

**ANALYSES OF THE BIO-PHYSICOCHEMICAL QUALITY OF SURFACE AND  
GROUND WATER IN THE TUBATSE MUNICIPALITY**

by

**MATHIPA MORONGWA MARY**

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**Supervisor:** Dr KLM Moganedi

**Co supervisor:** Prof P Masoko

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## DECLARATION

I declare that **ANALYSES OF THE BIO-PHYSICO-CHEMICAL QUALITY OF SURFACE AND GROUND WATER IN THE TUBATSE MUNICIPALITY**, submitted to the University of Limpopo for the degree of Master of Science (Microbiology) has not previously been submitted by me for a degree at this or any other University that it is my own work in design and in execution, and all the material contained therein has been duly acknowledged.

Signature: \_\_\_\_\_

Mathipa M.M (Miss)

Date: \_\_\_\_\_

## **DEDICATIONS**

I would like to dedicate this dissertation to my family for their love and support throughout my studies

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## ABSTRACT

Human activities are known to be the major contributors to contamination of natural water sources. This becomes a serious health risk when the communities rely on the same water sources for their household water needs. The current study investigated the bio-physicochemical quality of surface and ground waters found in a mining area in the Tubatse locality, for their suitability for drinking and other household use. The bacteriological analyses of surface waters showed dominance by genera of the phylum Proteobacteria, followed by Actinobacteria and Firmicutes. The isolates included the genera *Aeromonas*, *Pseudomonas*, *Cronobacter*, *Acinetobacter*, *Enterobacter*, *Pantoea*, *Serratia*, *Bordetella*, *Kocuria* and *Streptococcus*. This dominance pattern is proportionately similar to the pattern reported on human skin and of gut biota. *Enterobacter* spp. were the predominant species in the surface waters, followed by *Bordetella* spp. With regard to ground water, one sample was laden with coliforms whereas the other sample was free of coliforms. Physical quality parameters such as turbidity, colour and (total suspended solids) TSS of the surface and ground water samples were compliant with the set standards for drinking water according to South African water quality guidelines (2005). The concentrations of Zn, [SCN-], Cr, Co, Fe, Ni, Cu, H<sub>2</sub>O<sub>2</sub>, Cl<sub>2</sub> were determined in the surface and ground waters in dry and wet seasons as well. A non-significant decrease in the levels of Cl<sub>2</sub> and [SCN-] and an increase in TDS and Cr were observed in the wet season. All metal and chemical levels in surface and ground water, except Cr, were lower than permitted concentration for drinking water. However the concentrations of Zn, Fe and Co exceeded the normal expected concentrations of < 3.5 µg/L, 0.5 mg/L, < 0.01 mg/L and < 5 µg/L respectively. The sediments and soil samples were digested with aqua regia for Cu, Cr, Fe, Co and Zn analyses. An increase in the levels of Zn, Cr and Co in the wet season was observed. The concentrations of the heavy metals such as Co, Cu and Cr were higher in sediments at the sampling points closer to the mining sites. Cytotoxicity assay was performed with different concentrations, as detected in the water and sediment samples, of Zn, [SCN-], Cr, Co, and Fe on C2C12 (mammalian) cells. An increase in viable cells was observed after treatment with Cr (0.2, 0.45 and 0.9 mg/L), Zn and Fe (1.0 and 2 mg/L), Co (2, 5 and 10 mg/L). Only cells treated with SCN- (3.3, 5.4 and 7.2 mg/L) exhibited a significant decrease in viability. These results demonstrate that the water in the Tubatse municipality is

not suitable for drinking and other household purposes without prior treatment which will remove contaminating microorganisms and chemicals and heavy metals.

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## Chapter 1

### 1. INTRODUCTION

Water is one of the most important resources on earth. Freshwater is an essential societal requirement and it is consumed through domestic, industrial, agricultural and environmental withdrawals. The growing human population has an increasing demand for water, which has made this natural resource a scarce commodity in South Africa and worldwide. Out of the total water available on earth, only 0.16 % is suitable for human use (Mohd and Usmani, 2010). Approximately 880 million people still lack access to safe drinking water globally (Joint Monitoring Programme for Water Supply and Sanitation, 2008). It has been reported that almost 30 % of the South African population does not have access to an adequate supply of potable water, particularly in villages where people have to travel long distances between drinking water sources and their homes (Water Purifier Essential for Hurricane Preparedness, 2006). Thus a lot of the villagers still depend on surface and underground water for household purposes. Utilization of surface water for domestic purpose poses a huge health risk on the communities that rely on surface water due to lack of options for potable water.

Water quality describes the condition of the water, including chemical, physical and biological characteristics, usually with respect to its suitability for a particular purpose such as drinking or irrigation. Water quality is important for public protection not only that, water provides ecosystem habitats. Microbiological quality of drinking-water has been a concern when water was implicated in the spread of important infectious and parasitic diseases such as cholera, typhoid, dysentery, hepatitis, giardiasis, and schistosomiasis (WHO, 2005). Waterborne diseases, such as diarrhoea, cause 1.5 million deaths a year, prominently to children in developing countries (JMP, 2008). It is estimated that child mortality could significantly be reduced and general health be improved by providing access to safe potable water and by improving sanitation and hygiene (UNICEF, 2007).

Natural phenomenon or human activities including, municipal, commercial, industrial and agricultural activities can affect ground and surface water quality (Barret *et al.*, 2002). The occurrence of run-offs of effluent from mines into natural streams and rivers have been reported in several studies (Fallon *et al.*, 2002; David, 2003;

Runnalls and Coleman, 2003). Contamination by different pollutants from leaching of chemicals into ground and surface water might render the water unsuitable for consumption and put human and animal life at risk. Leakages of mine effluents have been previously reported (Economopoulos, 1993) and this is a serious concern for affected communities because of the potential health effect that results from exposure to chemicals. Depending on their persistence, chemicals discharged through the effluent from industrial sources to a surface water body may reach an intake of a drinking-water supply, and the chemicals accumulate in the bottom sediment of a water body (Economopoulos, 1993). Hence it is important to determine and analyse the bio-physicochemical properties of ground and surface water which are used as sources for domestic use in areas with mining activities.

Tubatse local Municipality is located in the Sekhukhune District Municipality of Limpopo province, South Africa. The municipality houses a number of platinum mines which are coded as TwH, Mrl and HcN. These are situated, some 40 km from Burgersfort(<http://www.angloamericanplatinum.com/media/pressreleases/archive/2001/06-09-2001.aspx>, 2015). The communities in the mining areas have little or no access to treated water provided by the local municipalities. Hence, they use surface and ground waters for their domestic needs. Health risks associated with consumption of surface waters are well documented (Huang *et al.*, 2008; Huang *et al.*, 2010; Murhekar, 2011). Surface waters generally have poor microbiological quality due to human activities and close interaction with the environment. The presence of chemicals and metal contamination are highly probable in surface waters in areas where there are mines and agricultural activities. There are three unnatural streams resulting from mining activities close to each of the mines housed in this district. All of these streams flow into the natural rivers in the area. The TwH, and HcN streams flow into the Motse river whereas the MrL stream flows into the Moopetsi river. Both the Motse and Moopetsi rivers flow into the Moopetsi-Motse river which in turn flows into the Lepelle river. A map of the study area is shown in Figure 1.1 and a schematic representation of flow of the water sources is shown in Figure 1.2 below. Table 1.1 shows the morphometric details of the closest estimates of the sampling points.

This study seeks to investigate the bio-physicochemical properties of ground and surface water commonly used for domestic purposes in the Tubatse municipality in the Sekhukhune district

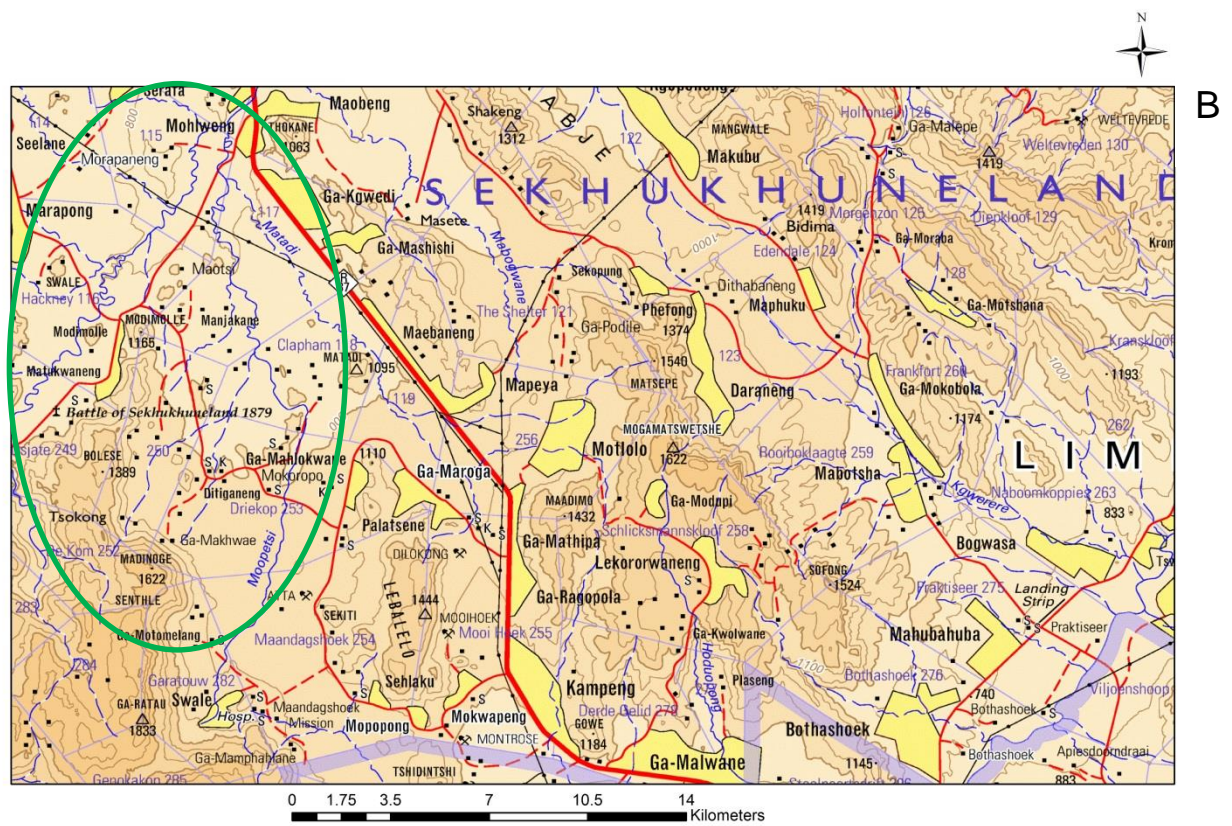
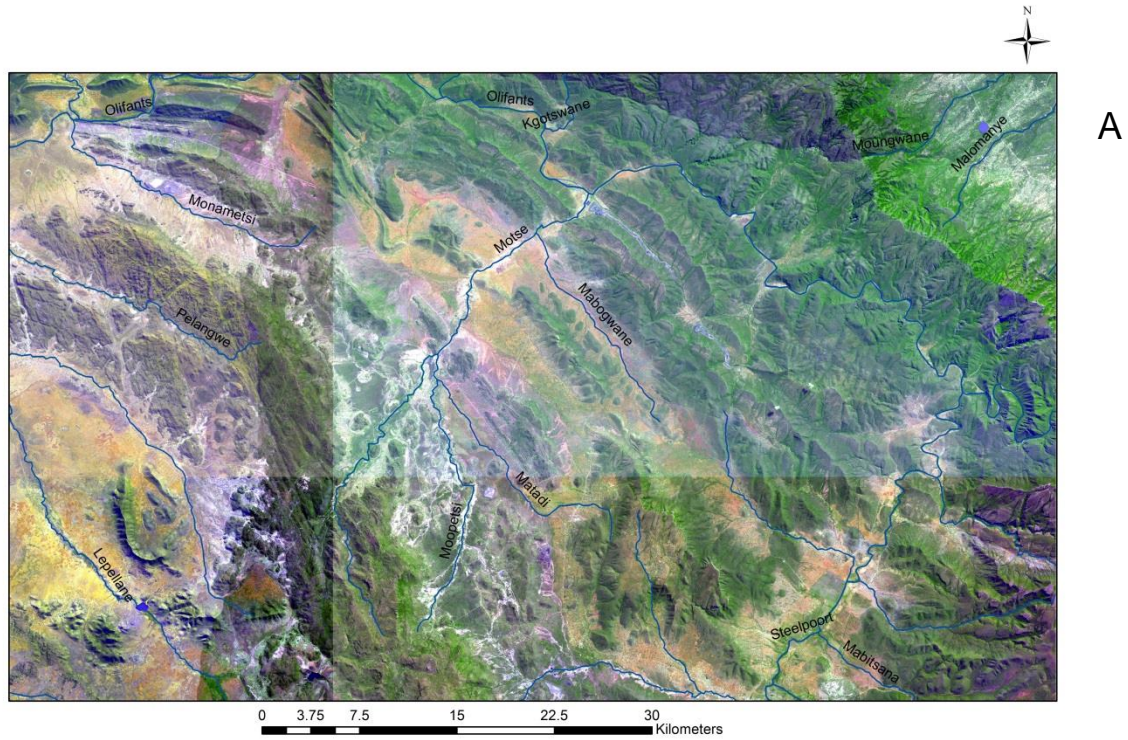


Figure 1.1 Maps of the study area. A- shows an aerial map that depicts the two rivers sampled, B- is a topographic map of the Sekhukhune district and the study area is shown within a green circle.



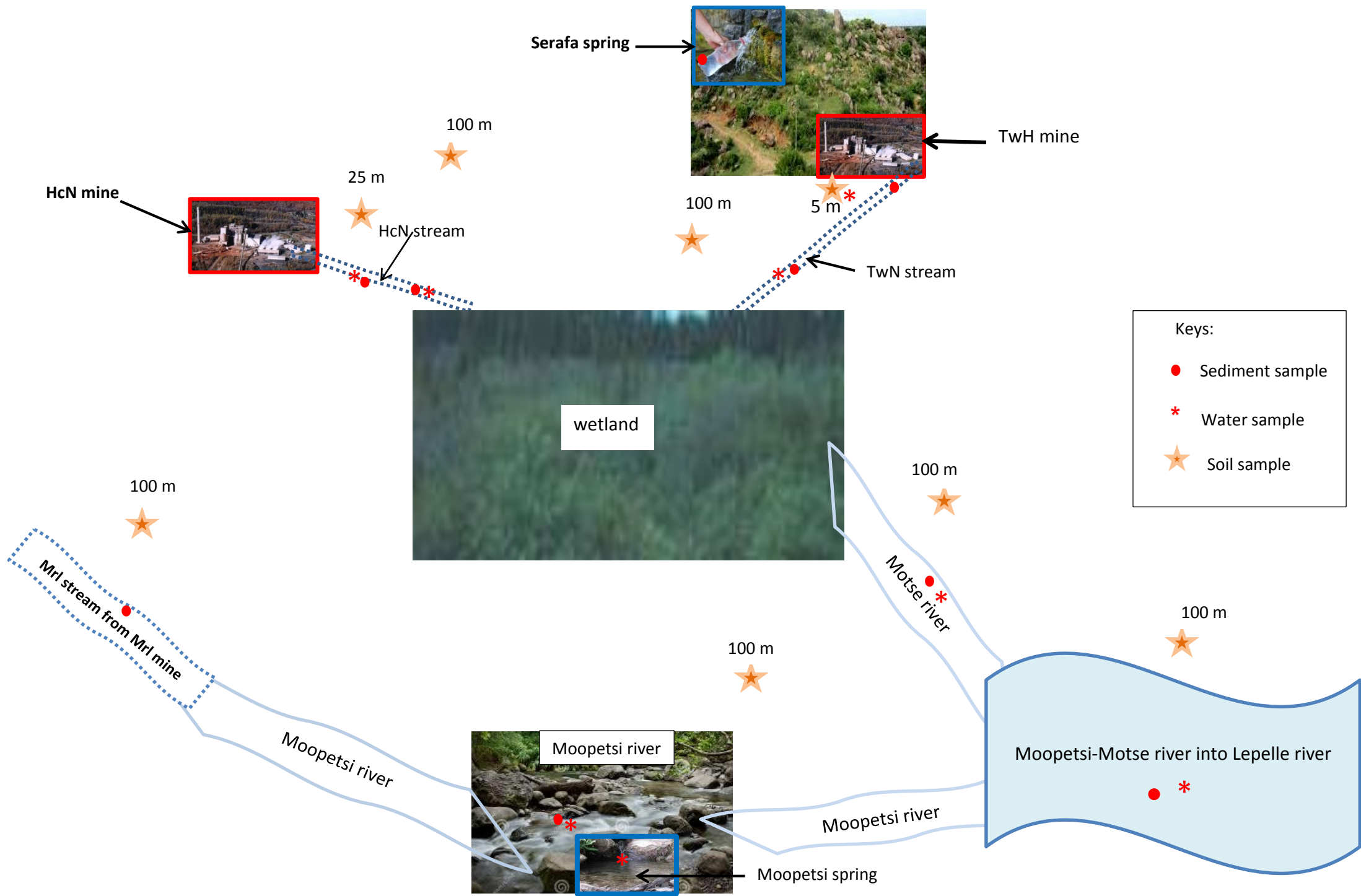


Figure 1.2 Schematic representation of the layout of the study area

Table 1.1 Morphometric details of the closest estimates of the sampling points

Site	Description	Latitude	Longitude	Elevation
1	Motse river natural surface water	E030°04.217	S24°24.916	751
2	Moopetsi river natural surface water	E030°04.176	S24°25.455	738
3	Moopetsi- Motse river	E030°04.354	S24°24.851	677
4	Moopetsi natural spring	E030°04.176	S24°25.455	738
5	Serafa natural spring water	E030°04.	S24°	
6	HcN stream resulting from mining activity	E030°03.245'	S24°26.874	725
7	TwH stream resulting from mining activity	E 030°01.826'	S24°24.593	803
8	MrL stream resulting from mining activity	E030°05.305	S24°30.078	788

## **Chapter 2**

### **2. LITERATURE REVIEW**

#### **2.1 The availability and importance of potable water**

Domestic water supply is one of the fundamental requirements for human life. Without water, life cannot be sustained beyond a few days and the lack of access to adequate water supplies leads to the spread of diseases (Howard and Bartman, 2003). The South African constitution states that everyone has the right to have access to an environment that is not harmful to their health or well-being (RSA, 1996). This includes a constant supply of clean, safe drinking water. Safe drinking water is water that is acceptable and has a pleasant appearance, taste and odour (Drinking water quality in South Africa, 2005).

Clean, safe drinking water is scarce. A large percentage of people without access to safe water is from the developing world, especially in rural areas and low-income communities. In South Africa, many people use raw water from surface or underground for drinking (Venter, 2001; Mackintosh and Colvin, 2003). The communities relying on such water sources are commonly poor and live in polluted environments with associated high health risks (Andreasen, 1996; WHO, 2003). In 2005, more than 95 % of the country's freshwater resources had already been allocated. The water quality of these resources has also declined due to increased pollution caused by industry, urbanisation, afforestation, mining, agriculture and power generation (UNICEF, 2007). Based on the water use and population growth of 2009, the Department of Water Affairs estimated a 1.7 % water shortage by 2025 (Meinhardt, 2009).

#### **2.2 Natural water resources**

##### **2.2.1 Surface water**

Surface water refers to water that is found in lakes, rivers, streams, ponds, and other natural water courses. It is also found in marine bays, estuaries, and oceans. Surface water supports various recreational activities such as swimming and boating, and provides habitat for aquatic life. Ideally, a clean, abundant supply of

surface water supports the health of humans and aquatic ecosystems, a strong economy, and provides a high quality of life. It is common that surface water sources will contain microorganisms, such as bacteria, viruses and parasites, that can cause cramps, nausea, vomiting, and diarrhoea, and in some cases more serious, or even fatal, illnesses when consumed (Anwar and Bureste, 2011).

Surface water sources should be protected as much as possible from contamination by harmful pollutants. Potential pollutants include microorganisms, inorganic chemicals, organic chemicals, and radionuclides (EPA, 1997). Completely protecting water source may not be possible because pollutants from the atmosphere can enter surface water through precipitation, and contaminated ground water can introduce pollutants through recharge. Surface-water characteristics can be affected by numerous physical variables such as topography, land cover, soil conditions, mineralogy and ground-water conditions, all of which may be affected by geological conditions (UNEP, 2006). In addition, stream flow is affected by numerous climatic variables which include timing, intensity, and amount of precipitation, as well as other variables affecting evaporative processes (UNEP, 2006).

### *2.2.1.1 Surface water properties*

The geological nature of the soil determines the chemical composition of the surface and groundwater. Water is constantly in contact with the ground in which it circulates, so equilibrium develops between the composition of the soil and that of the water; for example water that circulates in a sandy or granitic substratum is acidic and has few minerals and water that circulates in limestone contains bicarbonates alkalinity. Human activities commonly affect the distribution, quantity, and chemical quality of water resources. The range in human activities that affect the interaction of ground water and surface water is broad. Agriculture has been the cause of significant modification of landscapes throughout the world (APHA, 1989) Tillage of land changes the infiltration and runoff characteristics of the land surface, which affects recharge to ground water, delivery of water and sediment to surface-water bodies, and evapotranspiration (Zhang and Shan; 2008). Two activities related to agriculture that are particularly relevant to the interaction of ground water and surface water are irrigation and application of chemicals to cropland.

Point sources of contamination to surface water bodies include direct discharges from sewage-treatment plants, industrial facilities and storm water drainage.

#### *2.2.1.2 Contamination of surface water by human activities*

Threats to surface water quality can be caused by anthropogenic activities that include effluents and solid wastes from various industries and municipalities, indiscriminate use of toxic chemicals, pesticides, insecticides and fungicides, may lead to runoffs into the nearby streams and rivers, hence polluting the surface water. Excess rain or irrigation may contribute and facilitate runoff of these chemical pollutants which tend to percolate the ground and contaminate groundwater as well (Edgar, 1983).

Some of the pollutants such as lead (Pb), arsenic (As), mercury (Hg), chromium (Cr) nickel (Ni), barium (Ba), cadmium (Cd), cobalt (Co), selenium (Se), vanadium (V), oils, grease and pesticides are very harmful, toxic and poisonous. There are some minerals such as zinc (Zn), copper (Cu), and iron (Fe) which are useful for human and animal health in small doses beyond which they become toxic. For agriculture, elements like Zn, Cu, manganese (Mn), sulphur (S), iron, boron (B), together with phosphates, nitrates, urea, potassium are useful in prescribed quantities (Kant, 2005). Compounds such as cyanides, thiocyanides, phenolic compounds, fluorides, radioactive substances are harmful for humans as well as animals (SAWQG, 2005).

Some of the chemicals such as Cr, Ni, Ba, Co, cyanides, thiocyanides, phenolic compounds, and radioactive substances are not naturally found in surface water while the others such as magnesium (Mg), Zn, Cu and Fe are found at varying concentrations. Arsenic is normally found in unpolluted surface water at a concentration of less than 1 mg/L, Cd at < 0.1 mg/L, Se at < 10 mg/L, Va at < 1 mg/L, Pb found to be below 0.010 mg/L and Fe is normally between 0.001-0.5 mg/L (SAWQG, 2005). A rise in these concentrations suggests contamination and therefore the water is no longer suitable for drinking and other household use.

### 2.2.2 Ground water

Groundwater is water that accumulates underground. It can exist in spaces between loose particles of dirt and rock, or in cracks and crevices in rocks. The amount of water that seeps into the ground will vary widely from place to place, depending on the slope of the land, amount and intensity of rainfall, and type of land surface (UNEP, 2006).

As water seeps through permeable ground, it continues downward until it reaches a depth where water has filled all the porous areas in the soil or rock. This is known as the saturated zone (USEPA, 2006). The top of the saturated zone is called the water table. The water table can rise or fall according to the season of the year and the amount of precipitation that occurs. The water table is typically higher in early spring and lower in late summer. The porous area between the land surface and the water table is known as the unsaturated zone (USEPA, 2006).

Groundwater contamination can come from a number of natural and man-made activities. Spills and leaks of chemicals can result from accidents and inadequate monitoring and maintenance of mining plants (Malana and Khosa, 2011). The spills and leaks are especially problematic if proper procedures are not in place to clean up once they occur. Materials which can cause problems if spilled include gasoline, other petroleum products, hazardous chemicals such as arsenic (AS), mercury (Hg), Lead (Pb), Copper (Cu), cobalt (Co). Industrial wastes can create groundwater pollution problems that take years to resolve.

Landfills that are not properly constructed, liquid from decomposition of materials, or leachate, can leak out of the landfill into an aquifer. Leachate can contain high levels of bacteria, hazardous chemicals, metals, and ammonia (Mahmood *et al.*, 2011). Runoff water from landfills after rains can also carry pollution to groundwater recharge.

Septic systems can be a source of groundwater pollution if too many systems are located in an area. If a septic system is not working properly, it can contaminate groundwater with bacteria, viruses, and hazardous cleaning materials or household chemicals (USEPA, 2006). Even properly working well-maintained septic systems

can contribute nitrates to groundwater. These can show up in well water around the septic system (Pritchard *et al.*, 2005)

Misuse of fertilizers can cause groundwater pollution. Overuse can allow nitrates from fertilizer to seep into the water table. In sensitive groundwater areas, rainfall seepage can cause fertilizer to migrate and contaminate an aquifer (The Groundwater Foundation, 2007).

Underground injection wells are a method of waste disposal. Wastes disposed by this method include industrial chemicals, sewage effluent, cooling water, storm water, and salt water. Typically, injection wells inject wastes below sources of drinking water, but if injection wells have leaks or are used improperly, wastes can seep directly into a usable groundwater supply.

In many cases, the soil can remove bacteria, viruses and chemicals from water that percolates downward (Mahmood *et al.*, 2011). But not all soil types remove contaminants as effectively as others, and domestic and industrial waste can also exceed the soil's ability to remove chemicals and contaminants (The Groundwater Foundation, 2007). Some soils allow water to quickly percolate down to the aquifer. This generally means that less of the contaminants will be removed. As well, when the pollutants originate from an underground source, such as a storage tank or septic system, they may be very close to the groundwater and there is less time for entrapment of contaminants in the soil. The quality of the groundwater depends on the temperature, on the pressure which depends on how deep the groundwater is, (USEPA, 2001) the type of rock and soil and the residence time of the water.

Table 2.1 below compares the characteristics of surface water and groundwater based on the major analytical parameters.

Table 2.1 Comparisons of the characteristics of surface water and groundwater based on the major analytical parameters.

<b>Characteristic</b>	<b>Surface Water</b>	<b>Ground Water</b>
<b>Temperature</b>	varies with season	relatively constant
<b>Turbidity, Suspended solids (SS)</b>	Level variable, sometimes high	Low or nil (except in karst soil)
<b>Colour</b>	Due mainly to suspended solids	Due to dissolved solids
<b>Mineral content</b>	Varies with soil, rainfall, effluents, etc.	Largely constant, generally higher than in surface water from the same area
<b>Divalent Fe and Mn in solution</b>	Usually none, except at the bottom of lakes and ponds in the process of eutrophication	Usually present
<b>Aggressive CO<sub>2</sub></b>	Usually none	Often present
<b>Dissolved O<sub>2</sub></b>	Often near saturation level, absent in very polluted water	Usually none
<b>H<sub>2</sub>S</b>	Usually none	Often present
<b>NH<sub>4</sub></b>	Found only in polluted water	Often found
<b>Nitrates</b>	Level generally low	Level sometimes high
<b>Silica</b>	Usually moderate proportions	Level often high
<b>Mineral and organic micro-pollutants</b>	Can be present but liable to disappear rapidly once the source is removed	Usually none
<b>Living organisms</b>	Bacteria, viruses, plankton	Iron bacteria are frequently observed
<b>Chlorinated solvents</b>	Rarely present	Often present
<b>Eutrophic nature</b>	Often increased by high temperatures	None

Source: Clarke (2004).



## 2.3 Drinking water quality parameters

Drinking water must be potable and this is defined in law by standards and parameters for a wide range of substances, organisms and properties of water. These standards are set for public health protection and ensuring that water quality is acceptable to consumers. These parameters include microbiological, chemical, metal and physical properties of the water.

A supply of drinking water should be sufficient in quantity, must be potable and not dangerous to the health of the consumers. These requirements are all inter-related. The history of water-supply engineering has repeatedly shown that the provision of safe drinking-water is the most important step which can be taken to improve the health of a community by preventing the spread of waterborne disease (Momba and Kaleni, 2002; WHO, 2005).

### 2.3.1 Microbiological parameters

Microbiological monitoring provides a sensitive indication of the extent to which source protection, treatment and distribution can effectively provide barriers to the transmission of infectious agents of waterborne disease.

#### 2.3.1.1 *Microbial flora of ground and surface water*

Gram negative bacteria that are naturally found in ground water include species belonging to the genera *Pseudomonas*, *Azotobacter*, *Neisseria*, *Moraxella* and *Acinetobacter*. A study made by Bakwill and Ghiorse (1989) showed that 95 % of culturable bacteria isolated from water were non- streptomycete bacteria and more than 80 % were Gram negative rods (Balkwill and Ghiorse, 1989). Gram positive bacteria are not abundant in ground water systems. Gram positive bacteria include many important human pathogens such as *Micrococcus*, *Staphylococcus* and *Streptococcus*. Ground water systems also carry two major archaea bacteria which are the ammonia oxidizing archaea and methanogenic archaea (Bollmann *et al.*, 2014).

### 2.3.1.2 Microbial Indicators for water quality

All natural waters can be populated by transient bacteria. Among these are the human pathogens that gain entry to water from faecal contamination. The direct isolation of pathogens is impractical; instead public health inspectors determine the number of indicator bacteria. Indicator organisms are, therefore, generally used for routine monitoring of the potential presence of pathogens in water.

#### 2.3.1.2.1 Coliform and faecal bacteria

Coliform organisms have long been recognized as suitable microbial indicators of drinking-water quality, largely because they are easy to detect and enumerate in water (Allsop and Stickler, 1985). The term total coliforms refer to Gram-negative, rod-shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties, and are able to ferment lactose at 35 - 37 °C with the production of acid, gas, and alcohol within 24 - 48 hours (Atlas, 1996). They are also oxidase-negative and non-spore-forming. The coliform group also contains species which can multiply in relatively good quality drinking water such as *Serratia fonticola*, *Rahnella aquatilis*, and *Buttiauxella agrestis*. Coliform bacteria should not be detectable in treated water supplies; their presence suggests inadequate treatment, post-treatment contamination, or excessive nutrients in the water (James and Evison, 2005). In this sense, the coliform test can be used to assess treatment efficiency and the integrity of the distribution system. Hence, the coliform test is still useful for monitoring the microbial quality of public water supplies.

Thermotolerant coliforms are defined as the group of coliform organisms that are able to ferment lactose at 44 - 45 °C. They comprise the genus *Escherichia* and, to a lesser extent, species of *Klebsiella*, *Enterobacter*, and *Citrobacter* (WHO, 1993). Of these organisms, only *E. coli* is specifically of faecal origin, being always present in the faeces of humans, other mammals, and birds in large numbers, and rarely found in water or soil that has not been subjected to faecal pollution. Secondary indicator organisms are often used to ascertain faecal contamination. These include the

faecal streptococci and sulphite-reducing clostridia, especially *Clostridium perfringens*.

Faecal streptococci refer to the streptococci which are generally present in the faeces of humans and animals (Wiedenmann *et al.*, 2002). Taxonomically, they belong to the genera *Enterococcus* and *Streptococcus* (Madigan *et al.*, 2009). The genus *Enterococcus* has recently been defined to include all streptococci sharing certain biochemical properties and having a wide tolerance of adverse growth conditions (Tortora *et al.*, 2002). It includes the species *Enterococcus avium*, *E. casseliflavus*, *E. cecorum*, *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. malodoratus*, *E. mundtii*, and *E. solitarius* (Venter, 2001). Most of these species can generally be regarded as specific indicators of human faecal pollution under many practical circumstances (James and Evison, 2005). Certain species and subspecies, such as *E. casseliflavus*, *E. faecalis*, *Enterobacter liquefaciens*, *Enterococcus malodoratus*, and *Enterococcus solitarius* occur primarily on plant material (James and Evison, 2005).

#### 2.3.1.2.2 Heterotrophic bacteria

Heterotrophs are a group of microorganisms that use organic carbon sources to grow and can be found in all types of water. In fact, the majority of bacteria found in drinking water systems are considered heterotrophs (WHO, 2005). Heterotrophic plate counts may be used to assess the general bacterial content of water (Sobsey, 2002). They do not represent all the bacteria present in the water but only those able to grow and produce visible colonies on the media used and under the prescribed conditions of temperature and time of incubation.

Heterotrophic counts are often determined following incubation at 22 °C and 37 °C to assess the relative proportions of naturally occurring water bacteria unrelated to faecal pollution and of bacteria derived from humans and warm-blooded animals, respectively (SAWQG, 2005). The 22 °C heterotrophic count is used to assess the cleanliness and integrity of the distribution system and the suitability of the water for use in the manufacture of food and drink where high counts may lead to spoilage.

Any increase in counts in the test at 37 °C as compared with those normally found may be an early sign of pollution (Sobsey, 2002).

### 2.3.2 Physical parameters

The appearance, taste and odour of water determine what people experience when they drink or use water and how they rate its quality. The measurable characteristics that determine these largely subjective qualities are turbidity and colour.

#### 2.3.2.1 Turbidity

Turbidity is a measure of the cloudiness or murkiness of water due to suspended particles (Mahmood *et al.*, 2011). Turbidity can be caused by organic particles such as decomposed plant and animal matter, or living biological organisms' inorganic particles. Shallow or poorly-built wells or springs may be contaminated from surface water, especially during heavy rains or spring runoff (Aucamp and Vivier 1990). Turbidity in groundwater is mostly inorganic and caused by natural geological factors. Turbidity can range from less than 1 NTU to more than 1,000 NTU. At 5 NTU, water is visibly cloudy and at 25 NTU, it is murky. Microorganisms attach themselves to the suspended particles in turbid water (WHO, 2005). This prevents the water from being properly disinfected, and can increase the risk of gastrointestinal illnesses due to pathogenic microorganisms that evade disinfection.

#### 2.3.2.2 Colour

Colour in water can be due to natural minerals or it may be as a result of industrial or household effluents containing soluble coloured materials. Colour in ground water may be due to the presence of coloured organic matter, e.g., metals such manganese and iron (Oyedeji *et al*, 2010). The apparent colour of water is strongly related to turbidity. Other metal ions may on occasion give rise to colour in water. A change in colour is normally seen as a warning of quality changes (Sobsey *et al*,

1989). No direct health effects result from the presence of colour in drinking water except when colourants are toxic.

### 2.3.3 Chemical parameters

A number of chemicals, both organic and inorganic, including some pesticides raise concerns when found in drinking water from the health perspective because they might be toxic to humans (Agarwal and Manish, 2011) and can affect the aesthetic quality of water (Adnan *et al.*, 2010). Inorganic chemicals in drinking water usually occur as dissolved salts such as carbonates and chlorides attached to suspended material such as clay particles, or as complexes with naturally occurring organic compounds (Basavaraja *et al.*, 2005). Their presence may result from natural leaching from mineral deposits into water sources, land-use activities in catchments which lead to exacerbation of natural processes such as mobilisation of salts, carryover of small amounts of treatment chemicals and addition of chemicals such as chlorine and fluoride (Gupta and Saharan, 2009).

#### 2.3.3.1 *Total dissolved solids*

Dissolved solids (DS) in water supplies originate from natural sources, sewage, urban and agricultural run-off, and industrial wastewater. The amount of solids in water affects both removal and disinfection processes (WHO, 2005). Water containing DS concentrations below 1000 mg/L is usually acceptable to consumers, although acceptability may vary according to circumstances (SAWQG, 2005). Water with extremely low concentration of DS may also be unacceptable to consumers because of its flat, insipid taste and it is also often corrosive to water-supply systems.

#### 2.3.3.2 *pH*

The pH of water is a measure of the acid–base equilibrium and in most natural waters it is controlled by the carbon dioxide–bicarbonate–carbonate equilibrium (WHO, 2006). Although pH usually has no direct impact on water consumers, it is

one of the most important operational water-quality parameters. Careful attention to pH control is necessary at all stages of water treatment to ensure satisfactory water clarification and disinfection (SAWQG, 2005).

#### *2.3.3.3 Hardness*

Hardness is a measure of polyvalent cations in water (Bailey and Archer, 2000). Hardness generally represents the concentrations of calcium and magnesium ions, because these are the most common polyvalent cations (Oyedemi *et al.*, 2010). Other ions, such as iron and manganese, may also contribute to the hardness of water, but are generally present in much lower concentrations, particularly in surface waters. Hardness affects the amount of soap that is needed to produce foam or lather. Hard water requires more soap, because the calcium and magnesium ions form complexes with soap, preventing the soap from sudsing (Bailey and Archer, 2000).

### **2.4 Determination of bio-physicochemical quality of drinking water**

A water supply for domestic use should not contain any pathogens or a substance that may harm the consumers. Water analysis is the measurement of type and level of contaminants present in a water sample. The results of the analysis can suggest the level of treatment the water supply needs. There are different parameters as listed in 2.3 above that are evaluated in water analysis. Microbiological analysis determines the organisms that cause diseases whereas chemical and physical analyses identify elements in the sample that makes the water turbid, offensive and poisonous to consumers.

#### **2.4.1 Microbiological quality**

Microbial enumeration refers to the measurement of the number of bacterial cells per millilitre. Water microbiologists test water for the numbers of microorganisms to determine if the water is safe for human consumption. There are a number of

methods that can be used to determine the number of cells/unit and these methods can be divided into indirect and direct methods.

#### 2.4.1.1 Indirect methods

##### Most probable number method

The Most probable number (MPN) method is used to estimate the concentration of viable microorganisms in a sample by means of replicate liquid broth growth in ten-fold dilutions and is particularly useful with samples that contain particulate material that interferes with plate count enumeration methods. In an MPN enumeration procedure, multiple serial dilutions are performed to reach a point of extinction, which is a dilution level at which no growth is observable (Prescott *et al.*, 2005). A criterion, such as the development of cloudiness or turbidity in a liquid growth medium is established for indicating whether a particular dilution tube contains bacteria. The pattern of positive and negative test result is then used to estimate the concentration of bacteria in the original sample, that is, the MPN of bacteria, by comparing it to the observed pattern of results with a table of statistical probabilities for obtaining results (USP, 2009).

##### Turbidity

During exponential growth, all cellular materials increase in proportion to the increase in cell numbers. Thus instead of measuring changes in cell number over time one could instead measure the increase in protein, DNA, or dry weight of culture as a barometer of growth (Madigan *et al.*, 2009). A rapid and useful method of estimating cell growth in this way is by turbidity measurements. A suspension of cells looks cloudy (turbid) to the eye because cells scatter light passing through the suspension. The more cells that are present, the more light is scattered, and hence the more turbid the suspension. Total mass is assessed in a turbidimetric measurement. However, because cell mass is proportional to the cell number, turbidity is a measure of cell numbers in a growing culture (Hussong and Madsen, 2004). Turbidity is measured with a spectrophotometer.

#### 2.4.1.2 Direct methods

##### Viable count

The viable count method is a common procedure for the enumeration of living bacteria (Atlas, 1996). It is assumed that each colony arises from an individual bacterial cell. Therefore by counting the number of colonies that develop, as colony forming units (CFUs) and by taking into account the dilution factors, the concentration of bacteria in the original sample can be determined. Empirically, countable cultures with colonies 30 and 300 colonies are considered for microbial enumeration. Fewer than 30 colonies are not acceptable for statistical reasons and more than 300 colonies on a plate are likely to produce colonies too close to each other to be distinguished as individual CFUs (Water Quality Association, 2000).

A limitation of the viable plate count procedure in enumerating bacteria from natural environments is its selectivity. There is no set of incubation conditions and media composition that permits the growth of all bacterial types. The nature of the growth medium and time of incubation condition determines which bacteria grow and thus be counted (Standing Committee of Analysts, 2002). Exclusion of enumerating non culturable bacteria is also a limitation.

##### Total count

Bacteria can be enumerated by direct counting procedure, that is, by counting without the need to first grow the cells in culture. These procedures generally count all bacterial cells whether they are viable or not (Prescott *et al.*, 2005). Special counting chambers such as a hemocytometer or Petroff-Houser chamber are used. These chambers are ruled with squares of known area and constructed so that a film of liquid of known depth can be introduced between the slide and the coverslip. Consequently the volume of the liquid overlying the each square is known. To visualise bacterial cell, the cells are often stained (Fewtrell and Bartram, 2001).

Another approach in total counting is the use of electronic particle counter such as a Coulter counter. This instrument can register the magnitude and duration of the change in conductivity of a suspension of bacterial cell as they pass through a small



orifice and thus can register and record the number and distribution of the size of a cellular population. Such instrument permit a discrimination of particles and the size of bacteria can be counted automatically (Weenk, 1992).

Analyses of biological quality of water involve enumeration of heterotrophic bacteria, total coliform and faecal coliform. Each technique used in the enumeration of microorganisms is informed by the media used and the time of incubation. The temperatures engaged range between 20 °C and 40 °C, incubation times from hours to several days or weeks, with nutrient conditions from low to high (Standing Committee of Analysts, 2002).

#### 2.4.2 Physicochemical quality

Physicochemical analysis includes test for colour, turbidity and for toxins and elements which may be harmful to human beings.

##### Colour

The apparent colour of water is strongly related to turbidity. Most natural causes of colour are associated with the presence of dissolved organic matter, and iron and manganese compounds (Dey, 2005). Other metal ions may on occasion give rise to colour in water. Colour may be measured in Platinum-Cobalt (Pt-Co) colour units, using the visual colour comparison method, or by spectrophotometric methods, where the colour characteristics are reported as: Dominant wavelength (nm) and Purity (%) (Chavan *et al.*, 2004). The scale for Pt-Co colour goes from 0 to 500 in units of parts per million (ppm) of platinum cobalt to water. Zero on this scale represents distilled water (Hazen, 1896).

##### Turbidity

Turbidity in water is caused by the presence of suspended matter which usually consists of a mixture of inorganic matter, such as clay and soil particles, and organic matter. Turbidity is measured using a nephelometer in nephelometric turbidity units

(NTU). Turbidity is determined on the day of sample collection or samples may be stored in the dark for 24 hours, after which irreversible changes in turbidity may occur. Samples must be shaken vigorously before examination (Sobsey, 2002).

## pH

The pH of natural waters is influenced by various factors and processes, including temperature, discharge of effluents, acid mine drainage, acidic precipitation, runoff, microbial activity and decay processes. Water pH is measured electrometrically using a pH meter.

## Hardness

Total hardness is expressed as mg CaCO<sub>3</sub> /L. Total hardness is calculated from the calcium and magnesium concentrations (Skipton and Dvorak, 2009) as follows: Total Hardness = 2.50 x calcium conc. (mg/L as Ca<sup>2+</sup>) + 4.12 x magnesium conc. (mg/L as Mg<sup>2+</sup>).

## Total suspended solids

The total suspended solids concentration is determined either by the volumetric method or the gravimetric method. The volumetric method is preferentially used and excludes floating matter, whereas the gravimetric method includes floating matter (SAWQG, 2005). With the volumetric method, a well-mixed water sample is allowed to settle and the suspended matter is reported in units of mg/L while with the gravimetric method the solids are determined by filtering water through a pre-weighed glass fiber and water sample filtered through the filter and the glass fiber filter is dried and weighed again. Total suspended solid (TSS) is calculated as

$$\text{TSS (mg/L)} = (A-B) \times 1000/C,$$

-where A = final weight of membrane after filtration, B = initial weight of membrane before filtration, C = volume of water sample filtered (USEPA, 2001).

## Total dissolved solids

The total dissolved solids (TDS) test measures the amount of various inorganic salts dissolved in water. The TDS concentration is directly proportional to the electrical conductivity (EC) of water. Since EC is much easier to measure than TDS, it is routinely used as an estimate of the TDS concentration (SAWQG, 2005). TDS is measured in mg/L and it is equivalent to EC which is expressed in milli-Siemens per metre (mS/m), The TDS concentration can be measured as an estimate of the EC value or the dry weight of the salts after evaporation of a known volume of filtered water.

## **2.5 Influence of mines on natural water resources**

The type of wastewater contamination produced by a mining operation depends to a large extent on the nature of the mineralization and on the processing chemicals used to extract or concentrate minerals from the host rock.

### 2.5.1 Surface and ground water contamination by mining effluents

Heavy metal pollution is caused when such metals as arsenic, cobalt, copper, cadmium, lead, silver and zinc contained in excavated rock or exposed in an underground mine come into contact with water. Metals are leached out and carried downstream as water washes over the rock surface (Environmental Mining Council, 2000). Although metals can become mobile in neutral pH conditions, leaching is particularly accelerated in the low pH conditions (National Academy of Sciences, 1999). Processing chemicals pollution occurs when chemical agents such as cyanide or sulphuric acid used by mining companies to separate the target mineral from the ore spill, leak, or leach from the mine site into nearby water bodies (Mc Clure and Schneider, 2001). These chemicals can be highly toxic to humans and wildlife.

### 2.5.2 The effects of contamination by wastewater from platinum mines

The impact of mining on the environment varies greatly, depending on the methods employed to exploit the mineral deposit, the type and extent of the mineral resources, pollution control, the waste management and proper reclamation.

Acid mining drainage is probably the most severe environmental problem that occurs on mine sites. It happens where mineral and coal deposits contain sulphide minerals. When waste rock containing sulphides is exposed to air, these minerals are oxidized, releasing sulphuric acid (Economopoulos, 1993). The process is accelerated by bacteria such as *Thiobacillus ferrooxidans* that obtain energy from the oxidation reaction for their growth (Weeks and Wan, 2000). The release of acid can cause the pH of surface water and groundwater to become very low. Under these acidic conditions, metal concentrations in water can become very high due to the dissolution of elements from waste rock (Fallon *et al.*, 2002). The generated acid leaches and releases heavy metals such as lead, zinc, copper, arsenic, selenium, mercury and cadmium. High concentrations of iron and aluminium are also common in acid sulphate waters (Bigham *et al.*, 1996). In acid sulphate drainage waters and leachate solutions from mine waste tailings and soils, solubility appears to be controlled by a variety of basic aluminium/iron oxides, oxyhydroxides and sulphate phases (Karathanasis *et al.*, 1988; Monterroso *et al.*, 1994). Acidic water at mine sites often kills vegetation, and may cause fish deaths in rivers. Of the chemicals used to process ores, cyanide may be the most problematic due to its toxicity and the complexity of its chemical behaviour in groundwater (Economopoulos, 1993). Cyanide degrades rapidly into nontoxic chemical compounds when exposed to air and sunlight, but in groundwater it may persist for long periods with little or no degradation. Cyanide in the subsurface can react with minerals in soil and rock to form a wide range of metal cyanide complexes, many of which are very toxic (Weeks and Wan, 2000).

### 2.5.3 Determination of contaminants from mining effluents in water

Atomic absorption spectrometry (AAS) is an analytical technique that measures the concentrations of elements. Atomic absorption is sensitive that it can measure down to parts per billion of a gram ( $\mu\text{g dm}^{-3}$ ) in a sample. The technique makes use of the wavelengths of light specifically absorbed by an element. The wavelengths

correspond to the energies needed to promote electrons from one energy level to another, higher energy level. Atomic absorption spectrometry is used for environmental analysis such as measuring the levels of various elements in rivers, seawater, drinking water and, air(<http://faculty.rmu.edu/~short/chem3550/chem3550-references/RSC-AA-Leaflet.pdf>, 2015).

Atomic absorption spectrometry is widely used for determination of heavy metals and trace elements in different soil and water samples. Several studies have shown that the AAS is a reliable technique for detection of heavy metals. Adefemi and Awokunmi (2010) used this technique to detect levels of zinc (Zn), chromate (Cr), lead (Pb), copper (Cu), nickel (Ni) and iron (Fe) in river water. Le *et al.*, (2013) used the AAS technique to detect free heavy metal ion concentration of  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  in soil samples. Srikanth *et al.*, (2013) were able to analyse Pb, Cd, Cu, Zn and Hg in industrial effluent using AAS. Onder *et al.*, (2007) also used the AAS for analysis of heavy metals in grass and soil which included Pb, Cu, Zn, Co, Cr, V, Cd, and Ni.

Another method that is widely used for heavy metals detection is the induced coupled plasma mass spectroscopy (ICP-MS). The technique is based upon the spontaneous emission of photons from atoms and ions that have been excited in a refractive discharge (Voica *et al.*, 2009). Analyte elements are liberated as free atoms in the gaseous state (Matthias and Uwe, 1994) which are subsequently promoted to excited states and emit photons at ground state. These photons have characteristic energies that are determined by the quantized energy level structure for the atoms or ions (Moor *et al.*, 2001). Thus the wavelength of the photons can be used to identify the elements from which they originated. The total number of photons is directly proportional to the concentration of the originating element in the sample. This technique can detect metals such as Al, As, Cd, Cr, Cu, Mn, Ni, Pb, Zn, Hg and Cd according to (Matthias and Uwe, 1994; Voica *et al.*, 2009. Matthias and Uwe (1994) were able to detect Al, As, Cd, Cr and Cu in ground water samples.

A Nanocolor UV/VIS spectrophotometer is also used for the physicochemical and metal analysis of water samples (Kale *et al.*, 2010). It uses light in the visible and adjacent (near-UV and near-infrared [NIR]) ranges. The absorption or reflectance in the visible range directly affects the perceived colour of the chemicals involved. It

measures the intensity of light passing through a sample and compares it to the intensity of light before it passes through the sample.

## **2.6 Aim and Objectives**

The current study seeks to determine the bio-physicochemical quality of surface and ground waters for human use in the Tubatse municipality and to further assess the influence of mining activities on the quality of these water sources.

The objectives of this study include the following:

- i. Enumeration of coliform and heterotrophic bacteria in ground and surface waters.
- ii. Determination of the physical (turbidity and colour) and chemical (pH, DS, SS, properties of surface and ground waters.
- iii. Determination of the heavy metals, namely Zn, [SCN-] Cr, Co, Fe, Ni, SO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub>, Cl<sub>2</sub> in the surface and ground waters.
- iv. Determination of the heavy metals Zn, [SCN-] Cr, Co, Fe, Ni, SO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub>, Cl<sub>2</sub> in soil and river sediments.
- v. Detection and identification of the dominant bacterial contaminants.
- vi. Determination of the effect of detected chemicals and metals on human liver and kidney cell lines.

## **Chapter 3**

### **3. MICROBIOLOGICAL QUALITY OF GROUND AND SURFACE WATER**

#### **3.1 Introduction**

Microorganisms in drinking water are responsible for most waterborne disease outbreaks (UNEP/WHO, 1996). Organisms known to cause disease include bacteria, protozoa, and viruses; they thrive in the intestines of warm-blooded animals. They are transmitted to drinking water bodies through human and animal excreta.

It is very difficult, expensive, and time-consuming to directly test for individual bacterial pathogens in the water; indicator microorganisms are used instead. Indicator microorganisms such as heterotrophic bacteria, total coliform and faecal coliform are used to indirectly indicate the possible presence of pathogens in the water. Heterotrophic bacterial counts are used to indicate the general microbial quality of water. Heterotrophic plate count (HPC) procedures refer to a variety of simple culture-based tests that are intended to recover a wide range of microorganisms that feed on organic matter in water (Fewtrell and Bartram, 2001). Microorganisms recovered through HPC tests generally include those that are part of the natural (typically non-hazardous) microbiota of water; in some instances, they may also include organisms introduced due to lack of pollution. Though total coliforms are normally found in surface water and soil, however, faecal coliforms are found in the faeces of warm-blooded animals and their presence in water and soil indicate the potential presence of pathogenic microorganisms.

#### **3.2 Materials and methods**

##### **3.2.1 Sample collection**

Sterile 1L bottles were used to collect surface water samples from the Motse, Moopetsi and Moopetsi-Motse rivers and from the HcN, TwH and MrL streams, ground water samples from the Moopetsi and Serafa springs were also collected into sterile 1L bottles. Water samples were transported on ice to the Microbiology laboratory at the University of Limpopo and were processed within 6 hours of

collection. These samples were collected during the month of June. The analysis was performed in duplicates for every sample point.

### 3.2.2 Microbial enumeration

#### 3.2.2.1 Heterotrophic bacterial count

Membrane filtration (MF) technique was used for HPC analysis. Hundred millilitres of  $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$  dilutions of surface and ground waters were vacuum-filtered through a 0,45  $\mu\text{m}$  membrane filter (Pall Corporation) using a water filtration unit. The membrane filters were placed on R<sub>2</sub>A agar plates and incubated at 25 °C for 7 days and the duplicates were placed on nutrient agar (NA) plates which were incubated at 37 °C for 48 hours (WHO, 2003). Colonies were counted manually and different colonies, according to dominance, colour and morphological characteristics were picked and Gram-stained. Pure cultures were prepared and stored on TSA slants

#### 3.2.2.2 Total coliform count

Water samples were serially diluted at  $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$  with sterile distilled water and 100 mL thereof were filtered through 0,45  $\mu\text{m}$  filter membranes. Filter membranes were incubated on lauryl sulfate broth (Sigma-Aldrich) for 2 hours at 37 °C. Following incubation, the filter membranes were placed on m-Endo agar (Sigma-Aldrich) plates and incubated at 35 °C for 24 hours (UNEP/WHO, 1996). Pink colonies and metallic sheen colonies on m-Endo agar plates were enumerated, few well isolated colonies were picked and Gram stained and preserved on TSA slants.

#### 3.2.2.3 Faecal coliform count

m-FC medium containing rosolic acid (Sigma-Aldrich) was prepared for enumeration of faecal coliform. Hundred millilitres of the dilutions,  $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$  of each water samples were filtered through a 0,45  $\mu\text{m}$  filter membrane; filter membranes were placed on m-FC agar (Sigma-Aldrich) plates and incubated at 44.5 °C for 24 hours.



(UNEP/WHO, 1996). Blue colonies were enumerated as presumptive *E. coli* and were Gram-stained and stored on TSA slants

### 3.2.3 Biochemical characterisation of bacteria

Biochemical characterisation was performed to assist in the classification and identification of the bacterial isolates.

#### 3.2.3.1 Lactose fermentation test

Lactose test was performed to differentiate between groups of Gram negative rods that produce  $\beta$ -galactosidase enzyme. The organisms were inoculated in phenol red lactose broth containing lactose (Sigma) with inverted Durham tubes and then incubated at 35 °C for 48 hours. The medium changes to yellow when an acid is produced and a bubble is formed in the Durham tube when the organism ferments the lactose. In the absence of an acid the media remains red (Madigan *et al.*, 2009).

#### 3.2.3.2 IMViC tests

IMViC tests were performed for Gram negative rods which tested positive for lactose utilization. IMViC tests were used to differentiate between *Enterobacteriaceae* and the tests included indole, methyl red, Voges Proskauer and citrate tests.

#### Indole test

The bacteria were inoculated by stabbing on SIM media containing the amino acid tryptophan (Fluka) and the culture was incubated at 37 °C for 24 hours. Following incubation, Kovac's reagent (Sigma) was added to indirectly detect the presence of tryptophanase, an enzyme which hydrolyses and reduces tryptophan to indole as a by-product (Madigan *et al.*, 2009).

### MR-VP test

The organisms were inoculated into the MR-VP broth containing peptone (Fluka) and were incubated for 24 hours at 37 °C. After incubation methyl red indicator (Sigma) was added to the MR-VP media tubes allocated for the methyl red test. The broth commonly turns red within few seconds in the presence of an acid. For the Voges Proskauer test; 4 mL of 40 % of KOH (Fluka) and 3 drops 0.3 % creatin which turns the broth to pink in the presence of acetyl methyl carbitol was added to the MR-VP media tubes set aside for the VP test (Madigan *et al.*, 2009).

### Citrate test

The organisms were inoculated onto the Simmon's citrate agar (Sigma) plates which were incubated for 24 hours at 37 °C. After incubation growth was examined and it was indicated by blue colour. The media changed from green to blue when the pH increased to pH 7.6 due to the alkaline carbonates and bicarbonates produced during citrate catabolism (Madigan *et al.*, 2009).

### 3.2.3.3 SIM test

The SIM test was used to determine hydrogen sulfide (H<sub>2</sub>S) production, indole formation, and motility. SIM medium is used to differentiate members of the family *Enterobacteriaceae*. Bacteria which tested negative for lactose utilization were further tested for motility and production of H<sub>2</sub>S on SIM media (Fluka). Positive reaction was indicated by a black precipitate of ferrous sulphite (FeS) formed when H<sub>2</sub>S reacts with FeSO<sub>4</sub> following 24 hours incubation period at 37 °C. SIM media was also used to test for motility, i.e., to identify the organisms that have flagellae. The test organisms were stabbed on SIM media and incubated at 37 °C for 24 hours, and microbial motility was indicated by organisms growing away from the stabbing line (Madigan *et al.*, 2009).

#### 3.2.3.4 Catalase test

Gram positive cocci were tested for catalase production. The bacterial isolates were grown on NA and incubated at 37 °C for 24 hours. After incubation, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added to a colony on NA. Catalase production by the microorganisms was indicated by the formation of air bubbles when H<sub>2</sub>O<sub>2</sub> was added to a colony on nutrient agar (Madigan *et al.*, 2009).

#### 3.2.3.5 Bacterial identification

API 20E kit (Biomérieux) was used for identification of Enterobacteriaceae. Gram negative rods were inoculated on the API 20E kit and incubated for 24 - 48 hours. Following incubation, observations and identification were made following the manufacturer's instructions (Madigan *et al.*, 2009).

#### 3.2.3.6 Automated API system

The VITEK 2 system is a fully automated in vitro testing system. A suspension of a pure culture was prepared; the appropriate card (test kit) was selected. The card and the suspension were placed in the cassette which was placed in the VITEK incubator. The VITEK system analysed the card as growth of the organism occurs and gives an identity of the organism.

### **3.3 Results**

All natural waters can be populated by transient bacteria. Among these are the human pathogens that gain entry to water from faecal contamination. The results below show the microbial quality of the ground and surface water in the Tubatse municipality in the Sekhukhune district.

#### 3.3.1 Bacteriological analysis of ground and surface water

All the surface water samples were contaminated with heterotrophic and coliform bacteria. Ground water commonly had lower microbial load with regard to heterotrophs and coliforms when compared to surface water. Figure 3.1 shows the level of bacterial contaminants in the water samples.

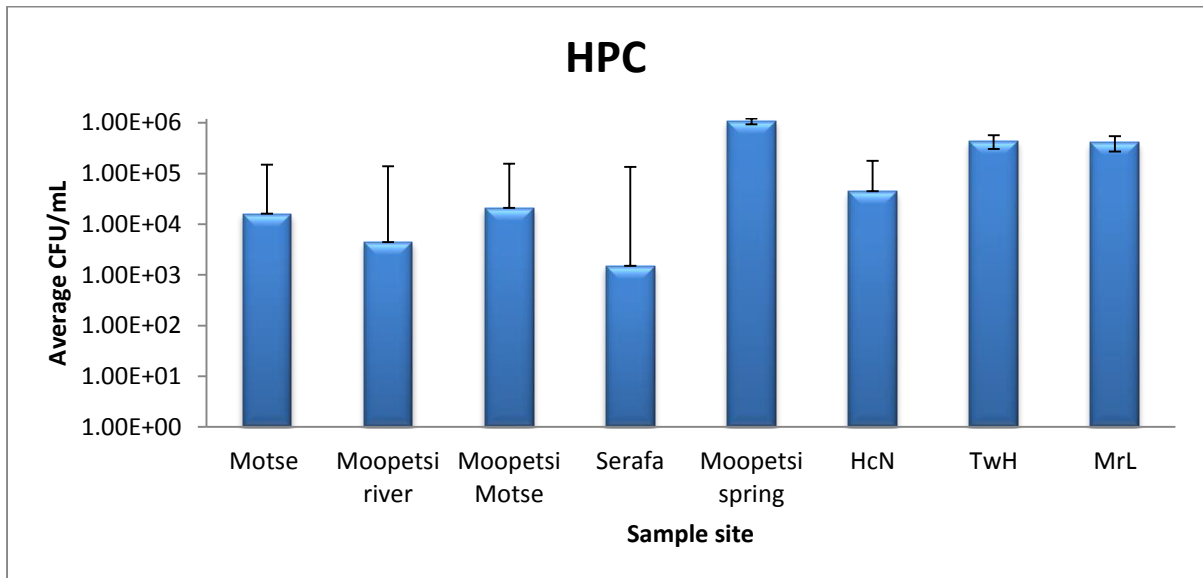


Figure 3.1: The level of heterotrophic bacteria in the water samples

Figure 3.2 Illustrates the level of coliforms in surface and ground water, with the Serafa spring showing zero coliforms.

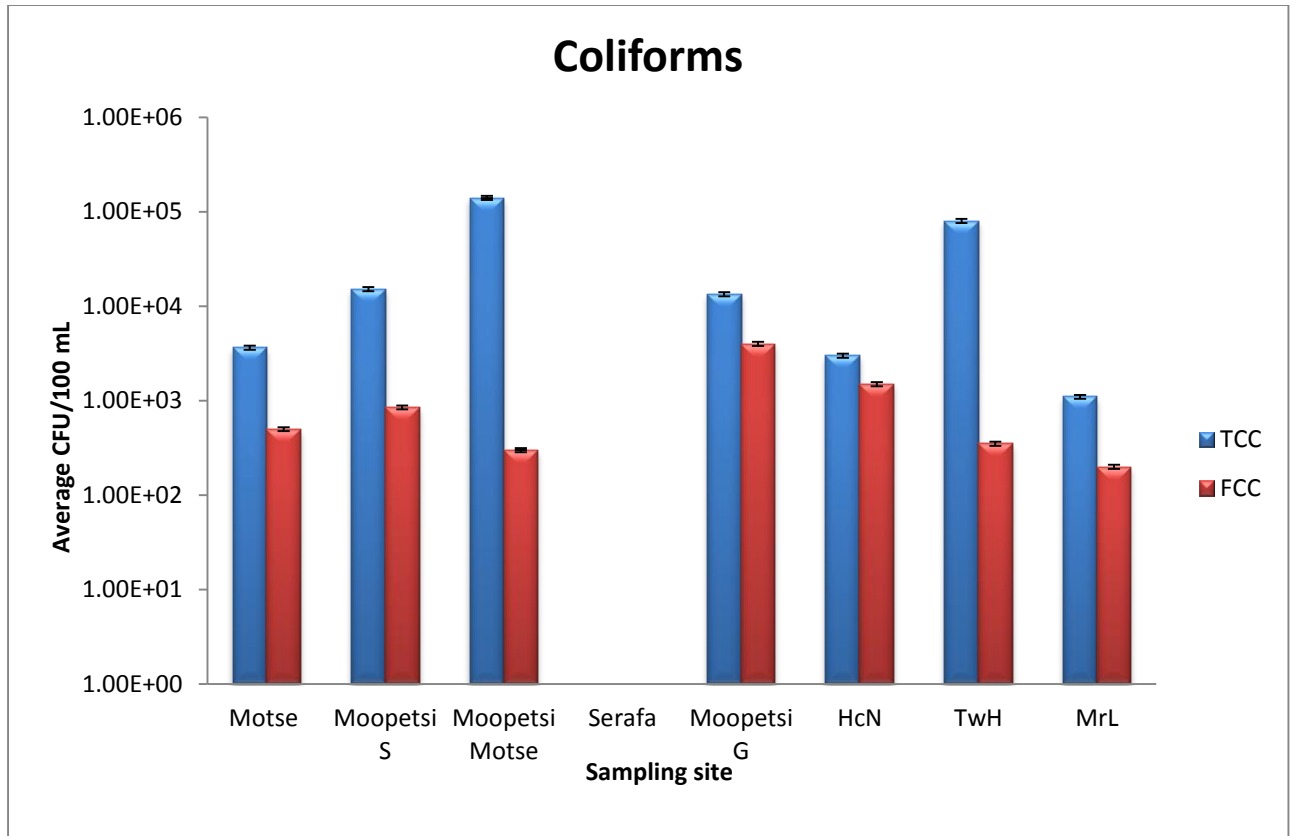


Figure 3.2: The level of coliform bacteria in the water samples.

Key: Moopetsi S =surface water

Moopetsi G = groundwater

### 3.3.2 Bacterial identification

Biochemical assays and API systems were used for characterization and identification of the dominant bacterial isolates. *Enterobacter* spp. were predominant in surface waters, followed by *Bordetella* spp. The identities of bacterial isolates are shown in Table 3.4 below. These organisms are commonly found in soil, water and normal gut of humans and are associated with health risks such as bacteraemia, meningitis, endocarditis, peritonis in humans and animals.

Table 3.1: Bacteria isolated from ground and surface water in the Tubatse Municipality

Bacterial Identity	Health risks
<i>Aeromonas hydrophila</i>	It is very toxic, it releases a toxin that can cause tissue damage
<i>Pseudomonas putida</i>	An opportunistic human pathogen.
<i>Pseudomonas luteola</i>	It is an opportunistic pathogen that can cause bacteraemia, meningitis, prosthetic valve endocarditis, peritonis in humans and animals
<i>Cronobacter sakazakii</i>	In infants it can cause bacteraemia, meningitis and necrotising enterocolitis
<i>Acinetobacter haemolyticus</i>	Can cause infection in debilitated patients in the hospital
<i>Enterobacter sakazakii</i>	Opportunistic pathogen that currently contaminates a wide spectrum of foods and poses a lethal threat to neonates, the elderly and persons with immune deficiencies
<i>Pantoea</i> spp.	Cases of septic (bacterial) arthritis and osteomyelitis (inflammation of the bone) caused by <i>Pantoea agglomerans</i> have developed after plant thorn injuries and wood splinter injuries. Most serious infections have occurred in persons with diminished host defenses. Meningitis of the newborn has also been documented ( <a href="http://www.gopetsamerica.com/bio/bacteria/pantoea.aspx">http://www.gopetsamerica.com/bio/bacteria/pantoea.aspx</a> )
<i>Enterobacter cloacae</i>	It is sometimes associated with urinary tract and respiratory tract infections
<i>Serratia odifera</i>	<i>Serratia</i> infection is responsible for about 2 % of nosocomial infections of the bloodstream, lower respiratory tract, urinary tract, surgical wounds, and skin and soft tissues in adult patients
<i>Kocuria rocea</i>	Has been implicated in occasional urinary tract infections in immunocompromised patients

<b><i>Streptococcus thoratens</i></b>	Unknown
<b><i>Streptococcus agalactiae</i></b>	<i>S. agalactiae</i> can be transferred to a neonate passing through the birth canal and can cause serious group B streptococcal infection
<b><i>Acinetobacter baumannii</i></b>	It is an opportunistic pathogen in humans, nosocomial infection.
<b><i>Bordetella spp.</i></b>	Can cause pertussis or whooping cough in humans and causes several diseases in other mammals, including kennel cough and atrophic rhinitis in dogs and pigs
<b><i>Acinetobacter calcaceticus</i></b>	Can be pathogenic and cause an opportunistic infection in patients with multiple underlying diseases

### 3.4 Discussion

South Africa is faced with a serious challenge of shortage of potable water. The most affected areas are the rural communities in the Limpopo province. These communities have now resorted to ground and surface water for drinking and household purposes. The quality of these water resources is not monitored. Several studies have shown that water is a source of various waterborne infectious diseases affecting numerous communities, particularly those in rural areas (Venter, 2001; Momba *et al.*, 2006; Murcott *et al.*, 2007). The current study investigated the microbial quality of the surface and ground water in the Tubatse municipality, in the Sekhukhune district. These water sources are used by the communities for drinking, bathing and other household uses.

Surface water was expectedly laden with high number of bacteria when compared to ground water, hence did not comply with the water quality standard set by SABS and WHO. Human and animal activities such as bathing in the rivers and cattle feeding, as witnessed during sampling, contribute to these high levels of microbiota in surface waters. Similar observations on high levels of bacterial contamination were reported in literature (Palupi *et al.*, 1995; Nevondo and Cloete, 1999; Obi *et al.*, 2002) and anthropogenic activities such as poor sanitary practices and domestic animal were reported as possible sources of contamination of the surface water (Nevondo and Cloete, 1999).

A study by Gao *et al.* (2003) indicated that the Proteobacteria are the dominant microbiota followed by Actinobacteria and Firmicutes on human skin. These phyla are also apparently present in the human gut and to a lesser extent in human faeces.

The bacterial isolates of surface water obtained in this study belong predominantly to the three phyla. The Proteobacteria include the genera *Aeromonas*, *Pseudomonas*, *Cronobacter*, *Acinetobacter*, *Enterobacter*, *Pantoea*, *Serratia* and *Bordetella*. *Kocuria* belong to Actinobacteria and *Streptococcus* to the Firmicutes. These clearly point to the dominance of Proteobacteria in surface waters and it is proportionately similar to the bacterial dominance pattern reported on human skin and of gut biota, although there is disparity in the type of genera observed in this study and those reported in literature. Bacteria such as *Pseudomonas* and *Streptococcus* are commonly known skin flora while *Enterobacter* and *Serratia* spp. are common human gut flora.

Ground water naturally does not contain coliforms. However, high levels of coliform bacteria were observed in the Moopetsi spring. This could be attributed to the sub-surface leaching of faecal material from sanitation facilities, such as pit latrines, improper disposal of wastes and also contamination from animal wastes. This potentially suggests that the soil in this area has become ineffective in removing bacterial contaminants since the area is dominated by pit latrines. Although the majority of the organisms isolated from the water can naturally be found in water and soil, they are associated with various health risks which are indicated in Table 3.1. These organisms include *P. putida*, *P. luteola*, *C. odifera*, *A. hydrophila* and *K. rocea*. The potential risk of diseases from consumption of this contaminated water is high and to mitigate the health impact of water-borne diseases in areas such as the ones under study, the surface and ground water could not be consumed without treatment. This study and others studies on domestic use and consumption of surface water in rural communities showed the inherent health challenges due to the presence of pathogens which render the waters not potable for human use (Obi *et al.*, 2002; Zvidzai *et al.*, 2007; Sabae and Rabeh, 2007), however, the waters are used without any household treatment due to lack of knowledge about potable water quality and no financial and material resources for household water treatment.



## Chapter 4

### 4. PHYSICOCHEMICAL PROPERTIES OF GROUND AND SURFACE WATER

#### 4.1 Introduction

Water quality and human health is threatened by unsanitary conditions through open drains which dispose wastewater into natural water bodies. According to WHO (2005), about 80 % of all the diseases in human beings are caused by water pollution. Natural water contains different types of impurities that are introduced into aquatic system by different ways such as weathering of rocks and leaching of soils, dissolution of aerosol particles from the atmosphere and from various human activities, including mining, processing and the use of metal based materials (Ipinmoroti and Oshodi, 1993 and Adeyeye, 1994). People lacking treated water are under serious threat due to undesired changes in the physical and chemical characteristics of ground and surface waters which they rely on for their household needs. In industrialised areas untreated effluents commonly pollute water and this tends to pollute the sediments and soil as well (Onianwa and Fakayode, 2002 and Fakayode, 2005).

#### 4.2 Materials and Methods

##### 4.2.1 Sample collection

Water samples were collected in sterile water bottles as highlighted in section 3.2.1. These samples were collected once in the dry season (month of June) and once in the wet season (October).

**N.B:** Two samples were collected from the HcN stream, one sample labelled HcN A (collected 25 M from the mine) and the other HcN B (collected 100 M from the mine)

##### 4.2.2 Physical properties of ground and surface water

###### 4.2.2.1 Total suspended solid

Glass fiber filters (Whatman) were weighed and the weights were recorded. Hundred millilitres of the water sample was vacuum filtered through a glass fiber filter membrane. After filtration the filter was dried at 105 °C and cooled to ambient temperature. The filters were weighed again and the weights were recorded. Total suspended solid (TSS) was calculated as:

$$\text{TSS (mg/ L)} = (A-B) \div C \times 1000,$$

where A = final weight of membrane after filtration, B = initial weight of membrane before filtration, C = volume of water sample filtered (APHA, 1992).

#### 4.2.2.2 True colour

A Nanocolor UV-VIS spectrophometer (Machenery-Nagel) was used to measure the true colour following the manufacturer's instructions (Standard Methods for the Examination of Water and Wastewater.1975).

#### 4.2.2.3 pH

pH was determined using test strips (Quantofix).

#### 4.2.2.4 Turbidity and Hardness

A Nanocolor UV-VIS spectrophometer (Machenery-Nagel) was used to measure the turbidity and hardness following the manufacturer's instructions (Niskanen *et al.*, 2006).

### 4.2.3 Chemical properties of ground and surface water

#### 4.2.3.1 Total dissolved solid

A gravimetric method was used to determine the total dissolved solids (TDS) in ground and surface water. A clear dry glass beaker was weighed and 100 mL of the water sample was poured into the beaker. The water was evaporated by gentle

heating. The beaker containing a precipitate was cooled and weighed again. TDS was calculated as:

$TDS \text{ (mg/L)} = (\text{weight of dish + solids in mg} - \text{weight of dish}) \div \text{sample volume dried in mL} \times 1000$  (Singh, and Kalra, 1975).

#### 4.2.3.2 Determination of metal and chemical content of water

A Nanocolor UV-VIS spectrophotometer (Machenery-Nagel) was used to screen for the presence of thiocyanate, chromate and chlorine in natural waters. Test kits (Quantofix) were used to detect and measure the quantities of cobalt, copper, peroxide, total iron, nickel and zinc found in the water (Kale *et al.*, 2010).

### 4.3 Results

#### 4.3.1 Physical properties of ground and surface water

##### 4.3.1.1 Physical properties of ground and surface water in the dry season

The level of suspended particles in ground and surface waters, as shown in Table 4.1, is generally low and conforms to the standards for drinking water (WHO, 2005 and SAWQG, 2005), while measures for turbidity and colour were high for the HcN stream.

Table 4.1 Physical properties of ground and surface water in the dry season

Sample	Turbidity (FAU)	Colour (1/m)	TSS (mg/L)
Motse river	1	0.2±0.1	0
Moopetsi river	<1	0.3±0	0.002
Moopetsi-Motse river	1	0.4±0	0.012
Moopetsi spring	<1	<0.2±0	0.012
Serafa spring	<1	<0.2±0	0.008
HcN stream	90	16.8±0.9	0.022
TwH stream	<1	<0.2±0	0.008

MrL stream	<1	0.6±0.07	0.003
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#### 4.3.1.2 Physical properties of surface water in the wet season

A change in the physical quality of water was observed in the wet season. There was a non-significant increase in the level of turbidity, colour and TSS in all of the sites as shown in Table 4.2 and Figure 4.1. The level of turbidity at Motse river, HcN stream and MrL stream was found to be above the permissible limits, whereas Moopetsi spring and Moopetsi-Motse were found to be compliant to the limits set by the WHO (2005) and SAWQG (2005) for drinking water.

Table 4.2 Physical properties of surface water in the wet season

Parameter	Turbidity (FAU)	Colour (1/m)	TSS (mg/L)	Hardness (ppm)
Motse	8	5.5 ± 0.7	0.02	14.6
Moopetsi S	<1	2.7 ± 0.4	0.02	15.9
Moopetsi-Motse	3	0.6 ± 0.14	0.003	9.8
HcN A	>100	>20	1.62	15.2
HcN B	36	4.85 ± 0.1	0.73	19.2
TwH stream	5	1.5 ± 0.14	0.01	1.1
MrL stream	17	4.5 ± 0	0.01	>20

#### 4.3.2. Chemical and metal properties of ground and surface water

##### 4.3.2.1 Chemical and metal properties of ground and surface waters in dry season

The concentrations of thiocyanate, zinc, iron and cobalt detected in the water samples exceeded the normal expected concentrations of < 3.5 µg/L, 0.5 mg/L, < 0.01 mg/L and < 5 µg/L respectively according to the South African drinking water quality guidelines (SAWQG, 2005). Chromium was found to be above the set permissible limit of 0.050 mg/L set for drinking water, whereas TDS was found be

within the permissible limits (SAWQG, 2005). Peroxide was only detected in the Moopetsi-Motse and HcN stream which are both surface waters. The concentrations of thiocyanate, TDS, Cl<sub>2</sub> were higher in surface water than in ground water sample. The levels of iron, zinc and cobalt were found to be the similar in both ground and surface water at the concentration of 2 and 10 mg/L respectively, as shown in Figure 4.1 below.

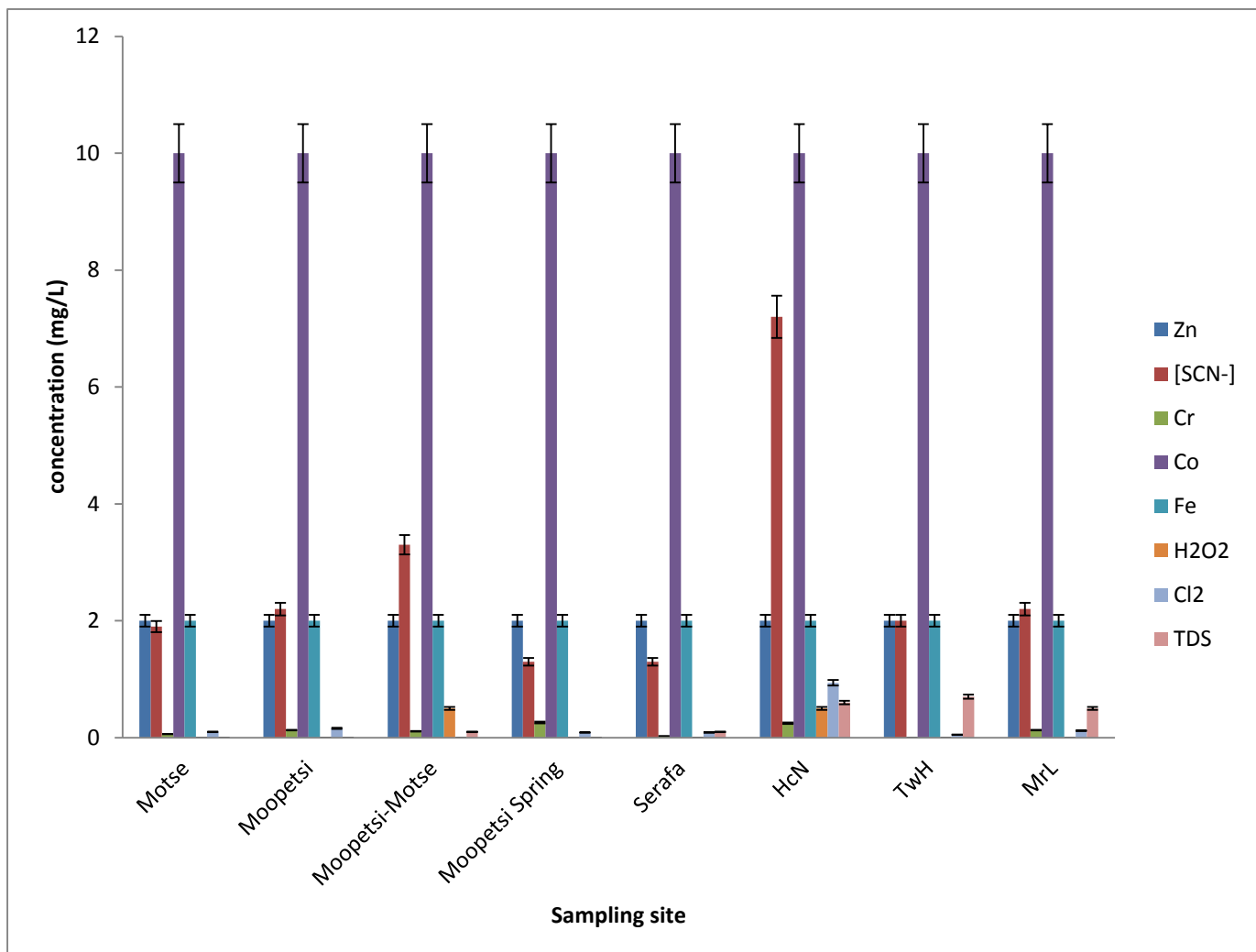


Figure 4.1 Chemical and metal properties of surface water in the dry season

#### 4.3.2.2 Chemical and metal properties of ground and surface waters in wet season

Fluctuations between the dry and wet seasons were observed. A non-significant decrease in the levels of Cl<sub>2</sub> and [SCN<sup>-</sup>] and an increase in TDS and Cr were

observed in the wet season. The concentration of zinc and iron were found to be constant in all the sites as shown in Figure 4.2 below.

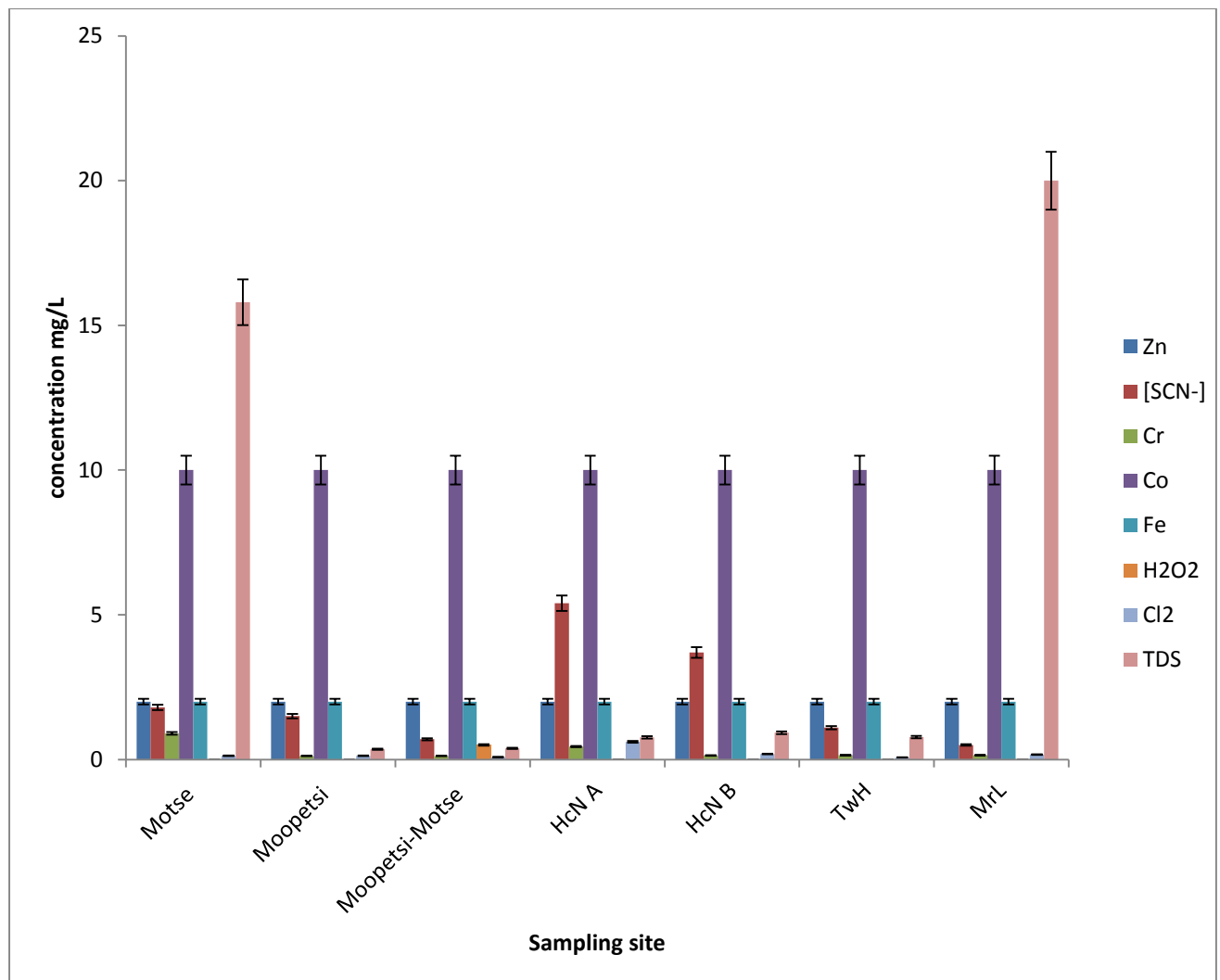


Figure 4.2 Chemical and metal properties of surface water in the wet season

pH is a term used universally to express the intensity of the acid or alkaline condition of a solution. Most of the surface water samples were slightly alkaline due to presence of carbonates and bicarbonates (Simpi *et al.*, 2011). The pH values of the majority of the water samples were found to be within the limit prescribed of between 6.5 - 8.5 by WHO (2005) as shown in Table 4.3 below.

Table 4.3 pH of the water samples

	Motse river	Moopetsi river	Moopetsi-Motse river	Moopetsi spring	Serafa spring	HcN stream	TwH stream	MrL stream
<b>Dry season</b>	9	8	8	8	7	8	8	8
<b>Wet season</b>	8	8	8	-	-	8	8	9

Key: - not determined

#### 4.4 Discussion

The physical quality of the water in Sekhukhune was generally good according to the permissible limits set by the WHO (2005) and SAWQG (2005) for drinking water. The HcN stream showed to have the highest level of turbidity, colour and TSS in both seasons. An increase in concentrations from the dry to the wet season in all the parameters was observed, this is contrary to the expectation that chemical and metal concentrations will be low in wet season due to the dilution effect as a result of rain. This was observed in a study by Dey *et al.*, (2005). However, other studies such as Pritchard *et al.*, (2005) and Moshood (2008) have reported on the increase in turbidity and TSS in the wet season. This increase in the above mentioned physical parameters may be due to improper disposal of waste materials and increased human, animal and mining activity. An increase in activities such as river bathing, water collections and cattle visits to the rivers is to be expected during wet seasons when there is more water in the rivers and streams than during dry seasons. Turbidity and colour do not have health implications but associated effects due to microbial contamination, or the ingestion of substances bound to particulate matter or presence of toxic chemicals that give off colour in solution can be harmful to humans. Microbial growth in water is most extensive on the surface of particulates; river silt also readily adsorbs viruses and bacteria (SAWQG, 2005). Consumption of highly turbid water that has elevated colour concentration may therefore pose a health risk to the consumers. According to the American Public Health Association (APHA, 1992), colour is strongly influenced by the presence of iron, manganese and

other metals either as natural impurities or corrosion products. A study by Kristzberg and Ekstrom (2011) showed that iron can explain variation in water colour (on average, 25 %), especially in surface water. Other studies such as (Canfield *et al.*, 1984; Pennanen and Frisk, 1984; Heikkinen and Ihme, 1995; Maloney *et al.*, 2005) have reported correlations between Fe and water colour. The concentration of iron obtained in the Sekhukhune district at all sampling points was found to be 2 mg/L, which is sufficient to produce colour in water noting that only 0.3 mg/L of iron is required to produce colour (Environmental health sheet, 2015).

The average concentrations of Zn, [SCN-], Cr, Co, Fe, H<sub>2</sub>O<sub>2</sub>, Cl<sub>2</sub> and TDS in the dry season were 2; 2.7; 0.12; 10; 2; 0.13; 0.2 and 0.25 mg/L respectively. The concentrations of [SCN-], Fe, Zn, Cr and Co were found to be above the normal expected limits. According to the EPA (1981), the mean cyanide concentration in most surface waters is not greater than 3.5 µg/L while Zn is usually below 10 µg/L and in groundwater it is between 10–40 µg/L (WHO, 2003). The concentration of dissolved Fe in unpolluted surface water ranges from 0.001 - 0.5 mg/L (SAWQG, 2005). Chromium is not normally found in water at concentrations greater than 0.01 mg/L (APHA, 1989). The total and dissolved cobalt concentrations in ambient, uncontaminated environments are generally low, found at < 5 µg/L (Shi *et al.*, 2013). The rises in the levels of the above mentioned chemical and metal properties may be from anthropogenic and natural sources.

Thiocyanate is released to water primarily from discharges of industrial waste waters from coal processing and extraction of gold, platinum and silver (Boucabeille *et al.*, 1994). The thiocyanate is formed from the reaction of sulphur donors that are present in coal and crushed rocks with the cyanide that is used in the processing of these materials and it is also found in mining waste waters where it results from the interaction of the cyanide anion (CN<sup>-</sup>) with sulphur (Boucabeille *et al.*, 1994). The sampling sites are in the surrounds of the platinum mines in the Tubatse municipality and the increased levels of these metals might be due to waste water from processing the ores. The HcN stream had the highest level of thiocyanate, followed by the Moopetsi-Motse river. However, the expectation was that the Motse river would be have higher level of thiocyanate than the Moopetsi-Motse as the HcN stream flows directly into it. The lower level of thiocyanate observed in the Motse river may be due to the entrapment of [SCN-] by the vegetation and wetland



between the HcN stream and Motse river. de Luís *et al.*, (2011) in their study on environmental impact of mining activities reported high levels of heavy metals such as  $\text{SO}_4^{2-}$ , Fe, Al, Cu, Pb, Zn, Cd, As and  $\text{CN}^-$  in the stream water surrounding the mining area, while Conca *et al.*, (2004) reported high levels of Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn, and Atibu *et al.* (2013) of Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Ag, Sn, and Pb which were all discharged from the mines and detected in the water bodies in the surrounding areas of the mines. These reports support the findings in the current study.

Iron is a metallic element found in the earth's crust, water percolating through the soil and rock can dissolve minerals containing iron and this might explain the elevated levels of Fe in the water. Grazulevicien, (2009) states that the quantity of Fe depends on the geology of an area; which explain the constant level Fe obtained in all the sampling sites.

The level of chlorine detected in the samples ranged from  $< 0.02$  to  $0.94$  mg/L in the dry season and  $< 0.05$  to  $0.61$  mg/L in the wet season. An increase in the concentrations of  $\text{Cl}_2$  in Moopetsi river, Moopetsi-Motse river, TwH stream and MrL stream were observed, while a decrease at Motse river was observed. The following water bodies Moopetsi river, TwH stream and MrL stream flows into the Moopetsi-Motse river which would account for the increase in the  $\text{Cl}_2$  in this particular river. The MrL stream is situated relatively closer to the mining plant which may account for the rise in  $\text{Cl}_2$  level as a result of recent contamination by the mining effluents discharged from the plant. Although  $\text{Cl}_2$  concentrations were above the expected levels for ground and surface water, they were found to be in compliance with the set standard of  $250$  mg/L for drinking water. These set limits governs a few countries such South Africa, USA, Canada, India ([http://www.safewater.org/PDF/resources/water\\_quality\\_guidelines\\_info/Regulations\\_Guidelines/comparisons](http://www.safewater.org/PDF/resources/water_quality_guidelines_info/Regulations_Guidelines/comparisons), 2015) and they are also regulated by WHO (2005).

The concentration of chromium in the samples ranged from  $< 0.001$ - $0.45$  mg/L. The Cr detected did not comply with to the set limit of  $0.05$  mg/L (SAWQG, 2005) in all the samples in both seasons. Fluctuations in Cr level in the dry and wet season were observed. Studies by Buggy and Tobin (2008); Griscom *et al.*, (2000); Wang *et al.*, (2005); Karak *et al.*, (2010); Shi *et al.*, (2013) reported that heavy metals accumulate

in sediments, while other researchers reported that heavy metals adsorbed in sediments can be desorbed back into overlying water (Niu *et al*, 2009). This would explain the fluctuations in Cr level observed which result from the change in water levels as seasons change. The increase in Cr level in wet season at Moopetsi river, HcN, MrL and TwH streams may be due to the heavy metal being desorbed back into the overlying water due to the increase of water level as suggested Niu *et al.*, (2009). The changes observed were not significant with the exception of the TwH stream, where Cr concentration increased from  $< 0.01$  which is below the permissible limits to  $0.15$  mg/L which is above the permissible (SAWQG, 2005). Studies by de Luis *et al.*, (2011), Tang *et al.*, (2013) and Resongles *et al.*, (2014) reported an increase in level of Cr in surface water due to contamination by effluents from the mines. These findings support the results obtained in the current study.

Concentrations of cobalt measured in surface and ground water was  $10$  mg/L which is above the normal expected levels. There is no documentation on the acceptable limits for cobalt concentration in drinking water. However, according to previous researchers the concentration of cobalt in surface water ranges between  $4$ - $50$   $\mu\text{g/L}$ . Maximum cobalt concentrations reported by Durum (1960) for Canadian freshwater surface were between  $4.0$  and  $5.1$   $\mu\text{g/L}$  in rivers found in Manitoba. Concentrations of cobalt in the range of  $1$  to  $10$   $\mu\text{g/L}$  were detected in streams close to populated areas, while concentrations in the range of  $11$  to  $50$   $\mu\text{g/L}$  were detected primarily in streams passing through mining districts and regions with heavy agricultural land use (Smith and Carson, 1981). This data suggest that the high concentration of cobalt detected in this study may be due to the mining activities surrounding these water bodies.

Copper and nickel were not detected in all the water samples (results not shown). The concentration of copper in surface waters is usually low, typically,  $0.003$  mg/L (SAWQG, 2005). This explains why copper was not detected in the water samples. However, other studies (Brown *et al*, 1995; Conca *et al.*, 2004 and de Luis *et al.*, 2011) have reported the presence of Cu and Ni in water bodies that surrounded by mines. These might be due to the type of chemicals and methods used in ore extraction. An elevation in concentration of the following parameters; thiocyanate, zinc, iron and cobalt from the expected normal concentrations in the water suggests

contamination by external sources such as effluents from the mining activity and improper waste disposal.

Literature has reported extensively on the effect of mining on the quality of water bodies that surround the mines, the resultant being an increased level of metals in the water and this is also influenced by the changes in seasons. Low concentrations of chemicals and metals were observed during dry season when compared to wet season. Deposition of metals in the sediments is probably high during dry seasons due to stagnant non-flowing low waters, and these metals get dissolved back into flowing waters during wet seasons. The communities depending on this type of water are at risk of developing terminal diseases such as gastrointestinal cancer which can be caused by exposure to high levels of Cr and elevated risk of foetal growth retardation caused by Fe. Based on the above mentioned observations, the water in the Tubatse municipality is unfit for human consumption due to the presence of heavy metals, and this can be attributed to the influence of mining plants around these water bodies as established in literature already.

## Chapter 5

### 5. HEAVY METALS IN SOIL AND SEDIMENTS DETECTION

#### 5.1 Introduction

Heavy metals with low solubility in water are easily adsorbed and accumulated in sediments (Jain and Sharma, 2006; Alvarez *et al.*, 2011; and Ma *et al.*, 2013). Sediments in river beds or lake bottoms therefore often represent a major repository for contaminants discharged into water bodies (Vandecasteele *et al.*, 2002 and Ma *et al.*, 2013). Heavy metals adsorbed in sediments can be desorbed back into overlying water under certain conditions, causing secondary pollution and potentially having toxic effects on organisms (Niu *et al.*, 2009). Heavy metal contamination of sediments can critically degrade aquatic systems (Suresh *et al.*, (2012). The accumulation of heavy metal in sediments is a cause of growing interest and concern to environmental scientists. Environmental problems due to heavy metal pollution of aquatic systems have been extensively documented (Griscom *et al.*, 2000; Buggy and Tobin 2008, Wang *et al.*, 2005; Karak *et al.*, 2010; Tang *et al.*; 2013; Shi *et al.*, 2013). These studies report the contamination of river waters and sediments with heavy metals such as As, Cd, Cr, Pb and Zn.

Heavy metals from industrial processes and mining activities are of special concern because they cause chronic poisoning in aquatic animals (Ellis, 1989). Heavy metals exist in water in colloidal, particulate and dissolved phases (Adepoju-Bello *et al.*, 2009) with their occurrence in water bodies being either of natural origin, such as through eroded minerals within sediments, leaching of ore deposits and volcanism extruded or as products of anthropogenic origin, such as through solid waste disposal, industrial or domestic effluents. Hence, there is always a need for and concern for the protection and management of surface water and groundwater quality. Heavy metals are priority toxic pollutants that severely limit the beneficial use of water for domestic purposes.

Powder X-ray Diffraction (XRD) is one of the primary techniques used by mineralogists and solid state chemists to examine the physico-chemical make-up of unknown material. With the aid of the atomic absorption spectrophotometer the soil

on the surrounds of the rivers under study and the sediments from the rivers were screened for metals.

## **5.2 Materials and methods**

### **5.2.1 Sample collection**

River sediments were collected in sterilised conical flasks. These samples were collected at the same site as the water samples in both the dry and wet season. The soil samples were collected 5 m, 25 m and 100 m away from the river site where water and sediments were collected.

### **5.2.2 Sediment analysis**

The sediments were dried at room temperature for 3 days. After drying the samples were crushed and sieved through a 200 mm diameter test sieve (Clear Edge) (UNEP/MAP. 2006).

### **5.2.3 Heavy metals detection in sediments and soil samples**

The sieved 2 g of soil samples were added to a 50 mL glass beaker, 15 mL of 32 % HCl and 5 mL of 55 % nitric acid ( $\text{HNO}_3$ ) were added to the soil sample. The mixture was incubated for 24 hours at ambient temperature. After digestion the samples were filtered through 110 mm filter papers (Whatmann) (Nemati, 2009). The filtrate was decanted into 100 mL conical flasks and distilled water was added to the filtrate to make a volume of 100 mL. The diluted sample was analysed in duplicates using the atomic absorption spectrometer (Varian) (Luo and Christie, 1998).

### **5.2.4 Statistical test**

A test of 2-way ANOVA and t-Test were performed to check the seasonal variations on the concentration of the metal detected in the sediments and soil.

## 5.3 Results

### 5.3.1 Heavy metals properties of the soil and sediments

Heavy metal contamination of aquatic bodies is a major environmental problem. Heavy metals are widespread and can persist in the environment, they are potentially toxic, and can become incorporated into the food web.

#### 5.3.1.1 Heavy metals in sediments in the dry season.

The ranges of the Cu, Zn, Cr and Co were 0.01 – 0.10, 0.05 – 0.62, 0.10 – 0.18 and 0.07– 0.17 mg/L respectively. The highest levels of Cu, Zn, Cr and Co were detected TwH stream, Motse and MrL respectively, as shown in Figure 5.1. Total metal concentrations at Motse river, Moopetsi river, Moopetsi-Motse river, Moopetsi spring, Serafa spring, HcN stream, TwH stream, and MrL stream were 0.35, 0.54, 0.23, 0.53, 0.79 and 0.33 mg/L respectively which followed the order: TwH stream > Moopetsi river > HcN stream > MrL stream > Motse river > Moopetsi-Motse river. TwH stream had higher metal content while Moopetsi-Motse river had the lowest metal content. The metal concentration followed the order Zn (1.27mg/L) > Cu (0.68 mg/L) > Co (0.53 mg/L) > Cr (0.29 mg/L).

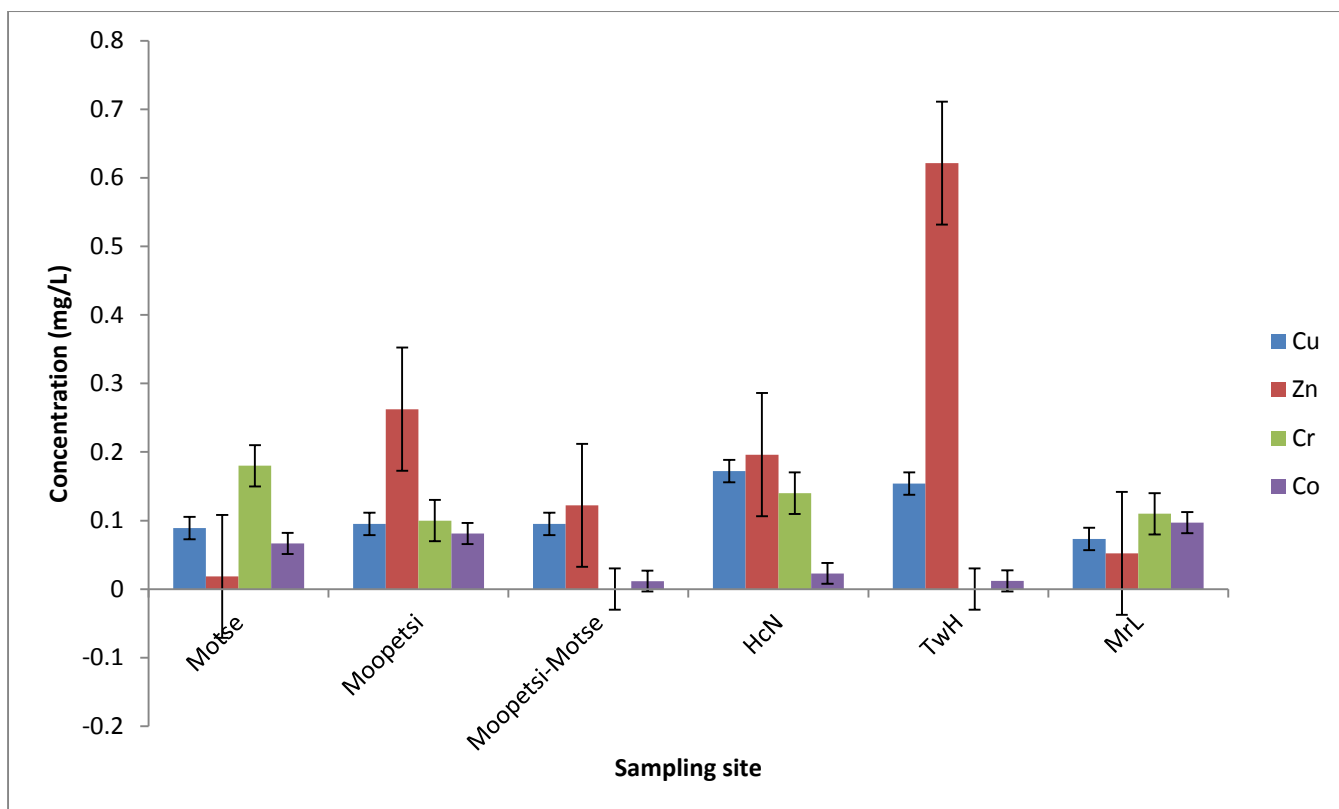


Figure 5.1 Heavy metals in sediments collected in the dry season.

### 5.3.1.2 Heavy metals in sediments in the wet season

The ranges of the Cu, Zn, Cr and Co were 0.04 – 0.98 mg/L, -0.02 – 0.65 mg/L, 0–0.2 mg/L and 0.23 – 0.49 mg/L respectively. HcN had the highest level of all the metals screened in this study as depicted in Figure 5.2 below. The total metal concentration at Motse river, Moopetsi river, Moopetsi-Motse river, Moopetsi spring Serafa spring, HcN stream, TwH stream and MrL stream were 0.38, 0.40, 0.74, 2.32, 0.77 and 0.77 mg/L which followed the order HcN stream > TwH stream > MrL stream > Moopetsi-Motse river > Moopetsi river = Motse river. The metal concentration followed the order Co (2.07 mg/L) > Cu > (1.76 mg/L) > Zn (0.93 mg/L) > Cr (0.62 mg/L).

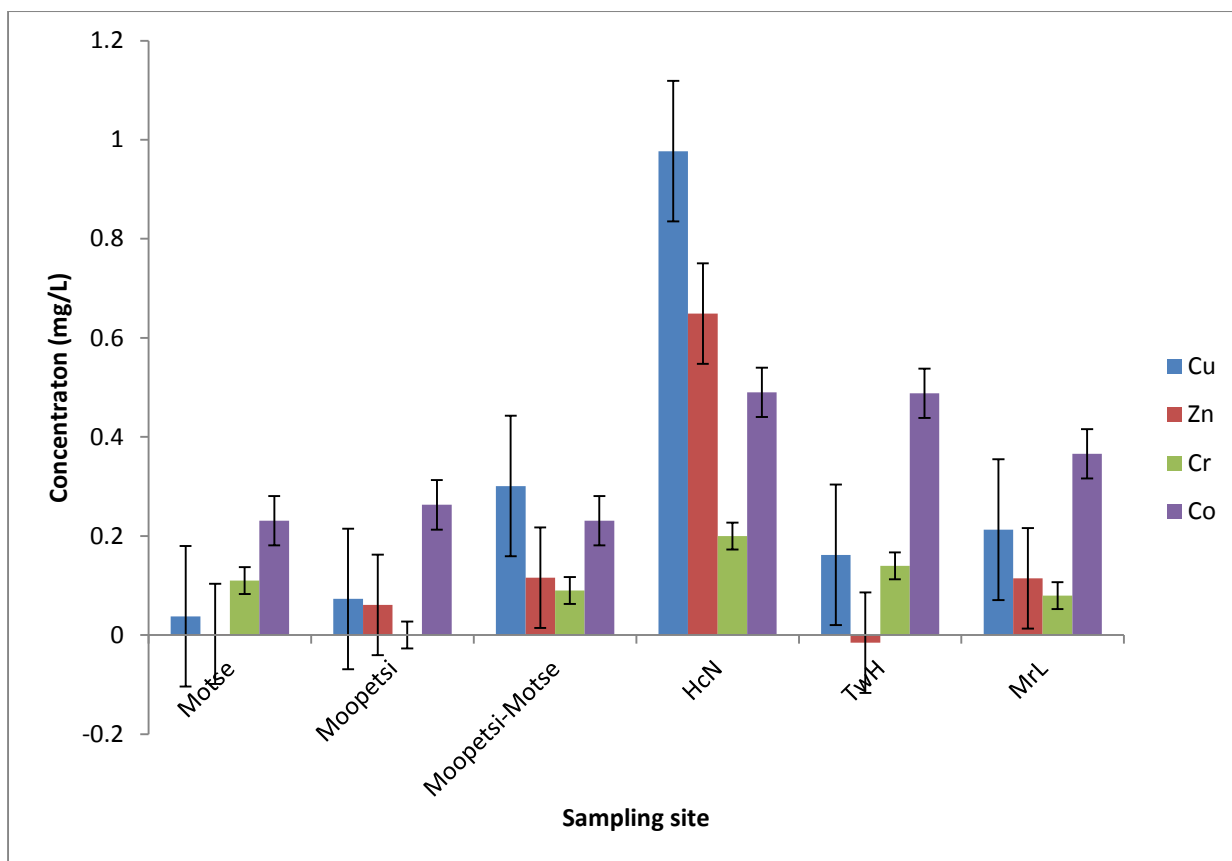


Figure 5.2 Heavy metals in sediments collected in the wet season.

The wet season had high total metal content and an increase was observed in the HcN stream, MrL stream and Moopetsi-Motse river while a decrease was observed in the TwH stream, Moopetsi river and Motse river. The sediments samples obtained closer to the mining area had a high metal content in both seasons with the exception of the Moopetsi river which was high in the dry season.

### 5.3.1.3 Heavy metals in soil samples in the wet season

The concentrations of the Cu, Zn, Cr and Co ranged from 0.045 – 4.65 mg/L, - 0.0001 – 2.6 mg/L, 0.03 – 0.28 mg/L and 0.16 – 0.53 mg/L respectively. Highest levels of Cu and Zn were detected at MrL stream, whereas Co and Cr were detected in the Motse river and Moopetsi spring respectively. Table 5.1 below shows the concentration of the metals screened in this study with the exception of Fe which was found to be above the detectable level



Table 5.1 Heavy metals in soil samples in the wet season

<b>Sample site</b>	<b>distance from the river/stream</b>	<b>Cu</b>	<b>Zn</b>	<b>Fe</b>	<b>Cr</b>	<b>Co</b>
<b>Motse</b>	100 m	0.19	-0.09	ADL	0.11	0.87
<b>Moopetsi</b>	100 m	0.21	0.30	ADL	0.28	0.53
<b>Moopetsi-Motse</b>	100 m	0.11	0.01	ADL	0.08	0.40
<b>HcN A stream</b>	25 m	4.65	2.60	ADL	0.12	0.34
<b>HcN B stream</b>	100 m	0.118	0.19	ADL	0.11	0.26
<b>TwH A stream</b>	5 m	0.18	0.07	ADL	0.03	0.52
<b>TwH B stream</b>	100 m	0.14	-0.0001	ADL	0.03	0.16
<b>MrL stream</b>	100 m	0.02	0.11	ADL	0.04	0.15

Key: ADL- Above detection limit

Figure 5.3 below is a representation of heavy metals in soil samples in the wet season. The highest metal concentration was observed in soil sample that was collected 25 m from the mine in the HcN region.

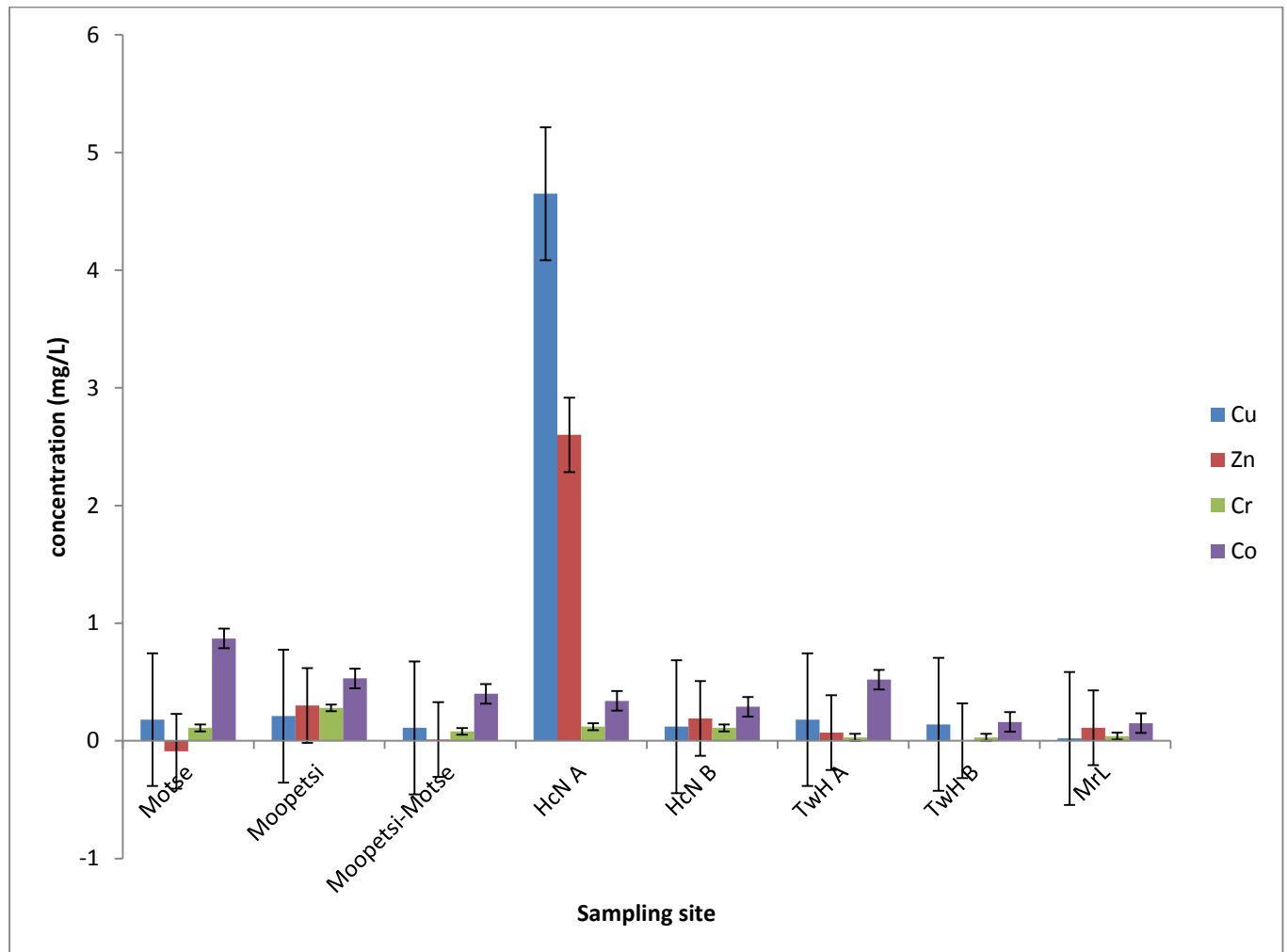


Figure 5.3: Heavy metals in soil samples in the wet season

The concentrations of the heavy metals such as Co, Cu and Cr in sediments and soil samples from the same area were found to be different. The concentration of Co was found to be higher in soil and lower in sediments at Motse river, Moopetsi river and Moopetsi-Motse river; and the opposite was observed in HcN, TwH and MrL streams as shown in Figure 5.4. The level of Co and Cu sediments was found to be higher than in the soil at the sampling points HcN, TwH and MrL (Figure 5.4) streams which are closer to the mine.

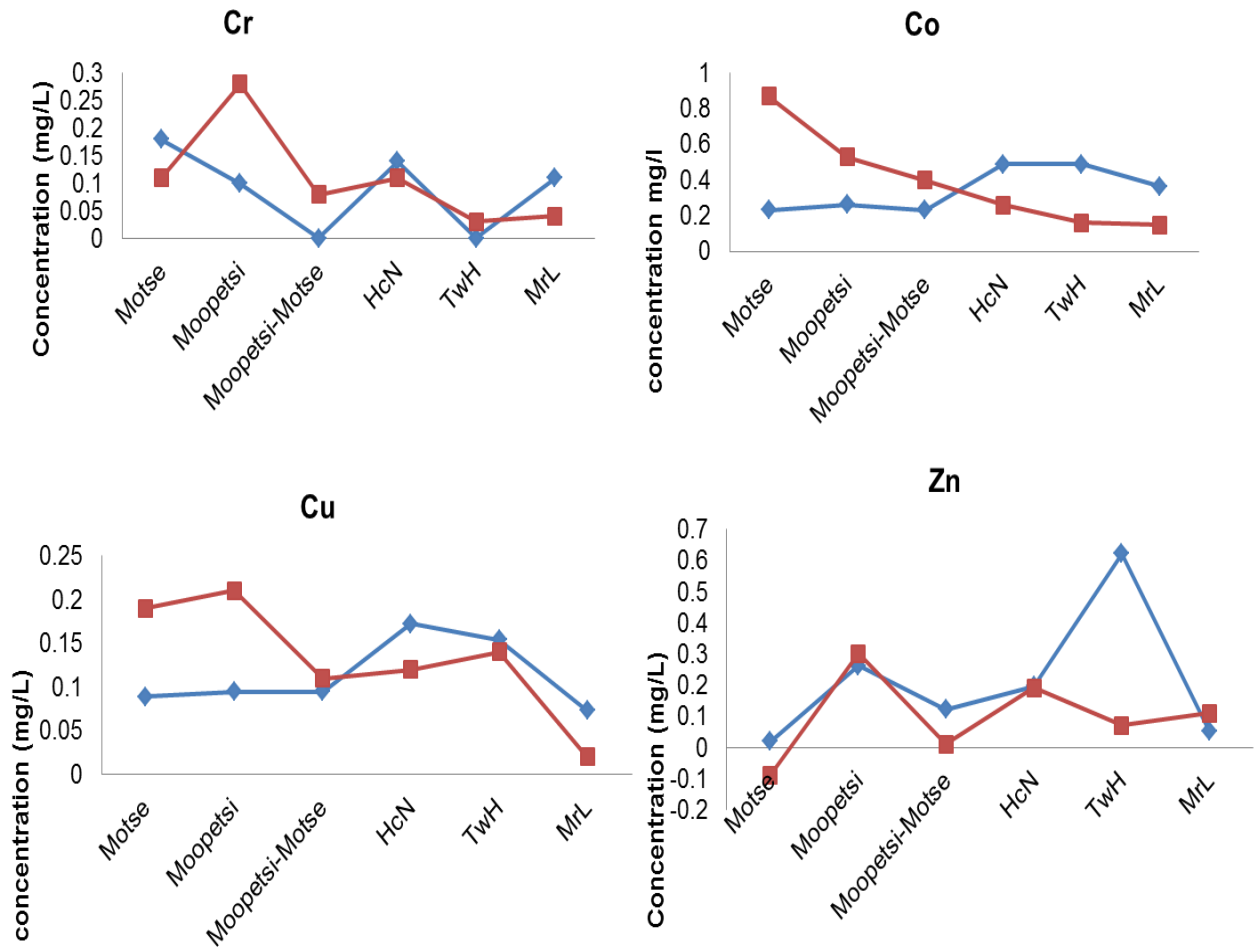


Figure 5.4 Comparison of the concentrations (mg/L) of heavy metals in soil (red) and sediments (blue).

## 5.4 Discussion

### 5.4.1 Heavy metals in soil and sediments.

A high concentration of potentially toxic heavy metals is an environmental hazard for the surrounding ecosystems. The increase in the levels of Zn, Cr and Co in the wet season implies an increase in the mining activity in this region during this season since the probable input of metals in this region are the mining plants. A study by Jena and Mohanty (2013) showed that soil composition and physicochemical characteristics strongly influence metal behaviour in the soil. They suggest that elements like Cu, Ni and Hg can be retained on organic matter thus being immobilised. This could explain the high level of Cu in HcN stream during both

seasons. Several studies have reported high metal concentration in river sediments situated near mining areas. A study by Resongles *et al.*; (2014) has reported high levels of Zn in soil and river sediments and Varol and Sen (2012) reported on high levels of Cr, Co, and Zn due to the pollution from mining activities which is in agreement with findings in the current study. Highest levels of Cu and Zn were detected in the HcN area. A study conducted by Boularbah *et al.*, (2006) reported soils in mining areas were highly contaminated with Zn and moderately contaminated with Cu, which is in agreement with the findings in the current study. Two soil samples were collected in the HcN; the samples collected nearer mine area, HcN (25 M) and the highest level of Zn when compared to the sample collected further away from the mine (100 M). This indicates possible contamination of the soil by the mining effluents containing Zn. Elevated levels of Zn in the stream water was observed at a higher concentration than that in the soil. In Moopetsi spring and Motse river, sediments had lower Cu and Co concentration when compared to soil samples. Anthropogenic sources such as the use of pesticides, insecticides, and fungicides may contribute to the elevation of Cu and Co concentrations in the soils collected away from the river. In fact, Cu soil pollution appears to be readily affected by anthropogenic factors (Martin *et al.*, 1993; Gray *et al.*, 2003). A study by Wei and Yang, (2010) showed that factors such as industrial emission and traffic emission contribute to the elevation of Cu and Co.

This study indicated that the river sediments and the soil in the Tubatse municipality were polluted with heavy metals, and the pollution was highest at sites closer to mines.

## Chapter 6

### 6. TOXICITY OF HEAVY METALS ON CELL LINES

#### 6.1 Introduction

With industrialization, environmental pollution with heavy metals becomes a major concern. A large number of chemical pollutants enter the aquatic environment each year. These pollutants, which include heavy metals, are known to be potentially toxic to biota and present a health threat to the public. Industries like mines discharge large amounts of waste water containing zinc, copper, nickel, mercury, cadmium, lead and chromium into the environment (Wang and Chen, 2009; Fu and Wang, 2011). Contamination of the environment by toxic metals constitutes a nuisance to the environment due to their persistent nature and tendency to spread into the ground, as well as surface water.

Heavy metal contaminants last for a long time in nature, and it can be bioaccumulated and bioaugmented through the food chain (Chen and Wang, 2008). Numerous studies have highlighted the toxicity of individual metals to living systems (Gollenberg *et al.*, 2010; Basile *et al.*, 2012; Bhattacharjee *et al.*, 2013; Taylor *et al.*, 2014). Toxic effects of heavy metals on living systems are varied, ranging from immune system dysfunction (Jadhav *et al.*, 2007), embryogenesis (Fathallah *et al.*, 2013), mortality in living organisms (Vellinger *et al.*, 2012), malfunction of neuronal systems (Rai *et al.*, 2013), cancers (Feki-Tounsi *et al.*, 2013), cytogenicity (Jadhav *et al.*, 2006; Le *et al.*, 2013; Varotto *et al.*, 2013), and induction of oxidative stress (Jadhav *et al.*, 2007). Further, potential pathological roles of metal ions are emerging in the form of premature aging and many other characteristic features of a wide range of diseases (Chowdhury and Chandra, 1986; Duruibe *et al.*, 2007). The current study aimed to determine the toxicity of the heavy metals (Zn, Fe, Co, Cu and SCN-) found in the water collected in the Sekhukhune district on human cell lines.

## 6.2 Materials and methods

### 6.2.1 Cell culture

The C2C12 (skeletal muscles) cells were maintained in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10 % FBS (foetal bovine serum). The cells were grown in 75 cm<sup>2</sup> tissue culture flasks at 37°C under a humidified atmosphere and 5 % CO<sub>2</sub> and were trypsinized and counted when the cells reached a confluent stage (Metzinger *et al.*, 1993).

### 6.2.2 Preparation of metal stock solutions

Stock solutions Cr (2 mg/L), Zn (5 mg/L), Fe (5 mg/L), Co (12 mg/L ) and [SCN<sup>-</sup>] (10 mg/L) were prepared in DMEM media containing 1 % DMSO (Dimethyl sulfoxide). They were prepared with reference to the concentrations found in water

### 6.2.3 Cytotoxicity assays

#### 6.2.3.1 Exposure conditions

The cells were grown in 75 cm<sup>2</sup> tissue culture flasks and, when the cultures were nearly confluent, were trypsinized and counted. All individual wells of a 96-well microplate were seeded with 100 µL of cell suspension containing 2x10<sup>5</sup> cells/mL for the cytotoxicity test. This seeding density was found to be optimal to achieve the desired confluency (60-70 % following 24 hours in culture). Twenty-four hours after cell seeding, the DMEM was removed and replaced by a fresh medium containing different concentrations of metals and the plates were incubated for 24 hours (Wise *et al.*, 2002). The cells were exposed to the following concentrations of the metals: Cr (0.2, 0.45 and 0.9 mg/L), Zn and Fe (1.0 and 2 mg/L), Co (2, 5 and 10 mg/L), and [SCN<sup>-</sup>] (3.3, 5.4 and 7.2 mg/L). The control used in the experiment was the untreated cells.

### 6.2.3.2 MTT assay

The growth of cells after treatment with different heavy metals was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] cell proliferation assay. At the end of the time point, 100  $\mu$ L of 0.05 % MTT reagent was added to each well and incubated at 37 °C for 4 hours to allow the formation of purple coloured formazan crystals. Formazan dissolving solution (100  $\mu$ L of 100 % DMSO) was added to each well, and the reaction mixture was incubated in the dark for 2-4 hours at room temperature. The colour intensity was then measured spectrophotometrically at 570 nm using a microplate reader (Berridge *et al.*, 1996).

## 6.3 Results

The C2C12 (skeletal muscles) cells were treated with increasing concentrations (highlighted in section 6.2.3.1) of heavy metals. Cell viability was estimated by measuring the capability of mitochondrial dehydrogenases to reduce MTT. Cell survival was expressed relative to a control in the absence of any treatment. An increase in viability on cells treated with Cr, Co, Fe and Zn was observed. Only cells treated with SCN<sup>-</sup> exhibited an almost 43 % decrease in viability as shown in Figure 6.1 below.

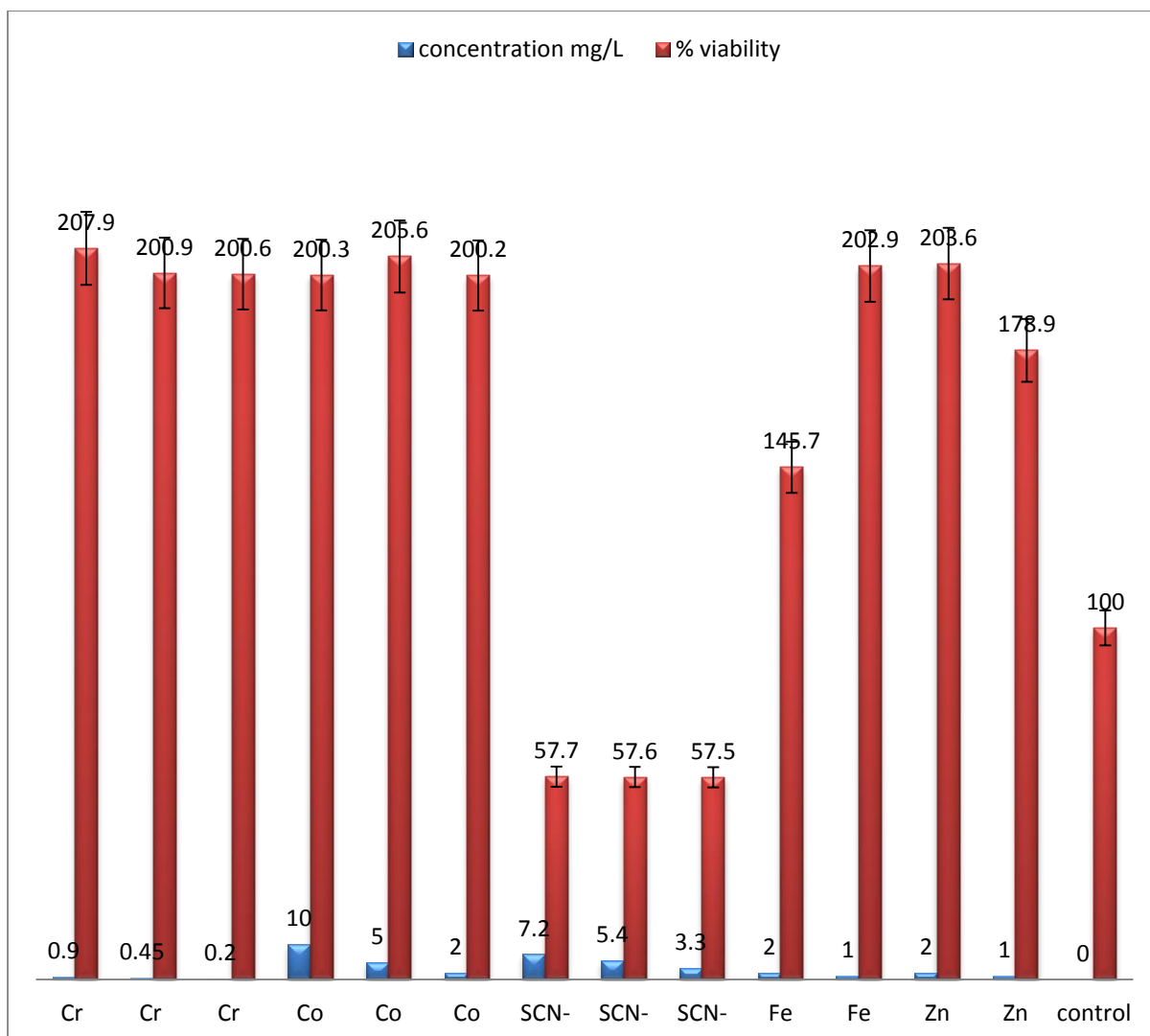


Figure 6.1 Percentage viability of C2C12 cells after 24 hour treatment with different concentrations of metals.



The data in Table 6.2 demonstrated no difference in activity between Fe and Zn. The CC<sub>50</sub> of Co was notably higher than for the other metals tested as shown in Table 6.2.

Table 6.2 CC<sub>50</sub> values of heavy metals revealed by MTT assay

Heavy metal	CC <sub>50</sub> (mg/L)
Zn	1.5
Fe	1.4
SCN-	3.7
Cr	4.8
Co	6.9

#### 6.4 Discussion

Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, damage to blood composition, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in slow progressing physical, muscular and neurological degenerative processes. The present study showed that the heavy metals tested in this study showed no lethal activity against the C2C12 cells as the percentage viability of the treated cells was higher than the control. Thiocyanate was the only compound tested that showed to be toxic to the cells as the percentage viability was lower than the control. In a study using laboratory animals (Brown *et al.*, 1995), thiocyanate has been shown to be of high acute toxicity and has been placed in Toxicity Category I (the highest of four categories) (EPA, 1997). Lanno and Dixon (1996) have shown that SCN<sup>-</sup> is toxic to fish cells as well. This is in agreement with the findings in this study. The other metals may not be exhibiting any toxicity due their solubility because all the metals used in the study were particulate metals with the exception of SCN<sup>-</sup>. A study by Smith *et al.*, (2014)

has shown that soluble cobalt is more toxic than particulate cobalt. This shows that the solubility of a particular metal plays a significant role in its toxicity. Furthermore, time of exposure is also an important factor when studying cell viability. A study by Rodilla *et al.*, (1998) has shown cytotoxicity of zinc on epithelial cells at lower concentrations after a 96 h exposure period. The MTT assay was used to calculate the CC<sub>50</sub> (50 % cytotoxicity concentration) of the metals detected in the water. There was no correlation between the percentage viability and CC<sub>50</sub> values for Zn, Fe, Cr and Co. The percentage viability obtained in the study suggests that the treatment with the metals encourage cell proliferation whereas the CC<sub>50</sub> suggests the opposite.

A comprehensive study on the metal states and the effect on human cells is hence necessary for understanding of the conditions, such as oxidation states, which make these metals harmful, and how toxicity occurs. An understanding of the mechanisms of their biochemical activities would explain the increase in cell viability exhibited by the metals in this study. With respect to thiocyanate, the CC<sub>50</sub> value correlated with the percentage viability obtained which confirms the toxicity of thiocyanate on the C2C12 cell line as stated above

## Chapter 7

### 7.1 GENERAL DISCUSSION AND CONCLUSION

Water is essential to life on our planet. The surface and ground waters have become contaminated with hazardous heavy metal ions and other impurities mainly due to human activities. Some metal ions are very toxic and can exert direct impact on human body resulting in several diseases. The current study investigated the influence of mining activities on the bio-physicochemical quality of surface and ground waters. The microbiological quality of the surface water was poor as pathogens such as *A. hydrophila*, *P. putida*, *P. luteola*, *C. sakazakii*, *A. haemolyticus*, *E. sakazakii*, *Pantoea* spp., *E. cloacae*, *S. odifera*, *K. rocea*, *S. thoratens*, *S. agalactiae*, *A. baumannii*, *Bordetella* spp. and *A. calcaceticus* were isolated. Physical quality of all the surface and ground water samples were found to be compliant with the set standards of 5 FAU, 15 Pt-Co and 250 mg/L for turbidity, colour and TSS respectively. The following chemicals and metals Zn, [SCN-] Cr, Co, Fe, SO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub>, and Cl<sub>2</sub> were found to be above the normal expected values and this suggests contamination from the surrounding mines. Most mining operations use metals, reagents, or other compounds to process valuable minerals. In platinum mining, certain reagents or heavy metals, such as cyanide bromine, cyanide, thiocyanate, nitric acid, sulphites and hydrochloric acid are frequently used in processing the ore and by-products such as iron, lead, copper; nickel and zinc are produced. These reagents and metals commonly leach, spill or leak from the mine site into nearby water bodies (McClure and Schneider, 2001). Researchers such as; de Luis *et al.*, (2011), Tang *et al.*, (2013), Resongles *et al.*, (2014) have reported elevated metal content in surface water resulting from mining activity for sulphides, copper and coal respectively. In the current study, the concentrations of Zn, Fe, and Cl<sub>2</sub> were found to be within the permissible limits set for drinking water. However, the concentrations of [SCN-] and Cr did not comply with the set limits whereas there are no documented standards for H<sub>2</sub>O<sub>2</sub>, and Co. Cobalt, Copper and Chromium were most dominant in the water bodies than in the corresponding soil samples, which suggest contamination of the water body by external factors and not as a result of geochemical properties of the area. Typical contamination of sediments in areas where mining effluents flow was previously documented by Resongles *et al.*, (2014) and Varol and Sen (2012). Heavy metals from the discharged from mining effluents

are widespread and persistent in the environment, potentially toxic, and can become incorporated into food webs. Heavy metals adsorbed in sediments can be desorbed back into overlying water under certain conditions, causing secondary pollution and potentially having toxic effects on organisms (Niu *et al.*, 2009). Heavy metal contamination of sediments can critically degrade aquatic systems. General chemical forms of metals in soils and sediments include: – water-soluble metals, as free ions, inorganic or organic complexes; exchangeable metals; – metals precipitated as inorganic compounds (Gambrell, 1994). As a result, the fate of metals in soils and sediments is governed by numerous processes, including sorption/desorption, precipitation/dissolution and complexation/ decomplexation (Salomons *et al.*, 1987). Any of the above mentioned processes might explain the concentrations of metals in water versus sediments. The flow rate of the river, flood frequency and duration, also affects the variability of metal accumulation within the sediments (Du Laing *et al.*, 2007; and Rinklebe *et al.*, 2005). In general, the elevation of Zn, [SCN-], Cr, Co, Fe, H<sub>2</sub>O<sub>2</sub>, and Cl<sub>2</sub> in surface water suggest contamination by external sources such as effluent from mining activity. The concentrations of Cu, Cr, Co and Zn in the sediments varied from the concentrations obtained in the soil which clearly indicates pollution of the water bodies by external factors which include effluents from the mine. The presence of pathogenic bacteria in the water present serious health risks to people relying on these water bodies for drinking and household purpose. The common flora of surface waters such as species of the genera *Pseudomonas*, *Bacillus*, *Aeromonas*, *Campylobacter* and *Klebsiella* were not predominant and some not detectable in these water bodies that were sampled in this study, however, prevalence of microbiota associated with human skin and gut were observed. Metal toxicity, in addition to human activity, could as well be implicated in this shift in the flora present in surface waters.

In conclusion the bio-physicochemical quality of the water bodies in the Tubatse municipality is poor due to the presence of pathogenic bacteria, elevation level of heavy metal content and due to the toxicity of SCN- on human cell lines.

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