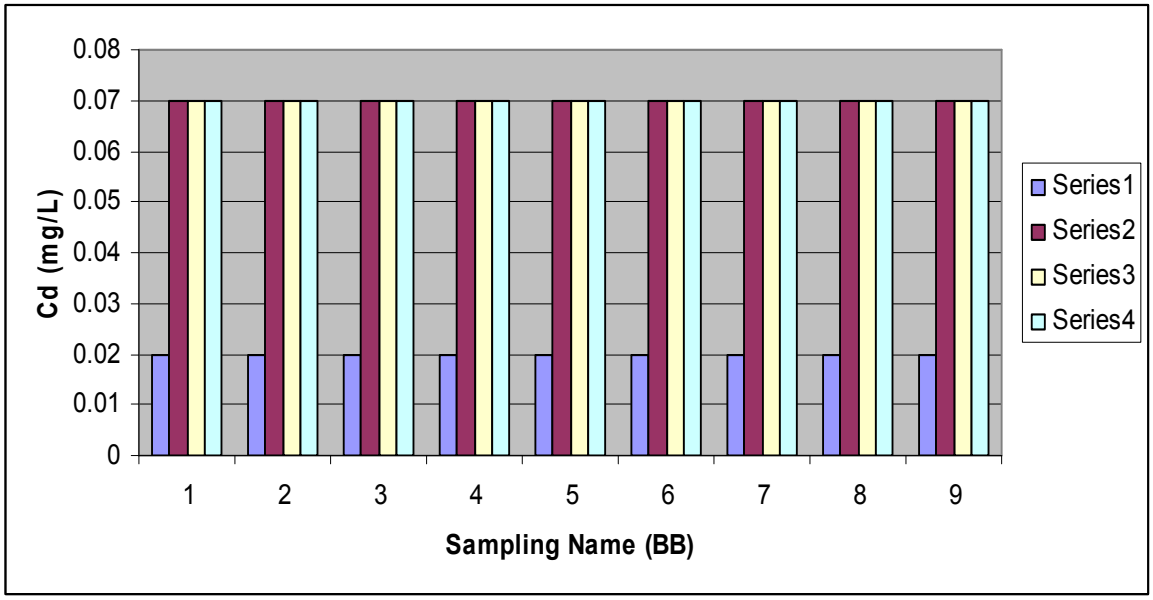

Figure 4.26: Seasonal variations of Cd per sampling points

From Figure 4.26, the levels of Cd were generally high in the months of July, November and January. From these results it could be concluded that rainfalls have no influence on Cd.

Table 4.44: The seasonal variation of Cu (mg/L) per sampling point

Cu (mg/L)				
Sampling point	Dry/winter seasons		Wet/summer seasons	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	0.01	0.04	0.04	0.04
BB2	0.01	0.04	0.04	0.04
BB3	0.01	0.04	0.04	0.04
BB4	0.01	0.04	0.04	0.04
BB5	0.01	0.04	0.04	0.04
BB6	0.01	0.04	0.04	0.04
BB7	0.01	0.04	0.04	0.04
BB8	0.01	0.04	0.04	0.04
BB9	0.01	0.04	0.04	0.04

From Table 4.44 above, the Cu levels for the winter season was constant at 0.01 mg/L in June and 0.04 mg/L in July while for the summer season the Cu levels were constant at 0.04 mg/L for both the months of November and January.

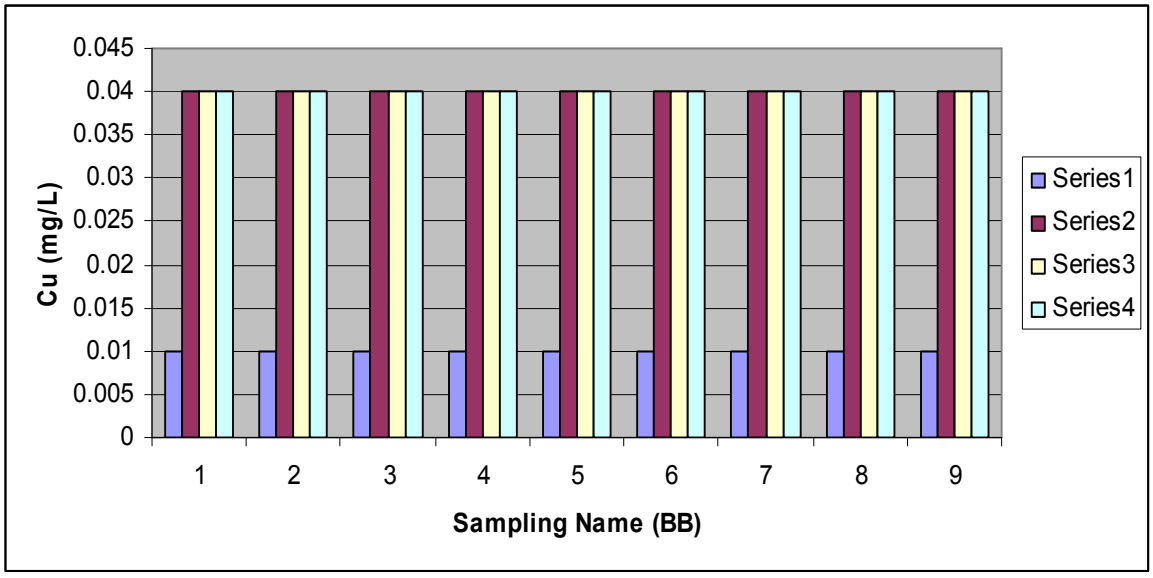

Figure 4.27: Seasonal variations of Cu per sampling points

From Figure 4.27, the levels of Cu were generally high in the months of July, November and January. From these results it could be concluded that rainfalls have no influence on Cu.

Table 4.45: The seasonal variation of Fe (mg/L) per sampling point

Fe (mg/L)				
Sampling point	Dry/winter seasons		Wet/summer seasons	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	0.19	0.08	0.13	0.09
BB2	1.37	1.47	0.41	0.41
BB3	0.03	0.11	0.1	0.16
BB4	0.03	0.05	0.05	0.05
BB5	0.01	0.05	0.06	0.12
BB6	0.06	0.05	0.31	0.09
BB7	0.07	0.09	0.39	0.18
BB8	0.1	0.1	0.91	0.31
BB9	0.11	0.13	0.49	0.26

From Table 4.45 above, the Fe levels for the winter season varied from 0.1 to 1.37 mg/L in June and from 0.05 to 1.47 mg/L in July while for the summer season the Fe levels varied from 0.05 to 0.91 mg/L in November and from 0.05 to 0.31 mg/L in January.

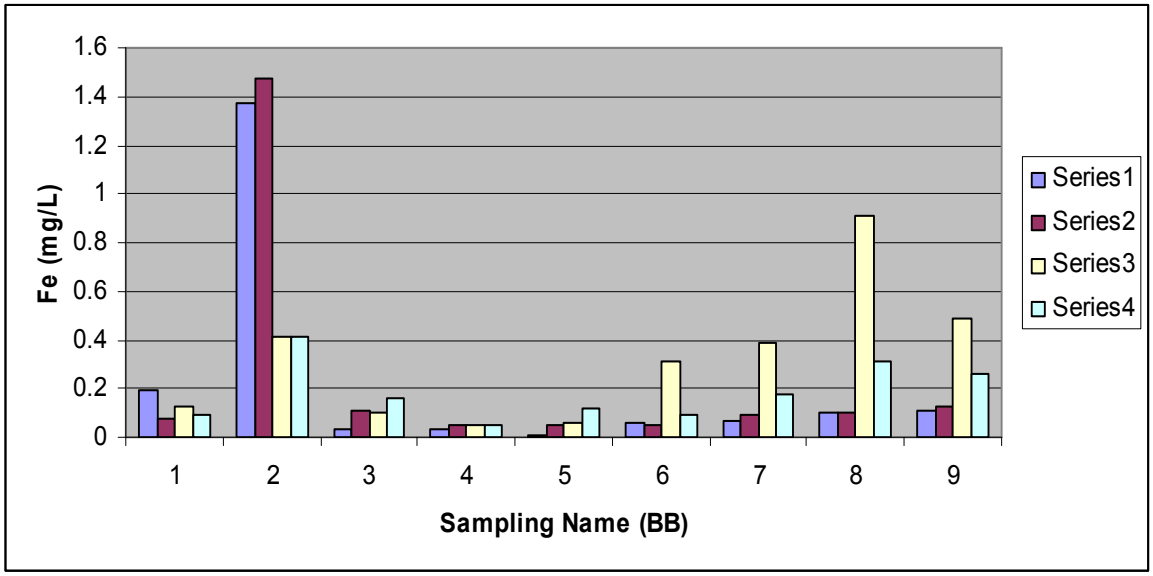

Figure 4.28: Seasonal variations of Fe per sampling points

From Figure 4.28, the levels of Fe were generally high at BB2, BB6-BB9 but showed that seasonal changes have no influence on Fe.

Table 4.46: The seasonal variation of Zn (mg/L) per sampling point

Zn (mg/L)				
Sampling point	Dry/winter seasons		Wet/summer seasons	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	0.07	0.06	0.06	0.06
BB2	0.07	0.06	0.06	0.06
BB3	0.07	0.5	0.06	0.06
BB4	0.07	0.06	0.06	0.06
BB5	0.07	0.06	0.06	0.06
BB6	0.07	0.06	0.06	0.06
BB7	0.07	0.06	0.06	0.06
BB8	0.07	0.06	0.08	0.06
BB9	0.07	0.06	0.06	0.06

From Table 4.46 above, the Zn levels for the winter season was constant at 0.07 mg/L in June and 0.06 mg/L in July while for the summer season the Cu levels were constant at 0.06 mg/L for both the months of November and January.

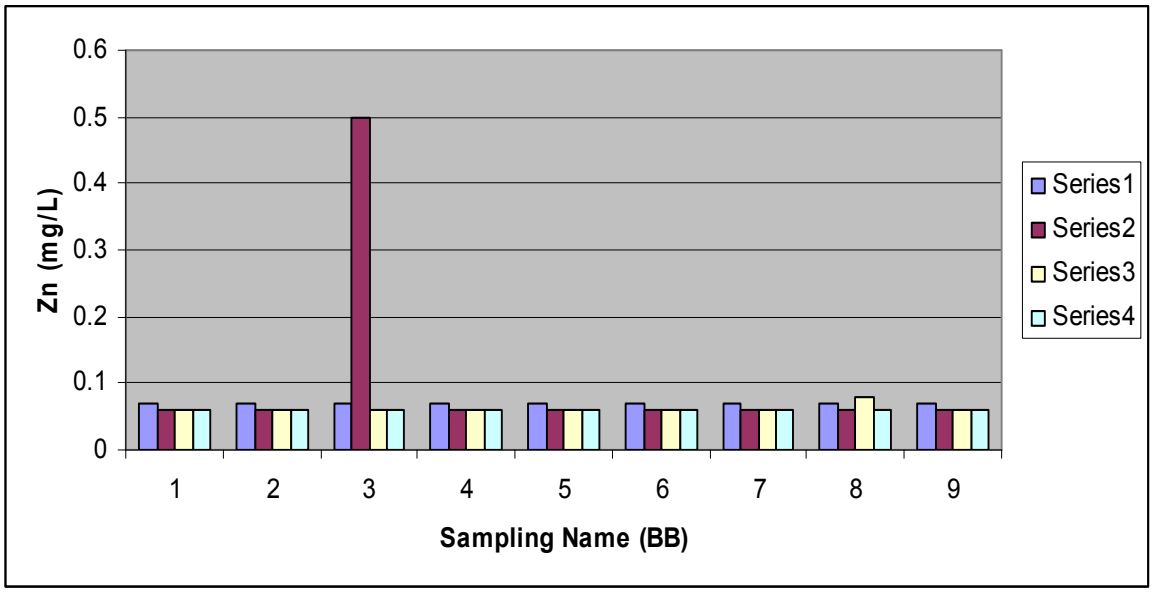

Figure 4.29: Seasonal variations of Zn per sampling points

From Figure 4.29, the levels of Zn were generally constant for all the points for the months of July, November and January with the exception of BB3 in July where the Zn levels were high. From these results it could be concluded that rainfalls have no influence on Zn.

Table 4.47: The seasonal variation of As (mg/L) per sampling point

As (mg/L)				
Sampling point	Dry/winter seasons		Wet/summer seasons	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	0.05	0.05	0.05	0.05
BB2	0.05	0.05	0.05	0.05
BB3	0.05	0.05	0.05	0.05
BB4	0.05	0.05	0.05	0.05
BB5	0.05	0.05	0.05	0.05
BB6	0.05	0.05	0.05	0.05
BB7	0.05	0.05	0.05	0.05
BB8	0.05	0.05	0.05	0.05
BB9	0.05	0.05	0.05	0.05

From Table 4.47 above, the As levels for the winter season was constant at 0.05 mg/L for both the winter and the summer seasons.

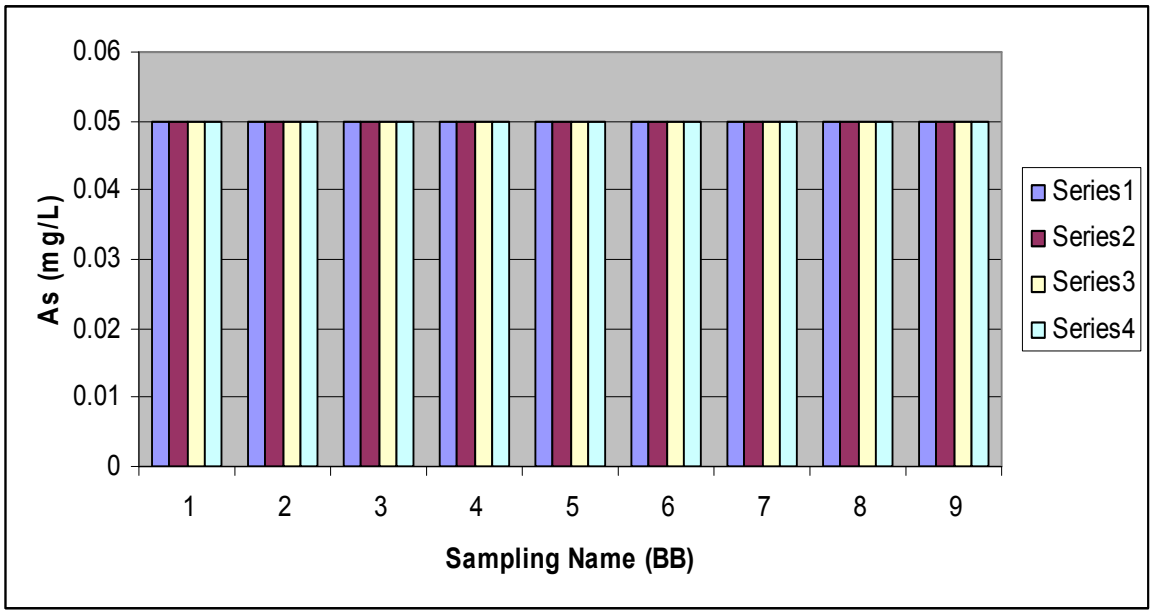

Figure 4.30: Seasonal variations of As per sampling points

From Figure 4.30, the levels of As were constant for both the winter and the summer seasons and therefore can be concluded that rainfalls have no influence on As.

Table 4.48: The seasonal variation of FC (cfu/100mL) per sampling point

FC (cfu/100mL)				
Sampling point	Dry/winter seasons		Wet/summer seasons	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	12	36	44	2
BB2	14	17	340000	50
BB3	4	0	340	5
BB4	2	1	16	3
BB5	63	31	280	19
BB6	420	10	2900	62
BB7	830	24	3600	51
BB8	320	850	9700	0
BB9	10	840	12500	240

From Table 4.48 above, the FC levels for the winter season varied from 4 to 830 cfu/100mL in June and from 0 to 850 cfu/100mL in July while for the summer season the FC levels varied from 16 to 340000 cfu/100mL in November and from 3 to 240 cfu/100mL in January.

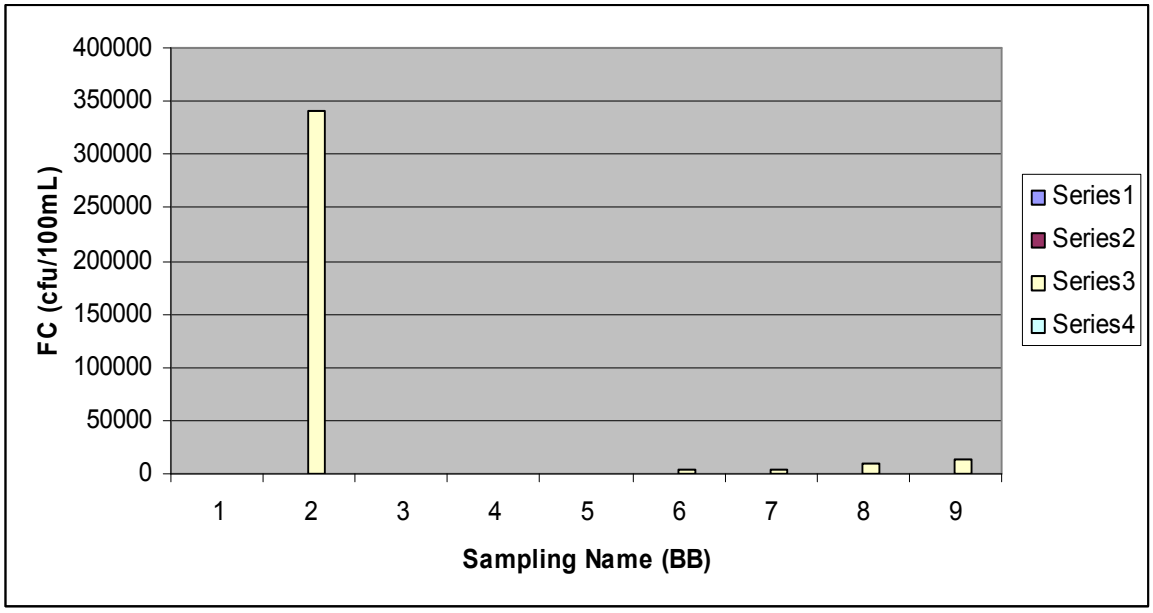

Figure 4.31: Seasonal variations of FC per sampling points

From Figure 4.31, the levels of FC were generally high in the month of November which is associated with high rainfall, this is due to the fact that more effluent is released from sewage works because of high volumes received in wet seasons, i.e. some of the inflow is from storm water channels.

Table 4.49: The seasonal variation of TC (cfu/100mL) per sampling point

TC (cfu/100mL)				
Sampling point	Dry/winter seasons		Wet/summer seasons	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	34	53	470	7
BB2	22	1700	3200000	160
BB3	11	4	5000	120
BB4	51	18	56	13
BB5	20	33	620	120
BB6	5400	180	18000	410
BB7	8600	1800	12000	350
BB8	4100	21000	66000	28
BB9	110	16000	92000	2200

From Table 4.49 above, the TC levels for the winter season varied from 11 to 8600 cfu/100mL in June and from 4 to 21000 cfu/100mL in July while for the summer season the TC levels varied from 56 to 3200000 cfu/100mL in November and from 7 to 2200 cfu/100mL in January.

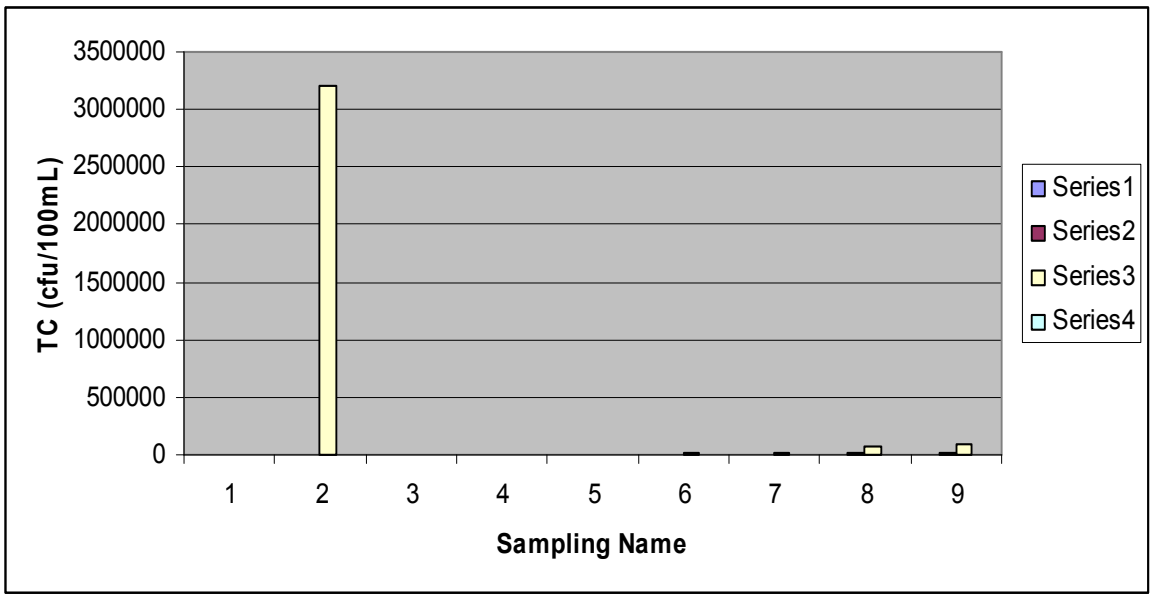

Figure 4.32: Seasonal variations of TC per sampling points

From Figure 4.32, the levels of TC followed a similar pattern as the one observed in figure 4.31, generally high in the month of November.

Table 4.50: The seasonal variation of *E. coli* (cfu/100mL) per sampling point

***E.coli* (cfu/100mL)**				
Sampling point	**Dry/winter seasons**		**Wet/summer seasons**	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	11	34	44	1
BB2	12	15	340000	50
BB3	3	0	340	5
BB4	2	1	16	3
BB5	50	31	280	17
BB6	290	10	2900	62
BB7	820	19	3600	50
BB8	280	850	9700	0
BB9	10	810	12500	210

From Table 4.50 above, the *E. coli* levels for the winter season varied from 3 to 820 cfu/100mL in June and from 0 to 850 cfu/100mL in July while for the summer season the *E. coli* levels varied from 16 to 340000 cfu/100mL in November and from 1 to 210 cfu/100mL in January.

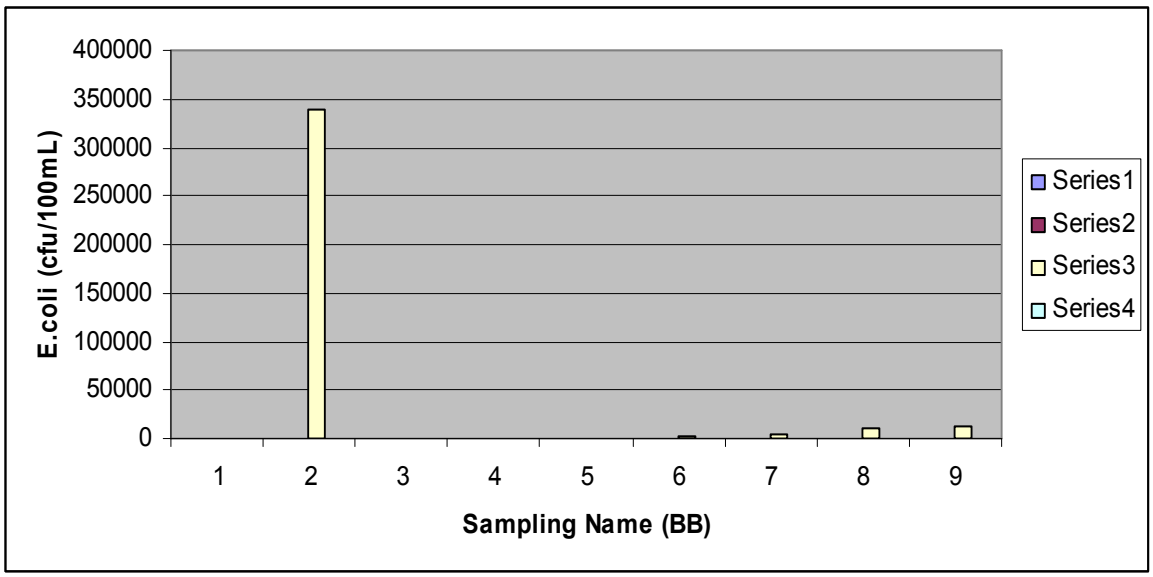

Figure 4.33: Seasonal variations of *E. coli* per sampling points

From Figure 4.33, the levels of *E. coli* followed a similar pattern as the one observed in figure 4.31 and 4.32, generally high in the month of November.

Table 4.51: The seasonal variation of Heterotrophic Plate Count (cfu/1mL) per sampling point

Heterotrophic Plate Count (cfu/1ml)				
Sampling point	Dry/winter seasons		Wet/summer seasons	
	5-Jun-08	17-Jul-08	20-Nov-08	13-Jan-09
BB1	4490	4600	17100	7100
BB2	156700	670000	1430000	14300
BB3	4570	8400	25700	1600
BB4	3150	3800	14000	2460
BB5	5500	5400	41200	4700
BB6	13390	4900	83000	8700
BB7	22000	22200	68000	32000
BB8	4300	3160	89000	213800
BB9	8900	25600	105000	32800

From Table 4.51 above, the Heterotrophic plate count levels for the winter season varied from 3150 to 156700 cfu/100mL in June and from 3160 to 670000 cfu/100mL in July while for the summer season the Heterotrophic plate count levels varied from 14000 to 1430000 cfu/100mL in November and from 2460 to 213800 cfu/100mL in January.

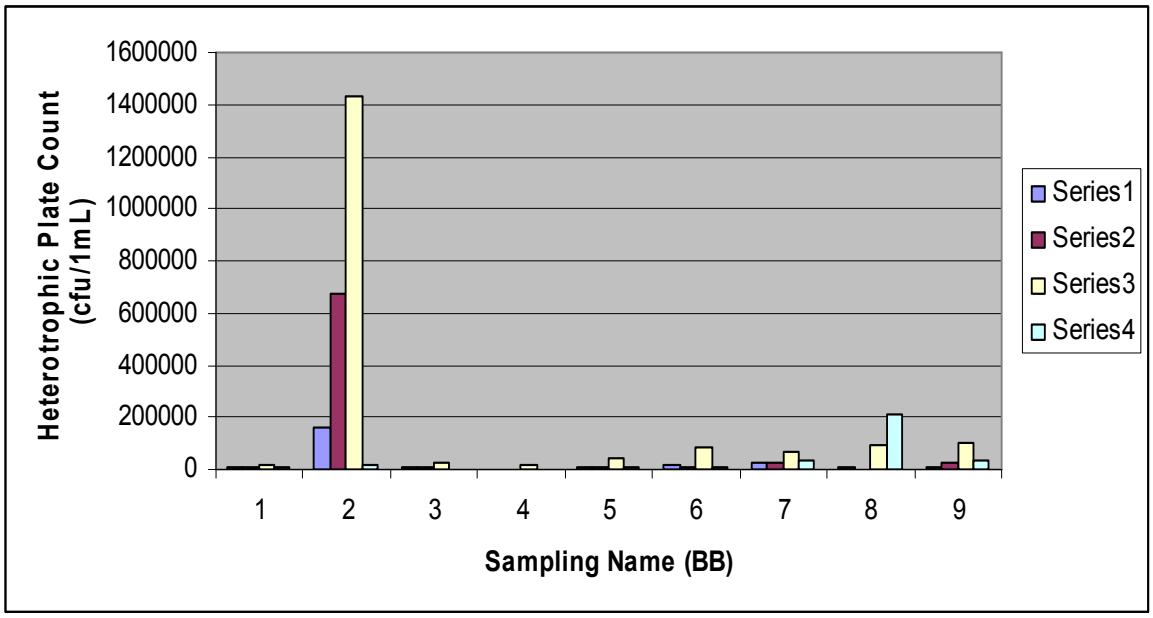

Figure 4.34: Seasonal variations of Heterotrophic Plate Count per sampling points

From Figure 4.33, the levels of Heterotrophic plate count followed a similar pattern as with all the microbial graphs, generally high in the month of November but in this graph the months of June and July were also high at BB2 and BB7&BB9.

4.5 Statistical evaluation/treatment of the results of analysed water samples

The importance of statistical handling and application of results obtained in environmental pollution and assessment studies cannot be over-emphasized. Statistical results assist in validating basic scientific concepts as well as in explaining and revealing relationships among the various parameters and variables involved in the results obtained.

Results of the statistical applications are presented in Tables 4.52- 4.54 The Tables showed selective and logical application of relevant statistical functions to results of the analyses. The sampling points and periods for statistic analyses were randomly selected to determine the analysis of variance (one-way ANOVA) which estimates variance based on a sample and provide information whether a set of data have a common mean or whether the set of data differ from the measured characteristic. F-test, a two-tailed probability test which specify whether the variances between two set or array of data are not significantly different (it provides the result of a test of the null hypothesis that these two set of data come from distributions with equal variances, and on the other hand, that the variances are not equal in the underlying distributions. A value of f close to 1 provides evidence that the underlying population variances are equal). The Pearson Moment Correlation Co-efficient (r) which indicate the extent to which two measurement variables or two set of data vary from each other. The value of any correlation coefficient must be between +1 and – 1. The results are as presented below:

Table 4.52: Statistical analysis for March/June sampling and August/January sampling using BB1

	Sampling periods			
Parameter	**13 Mar 08**	**05 Jun08**	**26Aug08**	**13Jan 09**
pH	6.8	7	7.4	6.8
EC (mS/m)	126	134	163	133
TSS (mg/L)	10	<10	80	10
DTS(mg/L)	866	746	1150	880
NO_3-NO_2-N)mg/l N	0.2	0.5	0.2	0.1
PO_4(mg/L)	0.6	0.1	0.8	0.1
SO_4 (mg/L)	344	309	410	330
COD (mg/L)	10	<10	<10	29
Variance	**83556.11**		**124483.097**	
F-Test	**0.966804**		**0.43465604**	
r (correlation coeff.)	**0.99936**		**0.99783725**	
Cd (mg/L)	0.07	0.02	0.02	0.07
Cu (mg/L)	0.04	0.02	0.01	0.04
Fe (mg/L)	0.05	0.19	0.1	0.26
Zn (mg/L)	0.06	0.07	0.07	0.06
As (mg/L)	0.05	0.05	0.05	0.05
Variance	**0.0023289**		**0.0049789**	
F-Test	**0.0038622**		**0.1018179**	
r (correlation coeff.)	**-0.155824**		**0.7735491**	
FC (cfu/100ml)	14	12	840	240
TC (cfu/100ml)	70	34	7100	2200
E.coli (cfu/100ml)	11	11	840	210
Heterotrophic Plate Count (cfu/1ml)	7000	4490	6000	32800
variance	**7460673.9**		**121888755**	
F-Test	**0.4851353**		**0.0283658**	
r (correlation coeff.)	**0.9999954**		**0.5127351**	

Table 4.52 above contained the analysis of variance, F-test and the Pearson moment correlation coefficient (r) for results of water analyses from sampling points BB1 at different periods of sampling as indicated in the Table. The variance, F-Test and correlation coefficient for the two set of physico-chemical parameters for March and June

2008 were 83556.11, 0.4851353 and 0.9999954 respectively. The values for November 2008 and January 2009 were 124483.097, 0.0283658, and 0.5127351 respectively.

For metals, the analysis of variance, F-Test and the correlation coefficient (r) for March and June was 0.0023289, 0.0038622 and -0.155824 respectively while that for November 2008 and January 2009 was 0.0049789, 0.1018179, and 0.7735491 respectively. With respect to microbial values, the analysis of variance, F-test and the correlation coefficient (r) for March/June were 7460673.9, 0.4851353 and 0.9999954 respectively, while those for November 2008/January 2009 were 121888755, 0.0283658, and 0.5127351 respectively.

Table 4.53 below shows the analysis of variance, F-test and the correlation coefficient (r) for the sampling point BB7. Statistical evaluation of the physico-chemical analysis for March and June indicated that the analysis of variance, F-test and the correlation coefficient (r) were 80669.94, 0.509373 and 0.999663 respectively, and for November and January; the values were 79095.623, 0.6983556, and 0.9923864 respectively.

For metals, the analysis of variance, F-test and the correlation coefficient (r) for March and June were 0.005321, 0.033618 and 0.540052 respectively while for November and January; the values were 0.0040722, 0.5830904, and 0.9460288 respectively. With respect to microbial analyses, the variance, F-test and correlation coefficient (r) values for March and June were 55541798, 0.250548 and 0.965515 respectively while for November 2008 and January 2009 was 124361091, 0.0054833, and 0.9994158 respectively.

Table 5.53: Statistical analysis for March/June sampling and August/January sampling using BB7

Parameter	**13 Mar 08**	**05 Jun 08**	**26 Aug 08**	**13 Jan 09**
pH	8	8.3	8.2	7.9
EC (mS/m)	112	131	132	116
TSS (mg/L)	10	10	49	32
DTS(mg/L)	730	942	892	784
NO_3-NO_2-N)mg/l N	1.1	0.7	1.2	0.3
PO_4(mg/L)	0.6	0.3	0.5	0.1
SO_4 (mg/L)	262	326	357	222
COD (mg/L)	27	11	10	29
Var		**80669.94**		**79095.623**
F-Test		**0.509373**		**0.6983556**
r (correlation coeff.)		**0.999663**		**0.9923864**
Cd (mg/L)	0.07	0.02	0.02	0.07
Cu (mg/L)	0.04	0.01	0.01	0.04
Fe (mg/L)	0.27	0.07	0.2	0.18
Zn (mg/L)	0.06	0.07	0.07	0.06
As (mg/L)	0.05	0.05	0.05	0.05
Var		**0.005321**		**0.0040722**
Ftest		**0.033618**		**0.5830904**
R (P m corr)		**0.540052**		**0.9460288**
FC (cfu/100ml)	820	830	78	51
TC (cfu/100ml)	6800	8600	250	350
E.coli (cfu/100ml)	820	820	78	50
Heterotrophic Plate Count (cfu/1ml)	10400	22000	3900	32000
Variance		**55541798**		**124361091**
F-Test		**0.250548**		**0.0054833**
r (correlation coeff.)		**0.965515**		**0.9994158**

Table 5.54: Statistical analysis for March/June sampling and August/January sampling using BB9

Parameter	13 Mar 08	05 Jun 08	26 Aug 08	13 Jan 09
pH	8	8.7	8.2	7.6
EC (mS/m)	108	127	134	106
TSS (mg/L)	27	<10	42	48
DTS(mg/L)	720	888	874	744
NO_3-NO_2-N)mg/l N	1.8	1	1.4	1.8
PO_4(mg/L)	0.5	0.3	0.5	0.8
SO_4 (mg/L)	240	322	333	31
COD (mg/L)	10	10	10	35
Variance	**77829.27**		**74050.392**	
F-Test	**0.485321**		**0.6430447**	
r (correlation coeff.)	**0.999483**		**0.9365593**	
Cd (mg/L)	0.07	0.02	0.02	0.07
Cu (mg/L)	0.04	0.01	0.01	0.04
Fe (mg/L)	0.31	0.11	0.1	0.26
Zn (mg/L)	0.06	0.07	0.07	0.06
As (mg/L)	0.05	0.05	0.05	0.05
Variance	**0.007366**		**0.0049789**	
F-Test	**0.067077**		**0.1018179**	
r (correlation coeff.)	**0.815263**		**0.7735491**	
FC (cfu/100ml)	200	10	840	240
TC (cfu/100ml)	420	110	7100	2200
E.coli (cfu/100ml)	80	10	840	210
Heterotrophic Plate Count (cfu/1ml)	13800	8900	6000	32800
Variance	**28668213**		**121888755**	
F-Test	**0.50174**		**0.0283658**	
r (correlation coeff.)	**0.999935**		**0.5127351**	

Table 4.54 above showed the analysis of variance, F-test and the correlation coefficient (r) for BB9. Statistical treatment of the result of the physico-chemical analysis for March and June 2008 indicated that the analysis of variance, F-test and the correlation coefficient (r) values were 77829.27, 0.485321 and 0.999483 respectively, and for November 2008 and January 2009; the values were 74050.329, 0.6430447, and 0.9365593 respectively. For metals, the analysis of variance, F-test and the correlation coefficient (r) for March and June 2008 were 0.007366, 0.067077 and 0.815263 respectively, and for November 2008 and January 2009, the values were 0.0049789, 0.1018179, and 0.7735491 respectively. With respect to microbial parameters, the

analysis of variance, F-test and the correlation coefficient (r) values for March and June 2008 were was 28668213, 0.50174 and 0.999935 respectively and those for November 2008 and January 2009 were 121888755, 0.0283658, and 0.5127351 respectively.

CHAPTER 5

DISCUSSION OF RESULTS

Various land uses, notably mining and agriculture contributed to the degradation of land, hence modifying water quality in many parts of the country (Hohls *et al*., 2002). On a national scale, however, land cover and the geology influence water quality predominantly. Discharges from sewage works, dewatering of the Grootvlei mine, presence of mine dumps and previous mining activities have shown to cause considerable pollution in the catchment. Combined with social developments, urban runoffs, dense settlements, these activities also have negative impact on the Blesbokspruit. Pollution emanating from abandoned mines and slimes dams is of long term in nature because pollution is mainly via seepages. Even though there are no hazardous waste disposal sites in the lower part of the Blesbokspruit, risk of contamination of the surface water still exist because the major hazardous waste disposal site (Holfontein) is located in the upper part of the Blesbokspruit. A downward massive runoff might lead to this scenario. Threat of underground water pollution and seepages to the Blesbokspruit exists if this site is not properly managed. Another risk of pollution arises as a result of transportation of hazardous waste from industries and mines along the N12, N3, N17 and regional roads where spillages can occur.

5.1.1 pH

There was a linear increase in pH values which ranged from 7.4-8.2 but these values were within the South African Water Quality guideline specifications. The pH values of monitored waters in Pearl River Estuary also varied slightly from 6.2 to 8.1 (Cheung et al., 2003), this results are similar to the ones obtained in this study meaning that the pH of the Blesbokspruit is acceptable. I was also recorded in the rivers located in Hong Kong that the pH values were similar to those reported by EPD, varying from 6.1-8.4. The situation was different from the results recorded by Johansson *et al*, (1995), where the pH values in the rivers of Southern Sweden vary mostly in the range 4 to 5, due to the median values for Zn and Cd in the rivers being 4 and 0.009mg/L respectively. In their observations, Johansson *et al*, (1995) reported that high concentrations of Zn and Cd in the rivers coincide with high water flow, thus transporting these metals in southern Sweden. These metals are suspected to originate from the industrial areas in the Southern cities of Sweden and have increased substantially due to the acidification. Low pH values of 5 were

also reported in the Perhonjoki River in Western Finland and has affected the hatchability of lamprey roe, this may be due to the fact that many of the rivers in western Finland flow through sulfide rich soils consequently, acid water with high aluminum and iron concentrations drains into the rivers during the snow-melt period and heavy rains (Myllynen *et. al.,* 1996)

5.1.2 Electrical Conductivity (EC)

The EC levels of the study area varied from 93-146.63 mS/m. Most of the EC levels were above 120 mS/m. A similar situation was observed in the Pearl River Estuary, South of China where most samples along the Estuary had EC at the range of 100-500 mS/m, with the exception of two sampling sites, which exceeded 2500 mS/m because of tidal currents entering into Deep Bay from the outer seawater. Very low EC was also found in waters at sampling points along Yuen Long Creek and Tin Shui Wai Nullah (Cheung *et al*., 2003). High levels of EC were recorded at BB1, BB3, BB4 and BB5 sampling sites. This could be as a result of the mining activities around these sampling points, especially BB1 where Grootvlei mine discharge waste effluent. From BB4 and BB5 sampling points, the impacts could be from Daggafontein mine, old mine dumps, tailing dams, Sub Nigel mine and other closed/abundant mining activities. Pekka *et al*. (2008) also recorded a mean EC of 75mS/m downstream of the mining activity which is nearly twice that in the middle of the Kola River. An increased value of EC at BB3 could be as a result of maize agriculture where runoffs transport fertilizers into the water resources. The high levels of EC recorded at these sampling points revealed the anthropogenic impact of various establishments which has direct impact/effect on the integrity of the Spruit.

In the River Yamuna in Delhi, India; EC was found to be 180.1mS/m at Kalindi Kunj downstream of Okhla landfill site and at 210.7mS/m at Nizamuddin Bridge downstream of Gazipur landfill site and may be taken as a water quality parameter of landfill leachate and landfill runoff in the river (Zafar and Alappat, 2004). Zafar and Alappat, (2004) also indicated that it was clear that closed landfills locations near river banks are also influencing the river water quality in a significant way, like operating landfills. The river water quality is influenced by the presence of landfill and it indicates that the leachate and runoff from Okhla landfill site and Gazipur landfill site find their way to river Yamuna.

5.1.3 Total suspended solids (TSS)

In Figure 4.2, it could be seen that there was a sharp increase in TSS generally from BB4. This has serious impact in that it decreases water clarity and light transmission which can interfere with fish populations. Agricultural activities at BB3 and BB6 would be impacted where runoffs transport sediments to the water resource. The sharp increase in TSS from site BB4 could be as a result of soil erosion and urban runoffs especially at Heidelberg (BB7). Possible release of effluent from improper treatment of sewage can also have an impact especially at site BB6, since this point is downstream of Herbert Bickley Sewage Works. Sullivan (1997) indicated that high TSS loads in India were also greatest from agricultural areas, in particular the Iroquois River Basin and tributaries to the lower Fox River. These are areas of intensive row-crop agriculture and fine, easily erodable soils.

Similar to the results obtained in this study, high levels of TSS obtained during the month of November 2008 which is associated with high rainfalls, showing the impact of soil erosion in the Blesbokspruit

5.1.4 Total Dissolved solids (TDS)

There was an indication that most of the solids in the Blesbokspruit are rather suspended that dissolved. This was highly observed at BB5 where there was a decrease in TDS as opposed to an increase in TSS. In general, values of TDS obtained in this study seemed not to be a problem in the Blesbokspruit; however in Texas the opposite was the case. Studies done by the Texas Commission on Environmental Quality (TCEQ, 2009) revealed that several streams in Texas, indicated elevated levels of total dissolved solids (TDS) far higher that the state of Texas' requirement that most streams, lakes, and bays be suitable for swimming, wading, fishing, a healthy aquatic environment, and use as a source of drinking water. The study suspected that high levels of TDS in the Petronila Creek watershed could be due to runoffs from contaminated soils and plugging abandoned oil wells.

Also of concern in the Cherry Creek, South Dakota, USA, the dissolved solids concentrations in the Cheyenne River ranged from 310 mg/L to 3182 mg/L with a mean value of 1686 mg/L and a standard deviation of 542 (Heakin, 1998). This mean value exceeds the stated Secondary Maximum Contaminant Level (SMCL) and the maximum value exceeds the standards for wildlife propagation and livestock watering. Berdanier and Ziadat, 2006, indicated that the flow and TDS concentrations in the Cheyenne River at

Cherry Creek were greater than those observed downstream of the Belle Fourche River indicating irrigation return flows to the Cheyenne River. In 2004 the TDS loading from the Belle Fourche was consistent with its loading from 2002 and its contribution served to dilute the higher TDS concentration in the Cheyenne caused by Rapid Creek's contribution (Berdanier and Ziadat, 2006). They further indicated that there appears to be a TDS load contribution between the Belle Fourche and Cherry Creek even at low flow conditions, which may be due to irrigation return flows however during the summer months, the TDS concentration in the Cheyenne River between Buffalo Gap and Wasta is controlled by loadings from Rapid Creek, operations of Pactola Dam, and concurrent choices of the Rapid City Wastewater Treatment Plant and irrigators downstream of Rapid City.

Studies done by Al-Jabbari *et al*. (1983) revealed that in the Almond River, Scotland, the dissolved solids level during low flows was about double of that during floods, these values were similar to the concentrations reported from rivers in humid temperate regions of North America. During high discharge, the salts have no time to become concentrated, so that the solute strength is less than that during low discharge due to dilution. This relationship has been recognized in this study where for all the sampling points, there was a decrease in the TDS values in the months of March and April 2008 followed by a continuous increase in the values for the months of June, July, August and October and another decrease from November and January 2009. This agrees with Al-Jabbari *et al*. (1983) and Berdanier and Ziadat, (2006) where during rainy seasons the TDS values goes down because of the dilution and increases as dilution decreases.

5.15 Nitrates

Nitrates is one major indicator of sewage contamination and results of analysed water samples along the Blesbokspruit varied from 0.8-2.10mg/L. Possible sewage contamination was also evident in the results where BB2, BB6, BB8 and BB9 showed elevated values of nitrates and all this points are downstream of sewage works. BB2 is a downstream point of Ancor sewage works situated in springs, BB6 is a downstream point of Herbert Bickley situated in Nigel, BB8 is a downstream point of Heidelberg sewage works situated in Heidelberg and Ratanda, a downstream point of Ratanda sewage works in Ratanda. A similar situation of high levels of Nitrates and phosphates exist in Yuen Long area Due to the growth of population, the quantity of sewage being treated at the Yuen Long sewage treatment Works and correspondingly the amount of effluent discharged into Yuen Long Creek has increased and the effluent quality has also

deteriorated the water resource (Cheung *et al*., 2003). However BB6 raises more concerns because it is the highly polluted point. This could be as a result of the combined impact from sewage works and nearby lawn farm runoffs to the Blesbokspruit. High levels of nitrates pose a risk of algal bloom in the water resource. Excessive levels of nitrate are also directly harmful to aquatic animals. Aquatic invertebrates and fish exposed to nitrate may be smaller, slower to mature, or have lower reproductive success. Under extremely high exposure levels, aquatic invertebrates and fish may die.

Higher nutrient concentrations were generally detected in the river courses that flow into Deep Bay, with maximum values of 2.2 and 2.9 mg/L for NO_3 +NO_2 -N and PO_4 respectively (Cheung *et al*., 2003). Abaychi *et al*. (1988) have found that the range of NO_3 +NO_2 -N and PO_4 in the Shatt al-Arab River of Iraq were 0.18-0.17 and 26.0-52.4 mg/L, respectively which is articulated from cultivated areas where the applied fertilizers leached into the groundwater through N mobilization and transformation after rainfall.

5.1.6 Phosphates

A decline in Orthophosphate values was visible at BB3 to BB6 with a steady increase afterwards. There was also high value of phosphate at BB2. All the points with increased Orthophosphate are downstream points of sewage works and this increase can therefore be associated with sewage effluent, posing eutrophication threat. Similar to the study done by Kinniburgh *et al*. (1997), where the phosphorus load to the River Thames peaked downstream of Swindon sewage works with the average load of 196mg/L. Colin, *et al*, (2006) reported PO_4 concentration range of 0.006 mg/L on the North Branch Potomac River and 38 to 0.087 mg/L on Antietam Creek at Poffenberger Road. Of all the monitoring points, Orthophosphate median concentration was highest at 31% of all stations that are located in the agricultural regions of Maryland, 26% of all stations and occurred in the North Branch Potomac and tributaries showed the lowest PO_4 median concentration (Colin *et al*., 2006).

5.1.7 Sulphates

High sulphate values obtained in areas within mining activities such as at the BB1 sampling point can be associated with the Grootvlei mine. Others are BB4 and BB5 where there were mine dumps, Daggafontein mine as well as the sub-Nigel mine. It is not surprising to see increased value of sulphate at these points since they are mining area

with old dumps around the water resources. Also visible at BB4 was the old river diversion that was done to accommodate the mining activity. Anderson, (2000) reported the high level of sulphate in the 11 sampling points along the Stonycreek River, and is considered to be highly degraded by AMD, primarily from abandoned mines. Currently, efforts are being made to restore the water quality in this river, mainly through the construction of passive treatment systems to treat abandoned-mine discharges.

Colin *et al*. (2006) conducted several researches to evaluate the water quality in the River of Thames and its tributaries. They found out that of the seventeen sampled stations located in the North Branch Potomac (NBP), tributaries to the NBP and MD stations contributing to the Ohio River drainage, the median sulphate concentration ranged for 30mg/L at the Youghiogheny River station below the confluence with the Little Youghiogheny to 412mg/L at Georges Creek. Stations with the highest median concentration (SO_4 >128.9 mg/L) comprise 25 % of all stations and were located in the uppermost NBP stations, Georges Creek and Braddock Run. Stations with lowest median concentration (SO_4 ≤56.5 mg/L) comprise 47 % of all stations sampled for SO_4 and were located in the Ohio River drainage, Savage River and Town Creek. Sulphate concentrations decreased at Cherry Creek, on the North Branch Potomac River at Bloomington, at US 220, at Pinto and at Moores Hollow Road. Concentration increased on the North Branch Potomac River at Old town Rd. and on the Little Youghiogheny River. Dissolved sulphate in surface water may also be derived from the dissolution of gypsum or the oxidation of sulphide minerals such as pyrite in association with mining of coal (Colin *et al*., 2006).

5.18 Chemical Oxygen Demand (COD)

There was no definitive pattern or trend with respect to the values of COD obtained in this study. But looking at the graphs, high values are visible at points downstream of the sewage works and this increase can therefore be associated with sewage effluent. Apsite and Klavins, (1997) reported the lowest mean values of water color and COD in the Tuliya River, Tebra River, and Venta River and the highest mean values were found in the Lielupe River Basin which was the most intensively farmed area in Latvia and can be assumed that it is caused by intensive use of fertilizers

5.1.9 Trace metals

Matsumoto *et al*., (2008) reported that heavy metals like Cd, As, Fe, Zn and Cu in the river stream which are the Trinity, the Colorado and the San Antonio rivers, Texas USA and several rivers of Japan which are the Tama, the Edo, the Tsurumi, the Ara, the Yamato, the Yodo, the Shonai, the Hiis are all below the recommended limits of Japan. However, the experimental results showed clear impact of human population in some bigger cities on heavy metal concentrations in the river sediments as compared to smaller cities with low human population. The rapid industrial development in Malaysia have seen incidences of toxic pollution from industry, with the maximum values of As concentrations from Juru River and Jejawi River being 5.98 mg/L and 3.84 mg/L respectively whilst the minimum values of Cu are 0.0 mg/L in both rivers (Abbas *et al*., 2008).

Pekka *et al*. (2008) recorded a two to five time higher levels of metals, i.e. As and Cu at points downstream of the mining activity as opposed to points in the middle of Kolo River. The values of Cadmium and Arsenic obtained in this study are relatively constant, hence, this may suggest that there may be no external factor that contribute to the cadmium and arsenic pollution in the catchment and can therefore be concluded to be the baseline of the catchment or perhaps impacts that happened long time ago altering the baseline. Copper level was also steady at BB2 to BB6 and high levels are visible at BB1 and BB7and BB8. At BB1, this can be associated with mining activity and surprisingly high levels at BB7 could be as a result of industrial effluent discharged into the sewage works.

Low values of iron obtained at BB1 was quite surprising however, this might indicate that the acid mine drainage at this point has been well managed. High value was evident at BB2 and a linear increase observed from BB6 to BB7 and decline to BB8. Since these points are downstream of sewage works, the impact can be associated with sewage effluent. Myllynen, *et al*. (1996) reported high levels of Iron in the range of 1.5-2.2 mg/L in the Perhonjoki River in Western Finland which affected the hatchability of lamprey roe. In the Lestijoki River, pH values as low as 4.9, iron concentrations as high as 7.3 mg/L have been reported (Jokela and Saastamoinen, 1988).

Dima *et al*., 2005 reported an increase in iron concentrations in the Ialomiþa River after it passes the Târgovitse city, an important industrial center of Romania. Vuori, 1995 also reported high levels of iron along the Zambezi River, Afon Coch, River Vidaa, Peat Mine

Ditch River and indicated that these levels are a contribution of mining activities and farming in these river basins.

The level of Zinc was generally steady but a sharply high value was recorded at BB3. The cause of this was unclear. Cheung *et al.*, (2003) indicated that Concentration of dissolved Cd and Cr along the Pearl River Estuary were low at most sites, similar to the low values found in this study, however the concentrations of Cu (0.068 mg/L) and Zn (10.7 mg/L) in some locations exceed the water quality criteria for protection of aquatic life, high levels of zinc was due to the fact that Zinc is primarily used for galvanizing iron and steel products, and 705 and 10 304 tons of Cu and Zn respectively were discharged into Pearl River from Guangzhou on an annual basis (Cheung, *et al*., 2003).

5.1.10 Microbial parameters

Microbial contamination is a problem world wide, more evident as the results shown alarming values of microbial contamination. Due to population growth, Yuen Long Creek suffered from sewage pollution as shown by high *E. coli* concentration exceeding 6·106 cfu/100 mL (EPD, 1997). From this study, disturbing results were observed at BB2, BB7 and BB8, indicating that the sewage works at these point really impact negatively on the water source. At site BB2, it can be inferred that chlorination process at the sewage work was either insufficient or totally absent due to the high values of microbial entities obtained. There is a direct correlation with regard to faecal coliforms and *E. coli* indicating human excreta finding their way into the water resource. Franklin *et al.*, (2004) also did a study on the San Juan River and found out that there appear to be a noticeable effect on *E. coli* levels in the San Juan River on the dates sampled. Of all inflows to the San Juan River sampled, few Largo Canyon appears to have had a noticeable effect and two clear inflows from off-channel wetlands near the community of Blanco also have had an effect. Higher *E. coli* results were observed at Largo Canyon due to additional mixing of suspended solids from with San Juan River water. Kutz Canyon also had an effect, as the *E. coli* results downstream of Kutz Canyon were generally greater than the results upstream (Franklin *et al*., 2004). These effects are suspected to be inflows from wetlands, irrigation return flow, urban runoff, seepage from leach fields, or some combination of these (Franklin *et al*., 2004).

The results from the studies done by the Northeastern University in 2008 indicated an increased concentration of *E. coli* in the Charles River after a period of little rainfall (NU,

2008). These finding do not agree with the results obtained in this study since for all the monitoring points, *E.coli* levels are up in the months of April and November and these months are associated with rainy seasons. In this case it can be concluded that *E. coli* contributions into the Blesbokspruit are as a result of effluent discharge from the sewage works which receives high loads during rainy seasons and as a result the discharged effluent is high. Similar results where observed by Schilling, *et al*, 2009, in the Raccoon River where an overall *E. coli* concentration were highest in the May to July, period that corresponds with periods of greater rainfall intensity and river discharge. The Raccoon River is used by the Des Moines Water Works to serve more than 400,000 people in central Lowa.

Generally, it could be concluded that the Blesbokspruit is not suitable for drinking, irrigational, recreational and even livestock drinking due to the level of E coli obtained in this study.

5.1.11 Statistical Analyses

From the outcome of the statistical treatment of results from Table 4.52, the variance values of 83556.11 and 12, 4483.097 for the periods of Mar/Jun 2008 and Nov 2008/Jan 2009 for physico-chemical parameters showed that the set of results do not have common mean; the F-Test revealed that the variances between the two arrays of data are significantly different. However, the closeness of r value to 1 for the Mar/Jun 2008 data showed that there was strong correlation between the set of data while that for Nov/Jan 2009 was averagely correlated. The results obtained for metals also revealed an uncommon mean of their values for the variance test; the F-test showed that the variances of the set of data are significantly different, while (r) for the Nov/Jan value showed average correlation with each other.

With regard to microbial values, the analysis of variance, F-test and the correlation coefficient (r) for March/June were 7460673.9, 0.4851353 and 0.9999954 respectively, while those for November 2008/January 2009 were 121888755, 0.0283658, and 0.5127351 respectively. These values also showed that the mean values are different, the variances of the set of data are significantly different as shown by the F-test while there was strong correlation between the Mar/Jun 2008 data as revealed by (r= 0.9999954).

The statistical evaluation of the parameters presented in Table 4.53 showed that the physico-chemical analysis for March and June of the year 2008 revealed similar pattern with respect to the statistical outcome obtained in Table 4.52 with the exception of (r). In this case, the mean differ, variances were significantly different but the (r) showed very strong correlation between the values. For metals under this category, the null hypotheses for the set of data for metals were all negative except for (r) that showed correlation (r = 0.946) between the Nov 2008 and Jan 2009 values. With respect to microbial analyses, only the correlation values (r) for both sets of data (Mar/Jun) as well as (Nov2008/Jan 2009) showed alignment with the hypothesis that the microbial values obtained are most likely from the same source.

From Table 4.54, the analysis of variance, F-test and the correlation coefficient (r) for the physico-chemical parameters at BB9 for March and June 2008 and for November 2008 and January 2009; again indicated that the mean of the values were not common; the variances were significantly different but very strong correlation existed between the values meaning that they are most likely from the same source. For metals, the negative hypothesis prevailed for all the statistical parameters. With respect to microbial parameters, there was very strong correlation in the set of data for microbial analysis during March and June 3008 as revealed by the (r) value. The values for the variance as well as the F-test showed negative null results.

Since the Department of Water Affairs and Forestry does not encourage drinking water directly from the water resource, the results will be assessed further against the In stream Water Quality Objectives for the Blesbokspruit, South African Water Quality Guideline: Aquatic Ecosystem, South African Water Quality Guideline: Agricultural use: Irrigation, and South African Water Quality Guideline: Agricultural use: Livestock watering.

5.2 Assessment of Results against the In-stream Water Quality Objectives for the Blesbokspruit

Table: 5.1: The results below are assessed against the In-stream Water Quality Objectives (IWQO) for the Blesbokspruit Catchment (www.reservior.co.za), (Only variables with objectives from the IWQO have been selected)

Sample Name	EC(ms/m)	TSS (mg/L)	NO_3-NO_2-N (mg/L)	PO4 (mg/L)	pH	SO4 (mg/L)	COD	Fe (mg/L)	FC (cfu/100mL)	*E. coli* (cfu/100mL)
BB1	146.63	19.25	0.26	0.79	7.4	379.5	15.75	0.078	20.63	19.13
BB2	93	12.88	1	0.96	7.4	118.63	34.75	0.73	44021	43999.13
BB3	137.34	28.75	0.26	0.59	7.7	337.75	21.88	0.12	52.88	52.5
BB4	142.63	11.25	0.16	0.61	8	353.13	15.63	0.04	15.88	14.13
BB5	141.75	18.13	0.16	0.58	8	369	18.63	0.13	61.75	59.13
BB6	134.36	22.5	2.01	0.5	8.1	326.25	16.13	0.1	772	719.63
BB7	122.5	31	1	0.55	8.1	297.75	20.13	0.18	1035.36	970.86
BB8	120	30.5	0.8	0.56	8.2	284.75	24.25	0.25	16878,5	1616.86
BB9	120	39	1.4	0.63	8.2	261.13	21.13	0.19	1936.25	1891.25

Colour coding

Ideal
Acceptable
Tolerable
Unacceptable

E.coli is not included in the IWQO; assumption is anything above 126 is unacceptable

From the IWQO, it is evident that Electrical Conductivity, PO_4^{3-}, FC and *E. coli* are the variables of concern. When looking at EC, it is evident hat mining activity in the catchment is having a major impact on the water resource as these points are mainly downstream of the mining activity or tailing dams. The other visible impact is due to sewage effluent, the unacceptable values of PO_4, FC and *E. coli* indicate poor sewage reticulation system within the catchment. Much effort in the catchment has been made to improve the water quality through the Blesbokspruit forum that is held quarterly and is evident as a reasonable amount of variables are within the acceptable and ideal objectives. Suspended solid in the Blesbokspruit is not a concern as with the Inner Deep Bay, South of China, high suspended solids in this region enhance pollutant dispersion

and they also exceeded the maximum values of water quality in relation to recreation (100mg/L) and wildlife propagation (20mg/L) (Frits, 1990).

5.3 Assessments of results against the South African Water Quality Guidelines

a) Aquatic ecosystem

The result below as presented in Table 5.2 revealed a comparative analyses of the mean levels of parameters from sampling point BB1 as compared to the recommended South African Water Quality Guideline for the Aquatic Ecosystem (DWAF, 1996d), (Only variables that are discussed in the above guidelines have been selected)

Table 5.2: Results of the comparison of the levels of parameters from BB1 and the South African Water Quality Guideline: Aquatic Ecosystem (DWAF, 1996d)

Variables	Mean Results BB1	Mean Results BB2	Mean Results BB3	Mean Results BB4	Mean Results BB5	Mean Results BB6	Mean Results BB7	Mean Results BB8	Mean Results BB9	Aquatic Ecosystem limits
Arsenic	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.02
Cadmium	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.0015
Copper	0.039	0.025	0.025	0.025	0.025	0.025	0.025	0.039	0.039	0.0015
Iron	0.078	0.73	0.12	0.04	0.13	0.1	0.18	0.25	0.19	0.0075
Zinc	0.065	0.065	0.12	0.065	0.065	0.065	0.065	0.068	0.065	3.6
Nitrates	0.26	1	0.26	0.16	0.16	2.01	1	0.8	1.4	10
pH	7.4	7.4	7.7	8	8	8.1	8.1	8.2	8.2	min 6 max 8
Total Suspended Solids	19.25	12.88	22.75	11.25	18.13	22.5	31	30.5	39	110
Unacceptable										
Acceptable										

Before water allocation, the water for reserve is set aside. The Reserve is defined in the NWA, 36 of 1998 as water set aside for basic human needs and aquatic ecosystem. When analysing the water quality of the Blesbokspruit against aquatic ecosystem requirements, it becomes evident that metals impact negatively on the water resource, thus not supporting aquatic ecosystem. From the Table above, it could be seen that the values of pH obtained from BB6 – BB9 were higher than the guideline range. Some measure of concern could be raised; however it does not seem to be an overly negative impact on the water resource. Arsenic has been reported to have a variety of adverse effects on both vertebrate and invertebrate aquatic organisms; the type and severity of adverse effects being dependent on the life stages of the organisms concerned. Exposure to arsenic results in reduced growth

and reproduction in both fish and invertebrate populations. Arsenic also causes behavioural changes such as reduced migration in fish (DWAF, 1996d).

Cadmium is easily absorbed by mammals, where it is concentrated by binding with the protein metallothionein. Many plant and animal tissues contain cadmium, but there is no evidence that cadmium is biologically essential or beneficial. Cadmium is chemically similar to zinc, and its physiological effects are often due to its replacement of zinc in some enzymes, thereby impairing enzyme activity. Cadmium is known to inhibit bone repair mechanisms, and is teratogenic, mutagenic and carcinogenic. The Blesbokspruit South Africa Soil analysis from the report of 26 October 1983 provided by Citrus Exchange indicated that the background Iron levels is 0.075 similar to the aquatic requirement of the SA Guidelines, it can therefore be concluded that the alarming levels of Iron in this case is anthropogenic. The report further indicates that the background for zinc is 0.063, also an anthropogenic impact in the Blesbokspruit. The levels of copper however indicate that the background is higher than the aquatic requirements and as such the possibility of aquatic organism's adaptation exists.

Cheung *et al*., (2003) concluded that the discharge of domestic sewage and industrial effluents seems to cause moderate nutrient and heavy metal pollution in Pearl River Estuary, the strong binding affinity of heavy metals results in low concentrations in water and high concentration in sediments, pollution problems at certain location, are mainly associated with major urban and industrial centers.

b). Agricultural use: Irrigation

The result presented in Table 5.3 below showed the comparative analyses of the mean levels of parameters from sampling point BB1 with the recommended South African Water Quality Guideline for the Agricultural Use (DWAF, 1996b), (Only variables that are discussed in the above guidelines have been selected)

The major concern in terms of the utilization of the water from the Blesbokspruit for the purpose of irrigation was the level of faecal and *E. coli* contamination more especially because most of the irrigated crops are edible crops. Field crops and vegetables can become contaminated with human and animal pathogens and parasites when irrigated with water containing these organisms. These organisms may be transferred to humans

when they are retained and survive on the surfaces of produce that are eaten raw, and to animals in their feed. The risk of Helminth (intestinal nematodes) and protozoan parasite (e.g. Giardia spp.) transmission by wastewater is also considered to be high (DWAF, 1996b). Poor sewage reticulation system has always been a concern throughout South Africa and all these unacceptable points are downstream of sewage works

Table 5.3: Results of the comparison of the levels of parameters from sampling point BB1 and the South African Water Quality Guideline: Agricultural Use (DWAF, 1996b)

Variables	Mean Results BB1	Mean Results BB2	Mean Results BB3	Mean Results BB4	Mean Results BB5	Mean Results BB6	Mean Results BB7	Mean Results BB8	Mean Results BB9	SA Guidelines: Agriculture: Irrigation
Arsenic	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	2
Cadmium	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.05
Copper	0.039	0.025	0.025	0.025	0.025	0.025	0.025	0.039	0.039	5
Iron	0.078	0.73	0.12	0.04	0.13	0.1	0.18	0.25	0.19	20
Zinc	0.065	0.065	0.12	0.065	0.065	0.065	0.065	0.068	0.065	5
Nitrates	0.26	1	0.26	0.16	0.16	2.01	1	0.8	1.4	30
pH	7.4	7.4	7.7	8	8	8.1	8.1	8.2	8.2	min 6.5 max 8.4
TSS	19.25	12.88	22.75	11.25	18.13	22.5	31	30.5	39	100
TDS	1001.3	590.25	952.25	970.25	1020.25	912.5	853	796.5	796.5	540
Faecal Coliforms	20.625	44021	52.88	15.88	61.75	772	1035.36	16878.5	1936.25	1000
E. coli	19.125	43999.13	52.5	14.13	59.13	719.63	970.86	1616.86	1891.25	1000
Unacceptable										
Acceptable										

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Table 5.4: The results below are assessed against the South African Water Quality Guideline: Agricultural use: Livestock watering (DWAF, 1996c)

Variables	Mean Results BB1	Mean Results BB2	Mean Results BB3	Mean Results BB4	Mean Results BB5	Mean Results BB6	Mean Results BB7	Mean Results BB8	Mean Results BB9	SA Guidelines: Agriculture: Livestock watering
Arsenic	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	1.5
Cadmium	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.02
Copper	0.039	0.025	0.025	0.025	0.025	0.025	0.025	0.039	0.039	5
Iron	0.078	0.73	0.12	0.04	0.13	0.1	0.18	0.25	0.19	50
Zinc	0.065	0.065	0.12	0.065	0.065	0.065	0.065	0.068	0.065	200
Nitrates	0.26	1	0.26	0.16	0.16	2.01	1	0.8	1.4	40
Faecal Coliforms	20.625	44021	52.88	15.88	61.75	772	1035.36	16878.5	1936.25	200
E. coli	19.125	43999.13	52.5	14.13	59.13	719.63	970.86	1616.86	1891.25	200
Unacceptable										
Acceptable										

c). Agricultural use: Livestock watering

The result presented in Table 5.4 below showed the comparative analyses of the mean levels of parameters from sampling point BB1 with the recommended South African Water Quality Guideline for the Agricultural Use (Livestock Drinking (DWAF, 1996c), (Only variables that are discussed in the above guidelines have been selected)

The major concern in terms of livestock watering on the Blesbokspruit was the possible faecal and *E. coli* contamination. The presence of pathogens like *E. coli* has adverse effects especially in young stock. The risk of infection in intensive domestic production systems such as piggeries and poultry, where the ratio of young: mature animals are high, is far greater than with extensive production systems

5.4 Ecological Status of the Blesbokspruit

Table 5.5: Below is the Ecological Status of the Blesbokspruit (Esterhyuse, *et al*., 2008)

Parameters	Upper Blesbokspruit	Mid Blesbokspruit	Lower Blesbokspruit
Habitat	Poor	Fair	Fair
Aquatic Invertebrates	Poor	Poor	Fair
Fish Population	Poor	Poor	Poor
Riparian Vegetation	Poor	Good	Poor
Water Quality	Poor	Poor	Poor

Definitions

River Health Indicators:

- Habitat > In-stream availability and habitat diversity

- Aquatic Invertebrates > A variety of organisms (snails, insect larvae, crabs and worms) requires specific habitat types and water quality for part of their life cycle
- Fish Population > Fish are good indicators of the long term influences on a river reach and general habitat conditions
- Riparian Vegetation > Healthy river banks maintain the form of the river channel, provide habitat for species (aquatic and terrestrial) and filter sediments materials and light
- Water Quality > the chemical, physical and bacteriological properties of water determine its suitability for use
- River Health Category:

  - Natural > No negligible modification of habitat and flora
  - Good > Some human related impact, biodiversity largely intact
  - Fair > Significant pressure from development and land use, sensitive species may be lost
  - Poor > Natural functioning disrupted, extensive use of river ecosystem

Anglogold Ashanti 2008 indicated that Ergo Daggafontein Tailings storage facility is the mega tailing dump lies just next to Blesbokspruit and dust from the dump is blown into the spruit. The findings in this report ties with studies done by Esterhyuse, 2008, the water quality in the Blesbokspruit is poor.

5.5 Conclusion

Water Quality Management is a world wide problem and South Africa is no different, this is motivated by the researches quoted in this study. The results obtained in this study indicate that the water quality in the Blesbokspruit is poor and does not support aquatic ecosystem even though it holds one of South Africa's important sites, The Blesbokspruit Ramsar Site. This then puts pressure to the country to react to the current situation to ensure that this status is maintained. The constitution of South Africa gives the every one the right to potable water, and this implies that water need to be treated before distributed to communities. Poor water resource management thus impact on the cost for treating this water to portable use before distribution.

In principle, the Department of Water Affairs and Forestry does not promote drinking water directly from the water resource, however, water resources play a major role in the African Religion and people utilise raw water for consumption as well as to perform African rituals. These raise a bigger challenge to the regulatory authority in balancing water use and water user requirements. The other concern is the groundwater pollution, the interconnection of surface and groundwater through infiltration makes the groundwater prone to pollution if the surface waters are allowed to be misused and mismanaged.

It is the reality of many mines, industries and other water users to discharge their effluent into the water resource, thus a need to investigate new approaches to clean the effluent before discharging. Water plays a major role in economic development and if not protected consequences are unbearable with financial loss and health impacts. The authorities therefore need to be stricter to industries to invest in new technologies rather that opting for the possible way out i.e. discharge.

The results obtained in this study indicate that the challenge lies with sewage works and these are managed by local municipalities. The concept of cooperative governance has failed and need to be substituted with stricter controls before the scares commodity of the country is lost. Awareness to communities to protect water resources need to be improved because thus far South Africa has not witnessed protest against resources pollution, this then allows those responsible for managing, protecting and conserving water resources to relax.

Naidoo (2009) reported on the Engineering news that, the National African Farmers' Union (Nafu) president, Motsepe Matlala told delegates attending the recent Water for Growth and Development (WFGD) Consultative Summit, in Johannesburg that the agriculture sector is facing an increasing decline in the quality of water as a result of a lack of maintenance and pollution by the industrial and mining sectors, He also said that this impacted on the quality of food, posed a high health risk and impacted negatively on export markets. From this summit, it is clear that polluted water resources threaten the food security in this country.

The Blesbokspruit is a tributary to the Vaal River, the core water resource of Gauteng Province. The results obtained from this study confirm that the Vaal River faces serious challenges as a reliable source of water for domestic, agricultural and industrial use. The use of incentives in overseas areas has proved to improve human behaviour and thus protect the environment. The plastic charges in South Africa have proved to reduce plastic litter and have improved human attitude towards the handling of plastics. The implementation of the Waste Discharge Charge system can also assist in improving water quality in South Africa; further environmental taxes also need to be investigated. Water is a scares commodity in South Africa and the NWA requires that it be Protected, Used, Developed, Conserved, Managed and Controlled. The Constitution of South Africa also informs the regulating authorities to protect the environment for current and future generations. We didn't inherit the Earth from our parents. We're borrowing it from our children Chief Seattle (1788 – 1866)

5.6 Recommendations

Based on the results obtained in this study, the following suggestions are recommended.

- Promotion of awareness about the dangers of polluted water streams and encourage responsibility,
- Promotion of Water Quality Monitoring and accessibility of results by the public
- Introduce flag status in catchments, municipalities and industries similar to the blue flag of beaches to encourage compliance
- encourages the need for authorisation of the sewage works and
- To promote compliance and protection of the water resource for future generations.
- Further studies to assess the extent of organic compounds pollution in the catchment is necessary and more especially the Persistent Organic Pollutants (POPs) and the Endocrine disruptors.

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APPENDICES

Appendix A

In-stream Water Quality Guidelines for the Blesbokspruit Catchment

In-stream Water Quality Guidelines for the Blesbokspruit Catchment **Effective: June 2003**

Variables	Measured as	Ideal Catchment Background	Acceptable Management Target	Tolerable Interim Target	Unacceptable
Physical					
Conductivity	mS/m	< 45	45 - 70	70 - 120	> 120
Dissolved Oxygen (O_2)	mg/l O_2		> 6.0	5.0 - 6.0	< 5.0
pH	pH units	6.5 - 8.5			< 6.5; > 8.5
Suspended Solids	mg/l	< 20	20 - 30	30 - 55	> 55
Organic					
Chemical Oxygen Demand (COD)	mg/l	< 20	20 - 35	35 - 55	> 55
Macro Elements					
Aluminium (Al)	mg/l		< 0.3	0.3 - 0.5	> 0.5
Ammonia (NH_4)	mg/l	< 0.1	0.1 - 1.5	1.5 - 5.0	> 5.0
Chloride (Cl)	mg/l	< 80	80 - 150	150 - 200	> 200
Fluoride (F)	mg/l	< 0.19	0.19 - 0.70	0.70 - 1.00	> 1.00
Iron (Fe)	mg/l	< 0.1	0.1 - 0.5	0.5 - 1.0	> 1.0
Magnesium (Mg)	mg/l	< 8	8 - 30	30 - 70	> 70
Manganese (Mn)	mg/l	< 0.2	0.2 - 0.5	0.5 - 1.0	> 1.0
Nitrate (NO_3)	mg/l	< 0.5	0.5 - 3.0	3.0 - 6.0	> 6.0
Phosphate (PO_4)	mg/l	< 0.2	0.2 - 0.4	0.4 - 0.6	> 0.6
Sodium (Na)	mg/l	< 70	70 - 100	100 - 150	> 150
Sulphate (SO_4)	mg/l	< 150	150 - 300	300 - 500	> 500
Bacteriological					
Faecal coliforms	counts/100ml		< 126	126 - 1,000	> 1,000
Biological					
Daphnia	% survival	100	90 - 100	80 - 90	< 80

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Figure 4.9: Variations in mean Cadmium (Cd) values of water samples across sampling points

Figure 4.10: Variations in mean Copper (Cu) values of water samples across sampling points

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Figure 4.14: Variations in mean Faecal Coliforms (FC) values of water samples across sampling points

Figure 4.15: Variations in mean Total Coliforms (TC) values of water samples across sampling points