



**ACCUMULATION OF SELECTED NUTRIENTS AND HEAVY METALS IN THE
KHUBELU RIVER CATCHMENT, MOKHOTLONG, LESOTHO.**

By

Bokang Shakhane (217137326)

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Supervisor: Dr N. Malaza

Co-supervisor: Dr L. Sibali

Cape Town Campus

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Abstract

Water is essential to all life forms. It is a fundamental enabler of socio- economic development and essential for maintenance of ecological integrity. A good quality water is therefore, a necessity to achieve all these sustainable pillars, thus calls for global, continental, regional as well as national communities to protect water resources against pollution. Freshwater sources are naturally scarce and not evenly distributed across the globe hence pollution and drought further reduce freshwater availability.

Presence of contaminants in freshwater systems is therefore, undesirable and necessitates all concerned and affected parties to act decisively to minimise the impacts. Close monitoring and studies on aquatic environmental pollutants are key more especially in areas like Khubelu River Catchment (KRC) where diamond mining and construction of large storage dams occur in close proximity. This study, therefore, seeks to determine levels of selected nutrients and heavy metals in the KRC, identify point and non-point sources of pollution as well as to evaluate possible impacts on the environment.

These objectives were achieved through use of various analytical methods. The flow injection analysis was used in analysing for ammonia, ion chromatography for nitrates and nitrites analysis and inductively coupled plasma optical emission spectrophotometry for selected heavy metals. The results indicated the concentration of nitrates/nitrites as N in the range from 0.13 – 167 mg/L i and 0 – 67.8 mg/L during dry and wet seasons respectively. Ammonia as N ranged from 0.17 – 1.89 mg/L and 0.15 – 0.68 mg/L in the dry and wet season respectively. Copper levels ranged from 0.039 – 0.219 mg/L and 0.011 – 0.029 in the dry and wet season respectively. Cadmium, arsenic and mercury were not detected in both sampling seasons whereas lead and chromium were only detected during dry season. The levels of lead ranged from 0 – 0.020 mg/L and that of chromium were found to lie between 0 and 0.046 mg/L.

Based on point and non-point sources, all the heavy metals detected were found to be non-point with the geology of an area supporting their natural occurrence. The nitrates and nitrites on the other hand proved to be mainly point sources because they were highest at Patising stream which carries some effluent from Letseng diamond slime dams. The downstream concentration levels confirm these as they are higher than the upstream levels. The presence of these nutrients upstream also confirms some contribution by land use activities such as animal droppings and crop farming as well as organic fertilisers thus minor component can be attributed to non-point sources. Ammonia is also distributed across all the sapling locations but highest at Patising still confirming the influence by the mine. Its presence in the upstream also confirms some contribution from non-point sources and natural processes of ammonia cycle.

The results obtained in the present study indicated that there was a definite pollution in the Khubelu River Catchment with respect to HMs and nutrients studied. Land use activities around the catchment were the route source of this pollution particularly nutrients because heavy metals proved to occur naturally within the KRC and the geology of the area supported that. These HMs (Pb, Cu & Cr) and nutrients (NO_3^- , NO_2^- & NH_3) can have serious health implications for both human beings and biota. It is therefore recommended that appropriate joint monitoring programme by the Departments of Environment and Water Affairs be done in order to minimise the possible impacts.

Detailed river health study that will investigate all drivers and response factors be done to evaluate all possible migration routes of these HMs and their impacts. Another study on determination of HMs in fish tissues such as liver and kidney might be helpful to investigate possible bioaccumulation effects of these HMs. Public health study on Patising community to investigate residents' health status with respect to the pollution in the catchment might be of importance. It might also worth investigating how the Patising stream might have impacted on the livestock of the community residing in the area because my observation and through communication with an area chief is that, the stream is no longer used for portable water instead the community uses water from the upstream confluence of Khubelu River and Patising.

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Dedication

- I thank Almighty God who has given me power, strength and wisdom to push this study to the end despite the obstacles I have encountered on my journey, His mercy was sufficient to take me through.
- Family and friends who have been supportive throughout this journey, special thanks to my wife, she has always been on my side to make this a success story and my boys, they have always been curious as to when Daddy is finishing his studies; this is the end of the road boys. Anti and uncle, your efforts could not be over emphasised, thanks guys.

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List of acronyms and abbreviations

<u>Abbreviation</u>	<u>Description</u>
ANFO	Ammonium Nitrate Fuel Oil
As	Arsenic
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
DNA	Deoxyribonucleic Acid
DWAF	Department of Water Affairs and Forestry
EDC	Endocrine Disrupting Chemical
EIB	European Investment Bank
EPA	Environmental Protection Agency
Fe	Iron
FIA	Flow Injection Analysis
GIT	Gastrointestinal tract
GPS	Global Positioning System
Hb	Haemoglobin
Hg	Mercury
HMs	Heavy Metals
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrophotometry
IGS	Institute of Groundwater Studies
Kcal	Kilo calories
KR	Khubelu River
KRC	Khubelu River Catchment
KR-DS	Khubelu River- Downstream

KR-US	Khubelu River – Upstream
LHDA	Lesotho Highlands Development Authority
MA	Millennium Ecosystem Assessment
MetHb	Methaemoglobinemia
MHz	Mega Hertz
NH ₃	Ammonia
NH ₄ NO ₃	Ammonium nitrate
NO ₂ ⁻	Nitrites
NO ₂	Nitrogen dioxide
NO ₃ ⁻	Nitrates
ORASECOM	Orange-Senqu River Commission
Pb	Lead
Ppm	Parts per million
PR	Patising River
PVC	Poly Vinyl Chloride
Q-GIS	Quantum Geographic Information System
ROS	Reactive Oxygen Species
SANS	South African National Standards
SDGs	Sustainable Development Goals
WHO	World Health Organisation
Zn	Zinc

Chapter 1

INTRODUCTION

1.1 Background and motivation

Water is the key driver and supporter of all life forms on planet earth (Meyer 2013; Goswami and Bisht 2017). It is the crucial driver of social and economic development and is also essential in maintaining the integrity of the natural environment (Goswami and Bisht 2017). Water resources can meaningfully provide essential environmental services such as fish, clean water (waste assimilation) as well as recreation provided they are kept in good quality state (Parker and Oates 2016, MA 2005). Furthermore, population growth, rising water pollution and the impacts of climate change ultimately give rise to competition between different water uses thus putting more pressure on water sources (Alcamo et al 2007; Meyer 2013). This background will therefore dwell more on water pollution focusing specifically on impacts of selected nutrients and heavy metals on water quality and the environment.

Nutrients mostly enter water streams through run-offs from organic and inorganically fertilised fields, municipal and industrial effluents, natural decomposition of nitrogen containing substances as well as diffusion of atmospheric nitrogen into water bodies (Holden, 2014). Once in streams, depending on their concentration, they can directly and indirectly threaten aquatic biota. Direct impacts include instances where these nutrients get ingested by aquatic organisms, fish species and human beings causing severe damage. Indirect impacts are cases where high concentration levels of nutrients trigger processes that in the end produce a very undesirable state for aquatic fauna and flora, eutrophication is a typical example for indirect impacts. Direct impacts will further be refined using fishes and human beings whereas indirect ones with eutrophication.

High concentration levels of nutrients in freshwater streams especially ammonia and ammonium end up being in fish through ingestion when eating and drinking and also through absorption through fish skin and gills (Eddy, 2005). Once ammonium is in fish system, it causes damage to gill tissues thus reducing blood oxygen-carrying capacity caused by progressive acidosis and in extreme cases, this situation results in the death of fish (EPA, 2013). High ammonia levels in aquatic environment can either impair ammonia excretion or result in a net uptake of ammonia from the environment. This increased ammonia net intake causes ionic imbalances which then lead to convulsions and death in fish species (Eddy, 2005).

In the case of human beings, high levels of nitrates and nitrites is very undesirable due to the fact that nitrites react with secondary amines to form carcinogenic compounds, nitrosamines (Porché, 2014). Nitrates in the presence of reducing agents get reduced to nitrites which are quite toxic. These nitrites because of their capability in forming methaemoglobin, are also known to cause blue baby syndrome in babies, oxygen deficiency (Fan, 2011; Porché, 2014). The rationale is that stable methaemoglobin compound is incapable of realising oxygen in areas with oxygen deficit against the normal process where haemoglobin reacts with oxygen to form oxyhaemoglobin which decomposes easily in oxygen deficient areas within the human circulatory system and as a result supplying body tissues and cell with oxygen (Porché, 2014; Khan *et al.*, 2012). Other carcinogenic effects of nitrites as indicated by Porché (2014) include gastric cancer.

In considering indirect impacts, eutrophication is known to be the consequence of high levels of nutrients in the water bodies (WHO, 2002). The presence of high levels of nitrates and phosphorus as phosphate in aquatic biome induces rapid growth of algae blooms. The algae cover the water surface and prevent diffusion of oxygen into water bodies. The situation worsens as more and more algae blooms cover the water surface and this results in shortage of dissolved oxygen. Consequently, the fauna and flora species in that aquatic environment will die and ultimately decay causing stinky smell, and overall process is eutrophication (WHO, 2002).

However, not only nitrates, nitrites, ammonium and ammonia have these environmental toxicological effects, but also heavy metals cannot be under-estimated given their persistence nature in the environment and toxicity even at very low concentrations (Govind and Madhuri, 2014). This poisonous effect is accelerated by the bioaccumulation nature of heavy metals in biological organisms which often results in higher concentrations of toxicants in an organism's biological system than is available in the environment (Govind and Madhuri, 2014). Freshwater ecosystems are therefore, threatened by presence of heavy metals in the environment because they turn to react with various components of surface water forming toxic insoluble complexes (Solomon, 2008). These toxic complexes can kill benthic organisms, like insects, worms and crustaceans reducing food availability for certain species like fish that feed on insects hence distortion of food chain (Solomon, 2008). In addition, low concentrations of heavy metals can cause chronic stress which may not necessarily kill individual fish species, but rather lead to a lower body weight and smaller size which makes it difficult for fish species to compete for food and habitat (Rosado, 2016).

Human beings are also the integral part of the system as they feed on contaminated fish, drink water contaminated with heavy metals. The impacts on exposure to elevated concentrations of heavy metals are quite severe ranging from cancer, respiratory, pulmonary, gastrointestinal and cardiovascular failures as well as nerve damage and sensory loss in the peripheral nervous system in the case of arsenic poisoning (Govind and Madhuri, 2014; Sharma and Agrawal, 2005; DWAF, 1996). Elevated levels of cadmium are associated with renal damage, chromium with lung and gastrointestinal cancer, copper with liver, kidney and red blood cell damage, mercury with neurological, kidney, gastrointestinal, genetic, cardiovascular, and developmental disorders, and death and lead with neurological impairment in fetuses and young children (Govind and Madhuri, 2014; DWAF, 1996).

Heavy metals end up in streams and other water bodies naturally through volcanic eruptions and erosion of rocks (Solomon, 2008; Sharma and Agrawal, 2005). They occur in various forms in the aquatic environment either as ions dissolved in water, vapour, minerals in rocks and in particulate forms. Anthropogenically, heavy metals can be released directly into streams through effluent from municipal wastewater treatment plants, industrial processes such as galvanizing, combustion of fossil fuels and mining effluent (Sharma and Agrawal, 2005). Heavy metals can also be released indirectly by surface runoff from roads and farming lands (Govind and Madhuri, 2014). Their presence in the environment, therefore, raises concerns over their potential to impacting human health, animals and the overall integrity of the ecosystem (Rai, 2008).

Impacts of nutrients and heavy metals on freshwater sources are very undesirable, not only on the resource itself but also on ecosystem health and integrity as well as general human well-being (MA, 2005). Close monitoring and studies on these aquatic environmental pollutants are key given the severity and magnitude of global community stress on accessibility to freshwater sources (WHO, 2015). It is important, therefore, that the little that is available is protected against pollution if we, the Basotho Nation are to do well in achieving the sustainable development goals; SDG 3 & 7 good health and well-being and access to clean water and sanitation (Salam *et al.*, 2017). Hence studies like the one conducted in this research are well aligned. Over and above this, countries like Lesotho where diamond mining and construction of large storage dams occurs in close proximities and or within the same system, studies of this nature will help in the enhancement of the sustainable management of the two sectors (water and mining).

1.2 Location of study area

The Khubelu catchment is situated in the north eastern highlands of Lesotho (ORASECOM, 2008). The catchment is a drainage basin of the Khubelu River which is a tributary to the Senqu – Orange River (ORASECOM, 2008). This is a south flowing river in Mokhotlong District with its sources at the Phofung (Mont-aux-Sources) and Sekhong (Mount Amery) mountains. (ORASECOM, 2008).

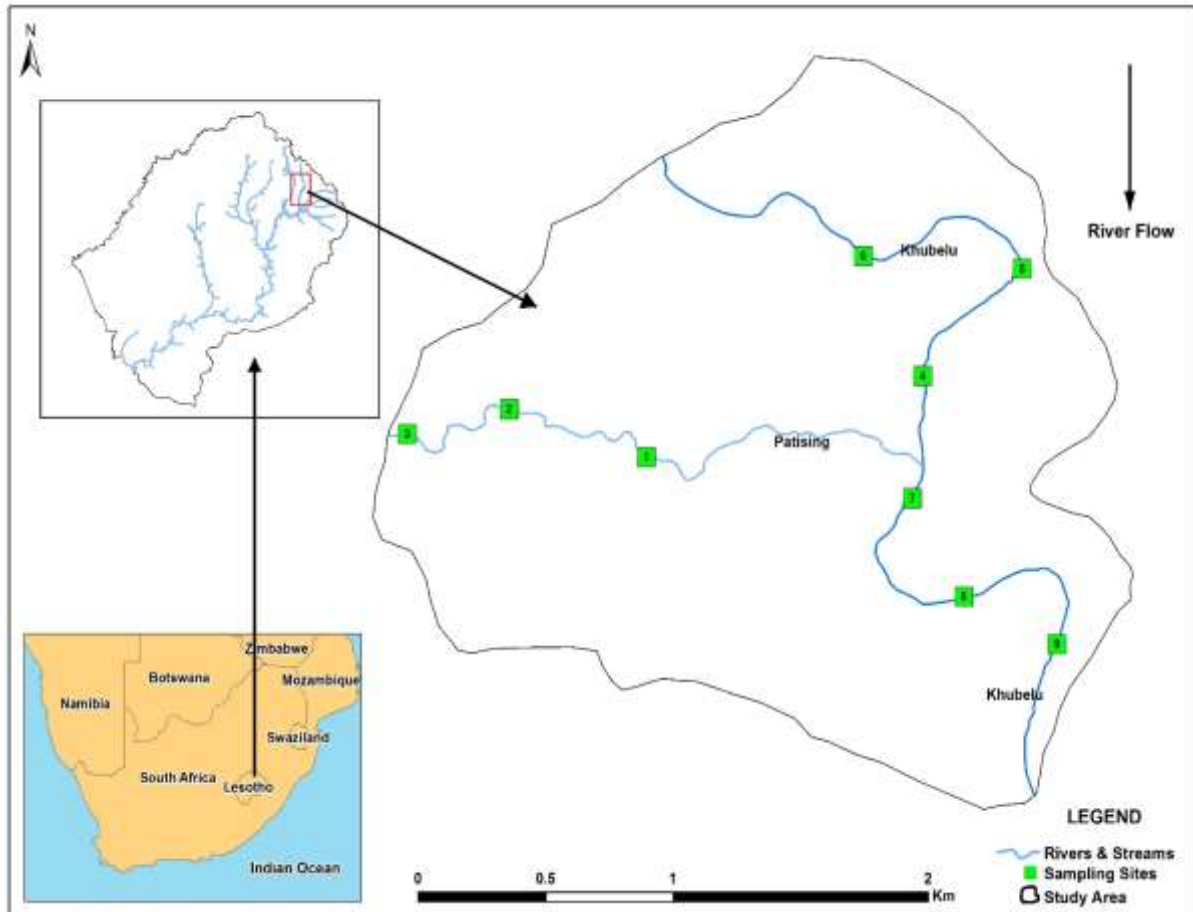


Figure 1.1: Study area map.

The study area can further be represented diagrammatically on an aerial photography as shown in figure 1.2 to give the reader a clearer picture of KRC.



Figure 1.2: Study area map (Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community, 2019).

1.3 Physiography, climate and vegetation

Lesotho is a land locked country with an approximated area of 30,344 km² between 27° to 30° East and about 28° to 32° South in the southern part of Africa (Mphale *et al.*, 2002). The country's climate is temperate and semi-arid with its hydrological year characterised by two seasons, summer rainfall season from October to March with maximum temperatures of approximately 40°C experienced in January and pronounced dry season from April to September with minimum rainfall in June and July (Sene *et al.*, 1998; Mphale *et al.*, 2002; Sharma and Makhoalibe 1987). Temperatures regularly drop below zero in the highlands during winter period usually around June and July and snow during this time is a common phenomenon though the water equivalent of this snow is usually less than 10% of the average annual rainfall (Sene *et al.*, 1998).

The country is divided into four physiographical zones, the lowlands, the foothills, the Senqu valley and the highlands (Mphale *et al.*, 2002; Sharma and Makhoalibe 1987). The KRC falls within the highlands and or Mountainous morphological unit which is in between 2000 to 3500

metres above the sea level (Mphale *et al.*, 2002; Sharma and Makhoalibe 1987). These highlands according to Sharma and Makhoalibe (1987) are believed to cover 2/3 of the country and have emanated from volcanic formations which makes them entirely basaltic in nature. The average land slopes in these highlands range from 8-10% while the channel gradients are in the range of 0.5-1% (Sharma and Makhoalibe 1987). The Khubelu catchment covers a total area of 852km² with the mean annual evapotranspiration of approximately 920mm and the average annual rainfall of 1168mm (ORASCOM, 2008). Shrubs, Oxalis species, Geum capensi (Geumcapensi), short sedge grasses, Helichrysum chionosphaerum, carex, Festuca and Festuca Caprina form the vegetation cover in the Khubelu catchment (ORASCOM, 2008). The dominant species at the Letseng area are Merxmullera (70%), Senecio (5%) and Oxalis (5%) (ORASCOM, 2008).

1.4 Land use

There are several land use activities happening within the KRC, chiefly amongst those is crop farming and livestock farming. Figure 1.3 below gives an overview of these activities through pictures taken during sampling exercise. The first two pictures, (a) and (b) illustrate sheep grazing on the riverbank and fields. The bottom right, picture (d) shows herd boys dipping their goats in an effort treat lice and curb. The bottom left, picture (c) shows fields along the Khubelu river, farming practice in this area is basically subsistence farming.



Figure 1.3: Land use within KRC.

1.5 Geological setting

The KRC is located within the Great Karoo Basin which consists of a large shallow basin of mainly sedimentary rocks that were deposited from approximately 200 million years ago (Van Rooy, 1993). The present-day remnant of the Karoo Basin underlies the whole of Lesotho as well as about 75% of the surface area of the Republic of South Africa. This basin is a near horizontal layered sequence of sedimentary rocks of continental origin capped by thick flood basalts of the Lesotho Formation, also referred to as the Drakensberg Basalts (Van Rooy, 1993). Grab (2005) also describes the Lesotho basalt as geochemically uniform and originate from the Pleistocene cold stages when enhanced frost wedging and nivation processes attacked the cross-jointed basalt outcrops. Knight (2017) describes the eastern mountains of Lesotho as having the bedrock that comprises of the flat-lying Jurassic basalts which have given rise to a relatively uniform initial land surface.

1.6 Statement of research problem

Water is an essential component of planet earth, covering approximately 70% of earth surface, with biological life and global economies having key hydraulic foundation (Holden, 2014). Despite this pivotal role, it is also a transport media for hazardous substances like heavy metals and nutrients which are known to pose threats to the ecosystem functioning and human well-being; Millennium Ecosystem Assessment (MA, 2005). Some of these toxic substances exist naturally while others are anthropogenic. Lesotho's economy is highly depended on availability of good quality water which is an essential input to industrial processes, mining and construction as well as revenue generation from royalties obtained from water sales to the Republic of South Africa (European Investment Banking, 2002). The Lesotho highlands water scheme, a conception resulting from good bilateral relations and transboundary policies, made it possible for the country to be advancing smoothly in terms of building large storage water dams intended primarily to generate revenue and boost country's economy; Lesotho Highlands Development Authority (LHDA, 2015). The phase II of the highlands water scheme is working on the construction of Poli-Hali dam, the third large storage dam apart from Katse and Mohale dams (LHDA, 2015).

The Poli Hali dam will receive water from the Orange-Senqu River which confluences with the Khubelu River prior the dam. There are two major activities occurring within the KRC namely agriculture (livestock and crop production) and mining. The use of fertilisers in crop production enhancement within the KRC and the application of pesticides on small stocks to treat lice and sheep cub are likely to introduce nutrients into the Khubelu River through run-offs during wet seasons. There is also likelihood of animal droppings and from human beings having the possibility of adding nutrients to the system during wet seasons. Besides these human and

animal activities, the Letseng diamond mine also has a potential to introduce heavy metals and nutrients into the Khubelu river system. Apart from the discussed possible anthropogenic sources of heavy metals, they also occur naturally in the environment and can be very toxic even at low concentration levels because of their persistence on environment and bioaccumulation effect on biological organisms (Govind and Madhuri, 2014).

The preceding discussions, therefore, raise concerns about the sustainability of water quality within the KRC thus calls for a constant water quality monitoring programmes and studies on environmental water quality pollutants specifically heavy metals and nutrients hence the study seeks to understand the concentration levels of selected heavy metals and nutrients within the KRC and evaluate the possible impacts in the environment.

1.7 Research questions

1. What are the levels of nutrients and heavy metals of interest in the Khubelu River Catchment (KRC)?
2. What are the threats posed by nutrients and heavy metals of interest within the KRC?

1.8 Aim and objectives

The aim of this study was to identify and determine the levels of nutrients (Nitrates, Nitrites and Ammonia) and heavy metals (Mercury, Lead, Arsenic, Copper, Chromium and Cadmium) in the Khubelu River Catchment. In order to achieve this aim, the project will:

1. Evaluate the impacts of selected nutrients and heavy metals accumulation to the environment.
2. Identify point and non-point sources of nutrient and metal contaminants.
3. Map and model the levels of selected nutrients and heavy metals within the KRC.

Chapter 2

LITERATURE REVIEW

2.1 Importance of freshwater sources

All life on Earth needs good quality water to survive. Poor water quality is detrimental to life more especially if it constitutes chemical and physical hazards because it puts at risk the health of individuals and populations as well as the entire ecosystems (Holden, 2014). Physical hazards such as drought and flooding, along with chemical hazards such as saline intrusion and pollution events impact on the health of many organisms including humans (Holden, 2014). Various stressors from both point and non-point sources of urban, agricultural and industrial sectors as viewed by Heathwaite (2010) compromise water quality resources, particularly microbial and nutrient pollution, which are believed to consist predominantly of pesticides and heavy metals.

A good quality water is, therefore, characterised by a thriving biota within its systems, high integrity often free or with minimal pollutants (Holden, 2014). A good quality water does not only maintain high ecological integrity and biological activities but also contributes significantly to the amount of freshwater availability on Planet Earth to support life (Heathwaite, 2010; Holden, 2014). This is quite key because an acceptable quality of water is capable of maintaining a system (could be a river, a lake or a dam) that is resilient enough to withstand perturbations and provide ecosystem services that are either provisioning, regulating, cultural and supporting for the betterment of human well-being (MA, 2005). It is estimated, therefore, that by 2025, 40% of the world's population could live in water scarce regions as a result of rising water demands and degradation of available water sources (Heathwaite, 2010). Globally, 18% of people have no access to safe drinking water, and about 2.4 billion people lack access to improved sanitation while other estimates indicate that water-related diseases kill a child every 8 s and 80% of all illnesses and deaths are in the developing world (Salam *et al.*, 2017; Heathwaite, 2010). The importance of these statistics are solely on emphasizing the role freshwater systems have in the natural regulation of freshwater quality and quantity

2.2 Previous studies

The magnitude and importance of water on Planet Earth, therefore, calls for all concerned individuals, organisations and institutions to carry out studies on water quality and quantity assessments as well as monitoring to protect and conserve this precious resource. Research has shown that oil spills and petroleum products can be a source of heavy metals traces into the environment (Akpoveta and Osakwe, 2014). A study conducted by Akpoveta and Osakwe (2014) confirmed the presence of some heavy metals in refined petroleum products. It is noted

from this study that oil spills and petroleum products from heavy duty construction equipment can introduce traces of heavy metals into freshwater systems.

Modaihsh study also reveals presence of heavy metals in artificial fertilizers (Modaihsh *et al.*, 2004), implicating an introduction of heavy metals into river systems through run offs from fertilized fields. Another study by Sen and Varol (2012) on assessment of nutrients and heavy metals contamination in surface water and sediments of the Tigris River, showed some level of contamination in this river and it was more pronounced on sediment samples. Contaminants were found to be total nitrogen, total phosphorus, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn and these pollutants were downstream of Ergani copper mine indicating the mine as a point source of pollution (Sen and Varol, 2012).

Previous study on Khubelu catchment was on the valuation of wetlands within the catchment (Moqekela, 2016). Another study was carried out by Matandare *et al.* (2019) on impacts of mining operations on water resources and ecosystems: The case of Letseng Diamonds in Lesotho. The study was a qualitative which was based on data collection using a questionnaire. This study was, therefore, unique because it was quantitative and evaluated water pollution by selected nutrients and heavy metals in the referred catchment taking into consideration point and non-point sources of possible pollution. Knowledge on levels of nutrients and heavy metals in Khubelu catchment will help the Government of Lesotho to manage its valuable limited water resources in a sustainable manner. To achieve sustainability, the activities of mining and agriculture need to be done in such a way that they don't cause water pollution in the catchment.

2.3 Mining Activities

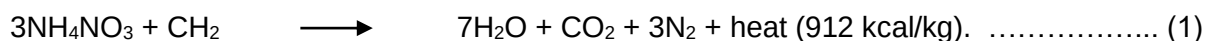
Diamond mining is known to cause nuisance to the surrounding environment in which it operates. The study on impact of Marange diamond mining operations on water quality in the Save and Odzi Rivers compiled by Zimbabwe Environmental Law Association (ZELA, 2012) indicates siltation and chemical pollution (heavy metals in particular) resulting from mining processes. Excavation works have the potential to expose minerals to the surface thus can easily enter streams during runoff periods. The presence of these pollutants in combination with high turbid water has deteriorated water quality in these rivers such that the majority of ecosystem services that these rivers used to provide are lost, these include provision of portable water for surrounding communities, irrigation, livestock and aesthetics to mention a few (ZELA, 2012). Analysis of heavy metals indicated high concentrations of iron, chromium and nickel particularly because these elements are the major constituents of Ferro-silicon, a chemical compound used in the diamond extraction process (ZEWA, 2012). In diamond

mining, Ferro-silicon is used as a dense medium separation material and is often used in granular rounded particles or in an angular milled form (Waanders and Rabatho, 2005)

Mining diamond does not only introduce Ferro-silicon metal constituents into the environment but nitrates as well. This often threatens the integrity of the river systems and the aquatic biota. Diamond mines often use nitrate based commercial explosives in their operations and these often contain ammonium nitrate mixed with fuel oil (Bailey *et al.*, 2011; Forsyth *et al.*, 1995). The loading practices applied by individual mines, their blasting efficiency as well as the presence of run-offs, controls the amount of nitrates entering the river systems. These nitrates often come from the waste rock disposal sites, spillages during explosive transportation, leaching of the explosive in wet blast-holes or undetonated explosives in the broken rock after blasting (Mahmood *et al.*, 2017; Forsyth *et al.*, 1995).

The commonly used explosives are grouped into three categories, Ammonium Nitrate Fuel Oil (ANFO), in the form of watergels/slurries as well as emulsions (Cranney and Sudweeks, 2012; Forsyth *et al.*, 1995). All these groups contain significant amounts of nitrogen, but have varying water resistance and as a result, have different capacity to introduce nitrogen into water systems. They can introduce nitrogen into the environment in the form of NH_4^+ and NO_3^- and also as nitrogen (N_2), ammonia (NH_3), nitrous oxide (N_2O), nitric oxide (NO) and nitrogen dioxide (NO_2) gases formed during detonation (Morin and Hutt, 2009). Nitrates and ammonia are generally the compounds of greatest concern for water quality degradation due to the potential human health hazard and impacts on aquatic life (Bosoi and Rose, 2009; Camargo and Alonso, 2006).

The chemical compositions of explosives can be highly variable depending on manufacturer's formulations which in most cases is not revealed (Morin and Hutt, 2009). The mostly used formulation is Ammonium Nitrate - Fuel Oil which is simplified as ANFO containing 94% NH_4NO_3 and 6% fuel oil (simplified as CH_2), and the explosive reaction is represented in equation (1) below according to Morin and Hutt (2009).



However, the combustion process as illustrated in equation (1) is not 100% efficient thus some traces of aqueous nitrogen species can be found in drainage systems (Morin and Hutt, 2009). This is due to the fact that factors such as explosives handling, spillages during handling, and the efficiency of a particular blast, drilling and packing of holes, sequences of detonations, and reliabilities of detonation play a major role in blasting inefficiencies (Morin and Hutt, 2009). In

some instances, the holes do not detonate and as a result, the explosives remain in the rock for later leaching during wet seasons (Bailey *et al.*, 2011; Morin and Hutt, 2009).

2.4 Agricultural Activities

On the global scale, nutrient transport is mostly from agricultural runoffs as compared to the natural nutrient cycles (Evans *et al.*, 2019; Carpenter, 1998). These nutrients enter the environment through fertilizer applications in crop production (Carpenter, 1998). The disturbances resulting from these additional nutrients on arable lands are the primary cause of diffuse water pollution from bad farming practices (Carpenter, 1998). Intensification of fertilizers and pesticides use as well as allied livestock activities has significant negative impacts on water quality (Díaz *et al.*, 2012). The most common water pollutants resulting from agricultural practices are nitrates, phosphorus, and pesticides (Pathak, 2015).

Increasing nitrate concentrations in the environment threaten the quality of drinking water (Holden, 2014). High nitrates levels in surface water reduce their provisioning environmental services' ability to support plant and animal life (MA, 2005). Agricultural pollution varies unpredictably over time and space and is mostly governed by rainfall patterns, land slope, soil characteristics, land use and crop choices, production techniques and the intensity of fertilizer use (Pathak, 2015). Intensive use of chemical fertilizers in farming and indiscriminate disposal of human and animal waste on land result in leaching of the residual nitrate causing high nitrate concentrations in surface water thus negatively impacting surface water quality (Parris, 2011; Pathak, 2015).

Agricultural activities do not only increase nutrients levels but also heavy metals as well. Sources of heavy metals from agriculture come mainly from inorganic and organic fertilizers as well as liming and pesticides application (Sharma and Agrawal, 2005; Modaihsh *et al.*, 2004). Inorganic fertilizers, phosphate in particular have variable levels of Cd, Cr, Ni and Pb. Cadmium is of particular concern especially in plants since it accumulates in leaves at very high levels which ultimately get consumed by animals and human beings (Begum *et al.*, 2008; Sharma and Agrawal, 2005). Cadmium enrichment also occurs due to sewage sludge, manures and limes while copper enrichment is mainly from animal manure (Begum *et al.*, 2008; Sharma and Agrawal, 2005).

2.5 Nutrients

2.5.1 Ammonia

Ammonia is one of the key pollutants in aquatic environment not only because of its toxic nature, but also of its ubiquity in surface water systems (Russo 1985). The sources for ammonia can either be natural or anthropogenic. Natural sources are mainly from decomposition of organic matter, gas exchange with the atmosphere, forest fires, animal waste, the discharge by biota, and nitrogen fixation processes (Latysheva *et al.*, 2012). Anthropogenically, ammonia is produced from mining industry where it is used for minerals extraction (EPA, 2004). It can also enter the aquatic environment through municipal effluent discharges, leaching of waste rock dumps contaminated with ammonium nitrate explosives as well as agricultural runoff (Latysheva *et al.*, 2012). It is important therefore that an introduction of ammonia into surface water systems be given considerable attention as this can threaten aquatic biota and general human well-being especially at elevated concentration levels (EPA, 2013).

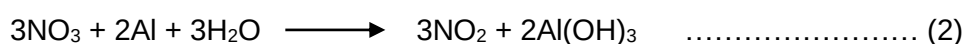
Chemically, ammonia exist in two forms in aquatic environment, ammonium ion (NH_4^+) and the unionized ammonia (NH_3) molecule, (EPA, 2013; Guan Bo, *et al.*, 2010). The toxic action of unionized ammonia on aquatic animals, particularly in fish causes damage to gill tissues and epithelium which ultimately leads to reduced oxygen-carrying capacity in fish and in extreme cases subsequent death (EPA, 2013). Despite most fish species being intolerant to elevated environmental ammonia levels, some species are ammonia-tolerant and use a variety of strategies to avoid ammonia toxicity (Ip and Chew, 2010; Randall and Tsui, 2002). Such strategies include amongst others production of amino acids, detoxification via glutamine and urea production as well as diffusion into water bodies (Ip and Chew, 2010; Randall and Tsui, 2002). Because of some negative impacts associated with ammonia toxicity, it is prominent therefore that human activities balance the ammonia concentrations in aquatic environment at safe levels for healthier aquatic ecosystem (Souza-Bastos *et al.*, 2015; EPA, 2013).

2.5.2 Nitrates and Nitrites

Nitrates can be introduced into the aquatic environment through natural processes such as decomposition of organic matter and nitrogen fixation process (Raymond *et al.*, 2004). It can also enter by human induced activities such as runoffs from agricultural lands, wastewater discharges and fossil fuels combustion (Grizzetti *et al.*, 2011). All these activities have detrimental impacts on the aquatic environment and human health (Grizzetti *et al.*, 2011). High nitrates levels in aquatic biome induces rapid growth of algae blooms which cover the water surface and prevent diffusion of oxygen into water bodies, causing anoxic conditions (Serediak

and Prepas, 2014). Consequently, the fauna and flora species in the affected environment will die and decay causing stinky smell, and overall process is eutrophication (Serediak and Prepas, 2014; WHO, 2002).

Drinking water which is contaminated with elevated nitrites is harmful to human health (WHO, 2011). This is due to the role nitrites play in the formation of nitrosamines, which are reported as carcinogens (Porché, 2014). Nitrite ions also play a role in “blue baby” syndrome and gastric cancer. Blue baby syndrome, also known as methaemoglobin, occurs when nitrite oxidizes haemoglobin into a very stable compound, methaemoglobin (Chan, 2011; Porché, 2014; Bryan and Grinsven, 2013). Methaemoglobin is therefore unable to transport oxygen and infants develop a blue skin colour (Chan, 2011; Porché, 2014). Excessive nitrites in drinking water are believed to be introduced by processes such as precipitation, groundwater movement and runoffs (Porché, 2014). Nitrates are also hazardous because they are converted to nitrites in the presence of reducing agents such as zinc, aluminium and cadmium or by reductase enzymes as illustrated in equation 2 below (Porché, 2014). These nitrites react with secondary amines to form carcinogenic compounds that are harmful to human health (Bryan and Grinsven, 2013; Porché, 2014). Despite the health hazards associated with nitrites, some health benefits are still observed, such as blood pressure regulation and maintenance of vascular homeostasis (Bryan and Grinsven, 2013; Hord *et al.*, 2009).



The toxicity of these nutrients have triggered the threshold values to be set by responsible institutions both globally and in South Africa. Table 2.1 summarises the recommended threshold values by South African National Standards (SANS: 241: 2015) and World Health Organisation (WHO, 2011).

Table 2.1: Nutrients standards (SANS 241, 2014; WHO, 2011).

Nutrients	SANS 241 (mg/L)	WHO standards (mg/L)
Ammonia as N	≤1.5	-
Nitrates as N	≤11	11
Nitrites as N	≤0.9	0.9

2.6 Heavy metals

According to Khayatzaheh and Abbasi (2010) heavy metals are defined as: (a) metals with atomic number 23 on a periodic table starting with vanadium and excluding rubidium, yttrium, caesium, barium and francium, (b) metals denser than 5 g/cm³ and (c) toxic metals. Nagajyoti

et al. (2010) on the other hand describes heavy metals in a general collective term, as the group of metals and metalloids with atomic density greater than 4 g/cm³, or 5 times or more, greater than water. The most common polluting heavy metals are arsenic, cadmium, chromium, copper, mercury, lead, nickel, and zinc (Ang, 2008). The first five are candidate heavy metals in this research. These are the most toxic and can occur naturally or be introduced by human activities (Varol and Sen, 2012; Rai, 2008). Arsenic in this study will be treated as a heavy metal following Nagajyoti definition.

Freshwater ecosystems are threatened by heavy metal pollution throughout the world. Heavy metals and other pollutants are introduced into rivers naturally through volcanic eruptions and erosion of rocks (Solomon, 2008). They occur in various forms in the aquatic environment either as ions dissolved in water, vapour, minerals in rocks and in particulate forms. Anthropogenically, heavy metals can be released directly into rivers through mining and smelting operations, untreated and partially treated effluent, disposal of toxic metal laden effluents, metal complexes from different industries and combustion of fossil fuels (Varol and Sen 2012; Rai, 2008). Heavy metals can also be released indirectly by surface runoff from roads and farming lands consequent to application of heavy metal-containing chemicals.

Once they have entered the aquatic environment, some heavy metals get distributed between the aqueous phase while others are adsorbed into bed sediments (Varol and Sen 2012). The adsorbed metals act as a sink and secondary source of contamination in water and aquatic biota. Such metal accumulation is influenced by pH, concentration of metals, anthropogenic inputs and other chemical parameters of the river. Under favourable conditions, metals can revert to the aqueous phase, posing multiple adverse effects on the aquatic ecosystem (Edokpayi *et al.*, 2016). The toxic nature of heavy metals is experienced when they first enter an organism and have contact with cells. They enter into fish by diffusion through gills and skin, by drinking contaminated water or eating sediments and by eating other organisms that have prior exposure to toxic metals; while they are introduced to humans by diffusion through lungs and skin as well ingestion of contaminated water and food (Solomon, 2008).

Contaminated sediments affect benthos, exposing worms, crustaceans and insects to high concentrations of toxins that kill benthic organisms, reducing food availability for larger animals such as fish (Edokpayi *et al.*, 2016). Some contaminants in the sediment are taken up by benthic organisms thus bio-accumulate and transfer to larger animals through the food web, they keep moving up the food chain and biomagnifying (Solomon, 2008; Edokpayi *et al.*, 2016). These heavy metals can also kill some crops or disrupt their nutrient uptake (Edokpayi *et al.*, 2016).

Heavy metals react with various components of surface water (carbonates, sulphates, organic, humic, fulvic compounds and amino acids) to form insoluble compounds which are harmless to aquatic organisms (Solomon, 2008). Some sink and accumulate in bottom sediments and when water pH declines during acidic episodes, these metals are released into the water column at levels toxic to aquatic biota (Solomon, 2008; Edokpayi *et al.*, 2016). On the other hand, low concentrations of heavy metals cause chronic stress which may result in lower body weight and smaller size, exposing them to competition for food and habitat (Rosado, 2016).

Multiple use of heavy metals for industrial, domestic, agricultural, medical and technological purposes led to their wide distribution in the environment, posing health and ecological risks (Tchounwou *et al.* 2014). The intensity of metal toxicity is influenced by a combination of factors that include chemical type, dose, route of exposure, age, gender, genetics, and nutritional status of affected individuals (Szczewski *et al.*, 2009; Tchounwou *et al.* 2014). Arsenic, cadmium, chromium, lead, and mercury are listed as the priority metals of public health significance due to their high toxicity even at lower concentrations (Nagajyoti *et al.*, 2010). These metals are also classified as human carcinogens (known or probable) by the U.S. Environmental Protection Agency, and the International Agency for Research on Cancer (Tchounwou *et al.*, 2014).

Human beings exposed to heavy metals may suffer from neurobehavioral disorders which include amongst others; fatigue, insomnia, depression, gastric symptoms, and motor symptoms (Rai, 2008). Heavy metals' exposure is also linked to developmental obstruction, multiple cancers, kidney malfunction, and death (Szczewski *et al.*, 2009). According to Rai, (2008), mercury intake through fish and aquatic foods can cause fatal brain damage, while cadmium causes renal dysfunction and bone disease (Itai-Itai). Some experiments conducted on animals exposed to heavy metals confirm presence of oxidative stress (Szczewski *et al.*, 2009).

2.6.1 Arsenic

Arsenic is evenly distributed in the environment with average concentration levels of approximately 2mg/kg and occurs as arsenates in combination with sulphides and other metallic ores (DWAf, 1996). It rarely occurs in the elemental form. Elevated levels are often experienced mainly because of the anthropogenic sources, mostly industries such as metallurgy, glassware, carpentry and ceramics, as well pesticide manufacturing (Govind and

Madhuri, 2014). On rare cases, geological outcrops of arsenic minerals occur. (DWAF, 1996; Govind and Madhuri, 2014). The toxicity of arsenic is mainly due to the metal being slowly excreted from the body hence has bioaccumulation effect.

Arsenic toxicity can either be chronic or acute. The chronic form is characterised by hyperpigmentation and cancer and its acute toxicity can cause death in extreme events (DWAF, 1996). Arsenic poisoning is expressed by nerve damage coupled with sensory loss of peripheral nervous system (DWAF, 1996). Other carcinogenic effects linked to arsenic poisoning include kidney, prostate, skin, liver, lymph, bladder and lung cancers (Govind and Madhuri, 2014). The arsenic induced cancer risks were mostly reported in smelter workers, arsenical pesticides producers and users (Govind and Madhuri, 2014).

2.6.2 Cadmium

Cadmium is a relatively soft, bluish-white metal resembling zinc in most of its properties, it is highly toxic with high bioaccumulation effects (Sharma and Agrawal, 2005). It is partially soluble under neutral or basic conditions and highly soluble at acidic pH (DWAF, 1996). It naturally coexists with zinc and lead and as a result, it is produced as a by-product of refining processes of these ores (Govind and Madhuri, 2014). Cadmium is mostly used in batteries and high quality rechargeable power sources (Flora and Agrawal, 2017). The cadmium coatings are known to provide good corrosion resistance particularly in stressful environments such as marine and aerospace, where there is a requirement for high safety and reliability (Govind and Madhuri, 2014). It is also used as a pigment, stabilizer in polyvinyl chloride and alloys as well as other electronic compounds and it constitutes various detergents, phosphate fertilizers and refined petroleum products (Flora and Agrawal, 2017; Govind and Madhuri, 2014).

The most commonly found cadmium ore is cadmium sulphide (DWAF, 1996). The sulphide, carbonate and hydroxide salts of cadmium are usually insoluble in water, whereas salts of cadmium chloride, nitrate and sulphate are highly soluble in water (DWAF, 1996). Cadmium reacts well with sulphydryl groups thus the tendency of cadmium to bioaccumulate in food chains. In the environment, the coexistence of cadmium and zinc is in the ratio of zinc to cadmium of 300:1 respectively (DWAF, 1996).

High concentrations of cadmium are toxic and can result in kidney malfunction (Agarwal *et al.*, 2011). Cadmium also causes gastroenteritis similar to the one caused by micro-organisms (DWAF, 1996). Elevated levels may also result in pulmonary diseases and lung cancer

(Govind and Madhuri, 2014). Bone defects also known as *osteomalacia* and *osteoporosis*, that are usually caused by acute cadmium exposure have been reported in humans and animals (Agarwal *et al.*, 2011). Besides, it is also known to cause increased blood pressure, increased prevalence of stroke and heart failure as well as myocardial diseases in animals (Agarwal *et al.*, 2011). The half-life of cadmium in the body is several decades, hence is important to avoid its exposure (Sarkar *et al.*, 2013; Flora and Agrawal, 2017; DWAF, 1996).

2.6.3 Chromium

Chromium exists as both trivalent Cr(III) and hexavalent Cr(VI) in drinking water, with the hexavalent form being highly toxic oxidised state of metallic chromium (Govind and Madhuri, 2014; Sharma and Agrawal, 2005; DWAF, 1996). It exists as a yellow dichromate salt in neutral/alkaline conditions; and the salt changes to orange under low pH (DWAF, 1996). The hexavalent chromium is highly water soluble (Bourotte *et al.*, 2009; DWAF, 1996). Its reduced forms, Cr(II) and Cr(III), are comparatively less soluble, have lower toxicity indices and do not pose a serious health hazard (DWAF, 1996).

Furthermore, chromium can exist in six oxidation states (from 0 to VI), but the trivalent and hexavalent forms are the most stable and readily available forms in aquatic environments (DWAF, 1996). Anthropogenic release of Cr(VI) comes from chemical industries and products, including paints, pigments, paper, electroplating and leather tanning (DWAF, 1996; Bourotte *et al.*, 2009). In addition, chromium is naturally occurring in aquifer minerals as the 21st abundant most element of the earth's crust (DWAF, 1996). Weathering of trivalent chromium containing minerals is a natural source of hexavalent chromium in groundwater (Shadreck and Mugadza, 2013). In drinking water, Cr(VI) occurs as an oxyanion (CrO_4^{2-}) while its trivalent has a very low solubility and can exist as various Cr(III) solids (DWAF, 1996).

Exposures to Cr(VI) through respiratory, oral, and dermal pathways are linked to skin ulcerations, lung and gastrointestinal cancers, while Cr(III) is considered a micro-nutrient and aids in the metabolism of glucose and lipids (Sharma and Agrawal, 2005). Low chromium levels can irritate skin and cause ulcer (DWAF, 1996). On the other hand, its chronic exposure can cause damage to kidney, liver, nerve and circulatory tissues (DWAF, 1996). It bioaccumulates in aquatic animals causing toxicity to fish and organisms that depend on fish up the food chain (DWAF, 1996; Govind and Madhuri, 2014). Govind and Madhuri (2014) report that introduction of hexavalent chromium salts into aquatic ecosystem causes low growth and survival rates in aquatic species.

2.6.4 Copper

Copper is an essential nutrient to plants, animals and humans due to its involvement in enzyme formation in humans (Sharma and Agrawal, 2005). Copper occurs as metallic copper (0), cuprous copper(I) and cupric copper(II) and its principal source in domestic water is the plumbing systems in areas with soft or acidic waters (Stern, 2010; DWAF, 1996). High concentrations of copper causes a bad taste to water the consumption of which is discouraged (DWAF, 1996). Copper is a naturally occurring metallic element that occurs in soil at an average concentration of about 50 parts per million (ppm) (DWAF, 1996). Blue-green stains left in bath fixtures are a sign of the presence of copper in water (DWAF, 1996). Other releases of copper to the environment include application of plant diseases and antifouling treatments, making coins as well as pigments (Ashish *et al.*, 2013; Govind and Madhuri, 2014).

In addition to being useful in manufacturing, copper is also a vital dietary nutrient, with only small amounts necessary for well-being. It appears in several enzymes, facilitates the absorption of iron, and helps to transmit electrical signals in the body (Ashish *et al.*, 2013). Ingestion of high concentrations of copper results in gastrointestinal disturbances and possible liver, kidney and red blood cells damage accompanied by severe mucosal irritation and corrosion; widespread capillary damage, central nervous system irritation and depression (DWAF, 1996). Copper toxicity symptoms include Wilson's disease, blue green diarrhoea and saliva, acute haemolysis and kidney malfunction (Sharma and Agrawal, 2005).

2.6.5 Mercury

Mercury has a rare geological occurrence and concentrations thus found at very low concentration levels in the environment. It occurs naturally exhibiting three oxidation states, the metal, mercury(I) and mercury(II) (DWAF, 1996; Kubán~ *et al.*, 2009). It is also found as organomercurials, the most important of which is methyl mercury (DWAF, 1996). The occurrence of mercury contaminants in water is predominantly site-specific and relates to identifiable site-specific discharges (DWAF, 1996). Mercury and organomercurial complexes are severely neurotoxic, their intake may occur through air, food or water with fish and fish products being the major source of mercury exposure (Rice *et al.*, 2014; DWAF, 1996). This is because mercury has a strong affinity for -SH and -OH groups and is therefore strongly associated with sediments and suspended solids where bacterial methylation occurs under anoxic conditions (DWAF, 1996). Methyl mercury readily accumulates in food chains and is the common form found in fish and mammal tissues (Abarikwu, 2013; DWAF, 1996).

Emissions from volcanoes, degassing of earth's crust, evaporation from natural water bodies and rock deposits are major sources of mercury (Govind and Madhuri, 2014). Mercury occurs

in the atmosphere as vapours and as volatilised organic mercury compounds from combustion of fossil fuels and municipal or medical waste (DWAF, 1996). Atmospheric mercury enters terrestrial and aquatic habitats through particle deposition, precipitation and industrial pollution (DWAF, 1996). These are mainly chloralkali industry, the paint, fungicide industries, pulp and paper manufacturing processes (DWAF, 1996). Mercury is also used in dentistry, in thermometers and electrical equipment. Metal mining indirect discharges mercury into the atmosphere (Govind and Madhuri, 2014). The Hg is mostly occurring in an inert form as a gas and persists in the environment (DWAF, 1996).

Methylated mercury bioaccumulates over a long time and concentrates in organisms, causing neurotoxicological disorders and renal disturbances (Govind and Madhuri, 2014; Sharma and Agrawal, 2005). Inorganic mercury toxicity is linked to tremors, gingivitis and minor psychological changes, together with spontaneous abortion and congenital malformation in humans (Govind and Madhuri, 2014; DWAF, 1996). Monomethyl mercury causes damage to brain and central nervous system, while foetal and postnatal exposures cause abortion, congenital malformation and development changes in young children (Govind and Madhuri, 2014; Raikwar *et al.*, 2008). It is noted from Govind and Madhuri (2014) that in Japan, of the 2252 people affected 1043 died from Minamata disease caused by high mercury pollution from a chemical plant. The Minamata disease is characterized by fatigue, loss of memory and concentration, constriction of visual field, cortical blindness (Sharma and Agrawal, 2005).

2.6.6 Lead

Lead is a highly malleable and ductile bluish-white soft metal with atomic number 82, atomic weight 207.19 g, specific gravity 11.34, melting point 327.5 °C and boiling point 1740 °C (Raikwar *et al.*, 2008). It is highly resistant to corrosion and mostly occurs as galena (lead sulphide), cerussite (lead carbonate) and anglesite (lead sulphate) (DWAF, 1996). It accumulates in sediments and soils, gets absorbed by vertebrates and deposits in the skeleton (DWAF, 1996; Raikwar *et al.*, 2008). Its many industrial uses result in lead contamination in water supplies (Govind and Madhuri, 2014; DWAF, 1996).

Lead exposure in young children should be minimised as continuous exposure to relatively low concentrations causes neurological impairment in fetuses and young children (DWAF, 1996). High levels of lead are related to problems in the synthesis of haemoglobin (Hb), effects on kidneys, gastrointestinal tract (GIT), joints and reproductive system as well as acute or chronic damage to nervous system (Govind and Madhuri, 2014). In adults, the neurological effects are less pronounced causing anaemia and lead colic (Sharma and Agrawal; 2005). Sharma and Agrawal (2005) are of the view that lead toxicity is results from direct interference

with enzyme activity and displacement of essential metals from metalloenzymes and the introduction of inorganic lead is by ingestion and adsorption through the gastrointestinal tract, respiratory tract and inhalation. The kidney, heart, renal tubular, reproductive system and liver are targets for lead toxicity before storage into the bones (Sharma and Agrawal, 2005; Agarwal *et al.*, 2011).

Table 2.2: Heavy Metal Standards (SANS 241, 2015; WHO, 2011).

Heavy Metals	SANS 241 (µg/L)	WHO standards (mg/L)
Arsenic	≤10	0.01
Cadmium	≤3	0.003
Chromium	≤50	0.05
Copper	≤2000	2
Mercury	≤6	0.006
Lead	≤10	0.01

Chapter 3

RESEARCH DESIGN AND METHODOLOGY

3.1 Research Design

This research involves two design techniques namely field and experimental designs. Field design is a technique where an activity is aimed at collecting primary data by direct observation. This technique makes it possible for the researcher to go to field work (KRC) and collect water and sediment samples for analysis in the laboratory and to do some observations. It also assisted the researcher in collecting data on site regarding physico-chemical water quality parameters such as temperature, pH, dissolved oxygen and conductivity. Another design technique that is employed in this study is the experimental design which basically uses manipulation and controlled testing to understand causal processes. Generally, one or more variables are manipulated to determine their effect on a dependent variable. In this instance, both top and bottom water samples were analysed for heavy metals and nutrients content in the laboratory using ICP-OES, Ion Chromatography and Flow Injection Analysis respectively. GIS software was applied to model the data.

3.2 Research Methodology

3.2.1 Ammonia analysis

For ammonia analysis, top and filtered bottom water samples were injected into a flow injection analysis carrier stream to which a complexing buffer, alkaline phenol and hypochlorite were added, figure 3.1 refers.

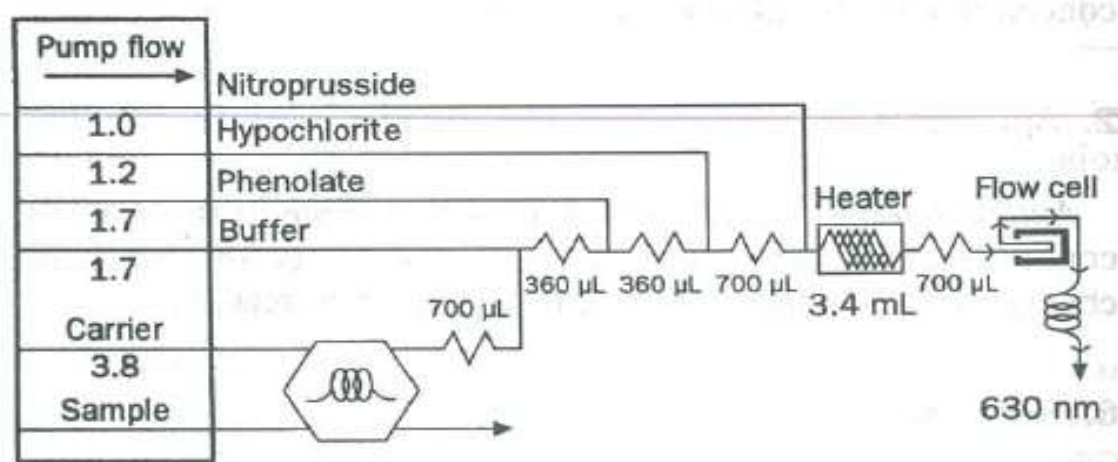


Figure 3.1: Flow injection analysis; Ammonia manifold (Eaton *et al.*, 2005).

The resulting reaction from figure 3.1, also known as Berthelot reaction produces the blue indophenol dye for which the blue colour is intensified by addition of nitroferricyanide (Nitroprusside). The resulting peak's absorbance is measured at 630 nm with the peak area being proportional to the ammonia concentration in the original sample.

3.2.1.1 Equipment and apparatus for ammonia analysis

Flow injection analysis equipment consisting of:

- FIA injection valve with sample loop
- Multichannel proportioning pump
- FIA manifold with tubing heater and flow cell
- Absorbance detector, 630 nm, 10 nm band-pass
- Injection valve control and data acquisition system

3.2.1.2 Reagents for ammonia analysis

All chemicals and reagents used were of analytical grade and they are:

- Disodium ethylenediamine tetra-acetate
- Sodium hydroxide
- Phenol
- Sodium hypochlorite
- Sodium nitroprusside (sodium nitroferricyanide)
- Ammonium chloride

3.2.2 Nitrate and Nitrite analysis

The method of analysis employed for analysis of nitrates and nitrites is ion chromatography, 4110B of the standard methods presented by American Public Health Association, American Water Works Association and Water Environment Federation (2005). Top and bottom filtered water samples were injected into a stream of eluent and passed through a series of ion exchangers. The anions of interest (nitrates and nitrites) were separated based on their relative affinities for a low capacity, strongly basic ion exchanger. The separated anions were then directed through a suppression device that provides continuous suppression of eluent conductivity and enhances analyte response. In the suppression chamber, separated anions are converted to their highly conductive acid forms and then measured by conductivity. Their identification was based on retention time compared to standards and quantification through measurement of peak area/ height which is proportional to sample concentration.

3.2.2.1 Equipment and apparatus for nitrate and nitrite analysis

- Ion chromatograph
- Guard column
- Analytical column
- Conductivity detector
- Pump
- Data acquisition system
- Sample injector

3.2.2.2 Reagents for analysing nitrate and nitrite

- Reagent water
- Borate
- Sodium gluconate
- Deionised water
- Gluconic acid
- Boric acid
- Glycerol
- Lithium hydroxide monohydrate
- Acetonitrile

3.2.3 Heavy metals analysis

Heavy metals analysis was performed using ICP-OES, method 3120B of the American Public Health Association, American Water Works and Water Environment Federation (2005) of the standard methods. The mode of separation of an ICP-OES is through an ICP source which consists of flowing stream of argon gas ionised by an applied radio frequency oscillating at 27.1 MHz. The ionised gas is inductively coupled by a water-cooled coil surrounding a quartz torch that supports and confines the plasma. A sample aerosol was generated in an appropriate nebulizer and spray chamber and was carried into the plasma through an injector tube located within the torch. Constituents atoms in the sample aerosol were subjected to very high temperatures that resulted in the complete ionisation and excitation of atomic emissions thus providing atomic emission spectra. Every sample was analysed by alternating each with the calibration blank. Rinsing was done for at least 60 s with dilute acid between samples and blanks. After introducing each sample or blank the system was allowed to equilibrate before starting signal integration. Careful examination was employed in each analysis to eliminate any carry-over memory effects that may be observed. In the cases where the carry-over memory was observed, rinsing was done until proper blank values were observed.

3.2.3.1 Equipment and apparatus for heavy metals analysis

- ICP source
- Spectrophotometer

3.2.3.2 Reagents and standards for analysing Heavy Metals

Reagents of ultra-high-purity grade are highly recommended; redistilled acids are acceptable. Except as noted, all salts must be dried at 105°C for 1 h and be stored in a desiccator before weighing. Use of deionized water prepared by passing water through at least two stages of deionization with mixed bed cation/anion exchange resins for preparing all calibration standards, reagents, and for dilution is highly recommended.

- Hydrochloric acid, HCl, concentrated and 1+1.
- Nitric acid, HNO₃, conc.
- Nitric acid, HNO₃, 1+1: Add 500 mL concentrated HNO₃ to 400 mL water and dilute to 1 L.
- Standard stock solutions for heavy metals of interest

3.2.4 Sampling

3.2.4.1 Top and bottom water sampling, preservation and storage

Top water samples were taken 5 cm below water surface while the bottom water samples were taken from the benthic zones in the river system. Care was taken to ensure that samples were taken as far away from riverbanks as is safe. The samples were collected using polyethylene ether bottles which were thoroughly rinsed with reagent water. There were three grab samples taken at each sampling location and then composited to a 1 L composite sample for laboratory analysis. The collected water samples were placed in a cooler box and ice chilled immediately after collection. Samples were transported to a laboratory where they were cooled to 4°C and analysed within 48 hours. All samples were properly labelled, with appropriate identifications considering their sampling locations together with GPS coordinates and insitu parameters recorded. Insitu parameters recorded are PH, temperature, dissolved oxygen and electrical conductivity using YSI 1001 PH Probe Kit while the coordinates were taken using Garmin GPS. Figure 3.2 below shows the sampler when taking insitu parameters at Patising stream.



Figure 3.2: Measuring insitu water quality parameters.

3.2.5 Mapping of Results

Coordinates from all sampling locations were recorded with a Garmin Global Positioning System and were used as an input data in conjunction with concentrations of analytes understudy in Arc-GIS software to map the levels of nutrients and heavy metals within the catchment. Kriging modelling was used to model the concentration levels of selected nutrients and heavy metals understudy. The colour intensity was used to denote the concentration magnitude of analytes understudied with darker colours representing presumably higher levels.

Chapter 4

RESULTS AND DISCUSSIONS

4.1 Results

This chapter presents results of the KRC study on surface water chemistry particularly on selected nutrients and heavy metals. Nutrients that were of interest are ammonia, nitrates and nitrites while heavy metals included copper, chromium, arsenic, cadmium, lead and mercury. Results are presented in chart format, tables as well as maps modelled with Arc-GIS. The obtained levels are compared to SANS: 241:2015 and WHO 2011 standards and possible risks inherent are discussed, giving special attention to human health and ecological aspects (aquatic fauna). The insitu water quality parameters were also measured during sampling and they are temperature, PH, dissolved oxygen and electrical conductivity, refer to appendix 2A and 2B

4.1.1. Nitrates/nitrites

The concentration levels of nitrates/nitrites for both top and bottom samples as well as dry and wet seasons are represented in Table 4.1 below. Their spatial distribution within the study area follows from figure 4.2 to figure 4.5 below. The highest levels (include these levels) of nitrates /nitrites were observed at Patising tributary.

Table 4.1: Nitrates/nitrites concentration during dry and wet seasons in Khubelu River catchment.

Sampling Points	Nitrate/Nitrite as N (mg/L)-dry season	Nitrate/nitrite as N (mg/L)-wet season
KR DS - Bottom	10.04	2
KR DS- Top Sample	14.98	2.9
KR US- Bottom	0.4	<0.1
KR US- Top Sample	0.13	0.47
Patising Bottom	167	65.95
Patising Top Sample	114.1	67.8

NB: KR- Khubelu River, DS- Down Stream, US-Up Stream.

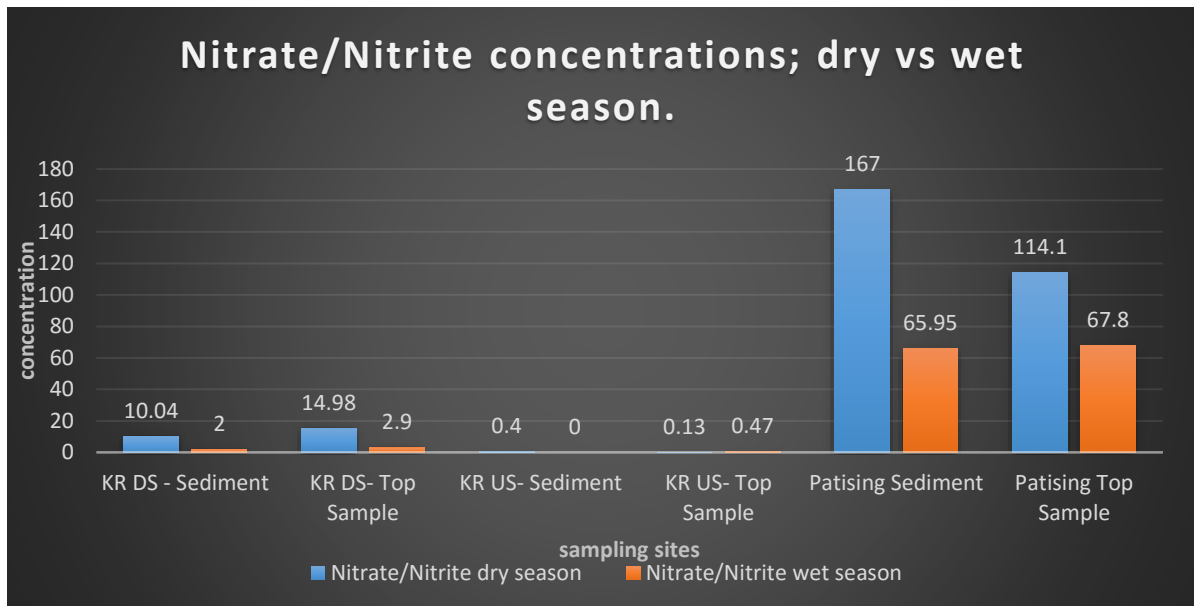


Figure 4.1: Comparison of nitrates/nitrites concentration levels for dry and wet seasons.

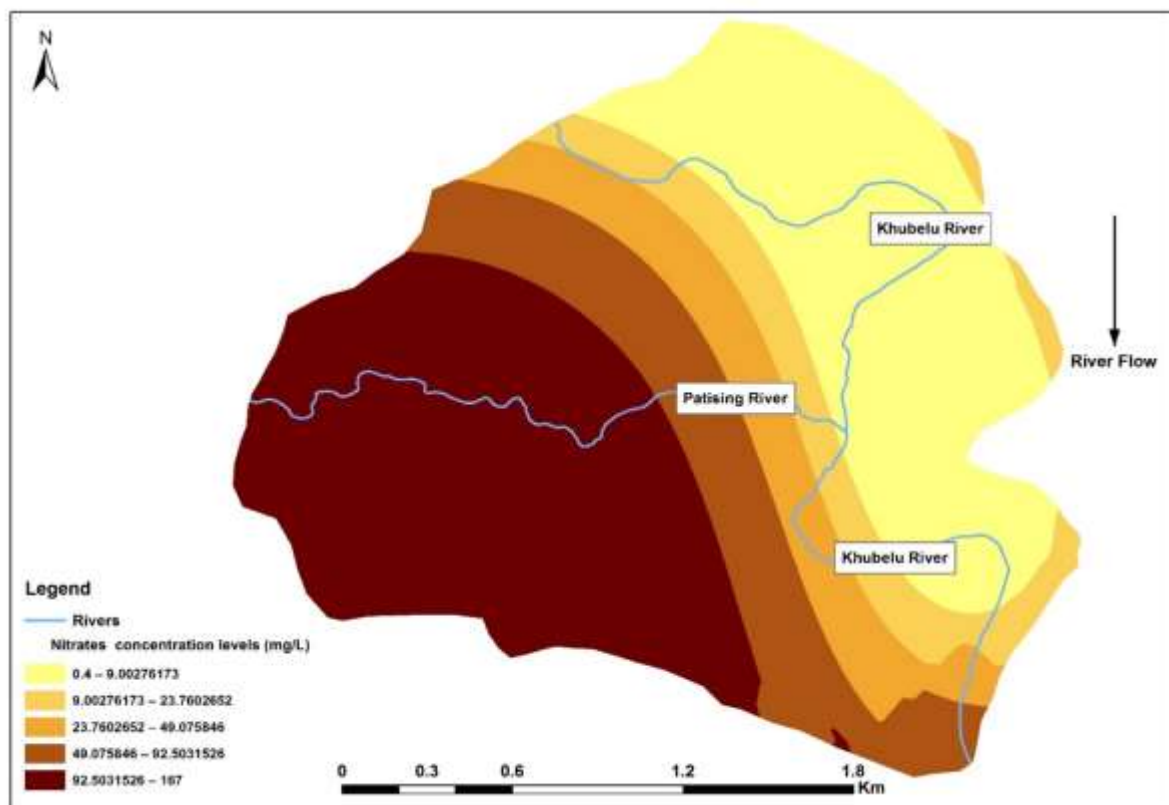


Figure 4.2: Distribution of nitrates/nitrites concentration for bottom samples during dry season

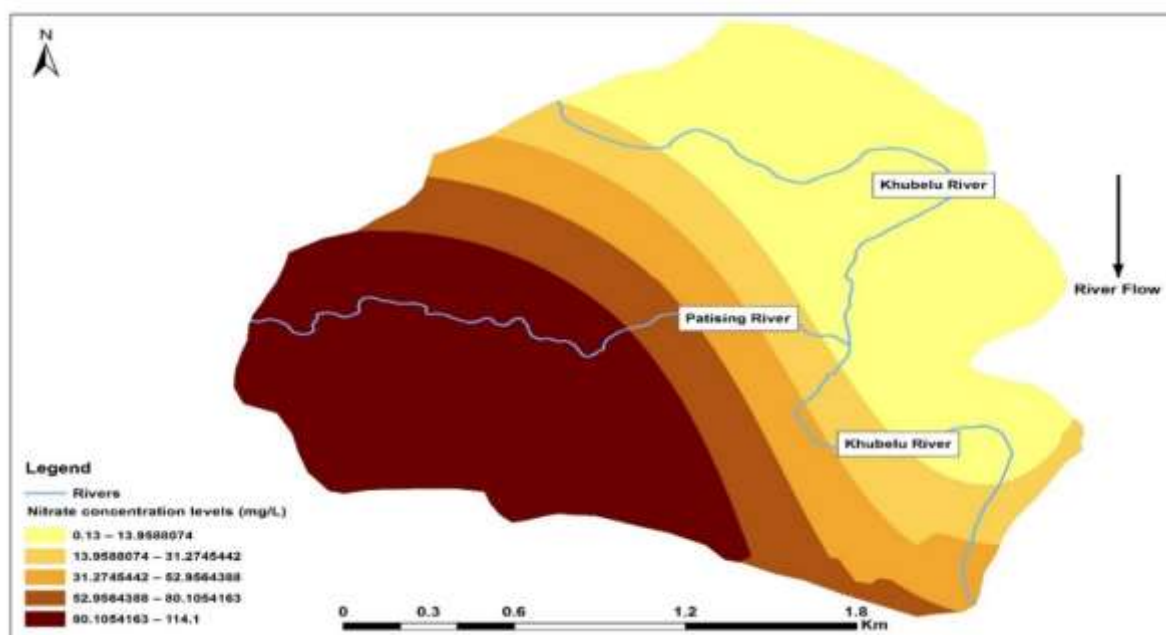


Figure 4.3: Distribution of nitrates/nitrites concentration levels for top samples during dry season.

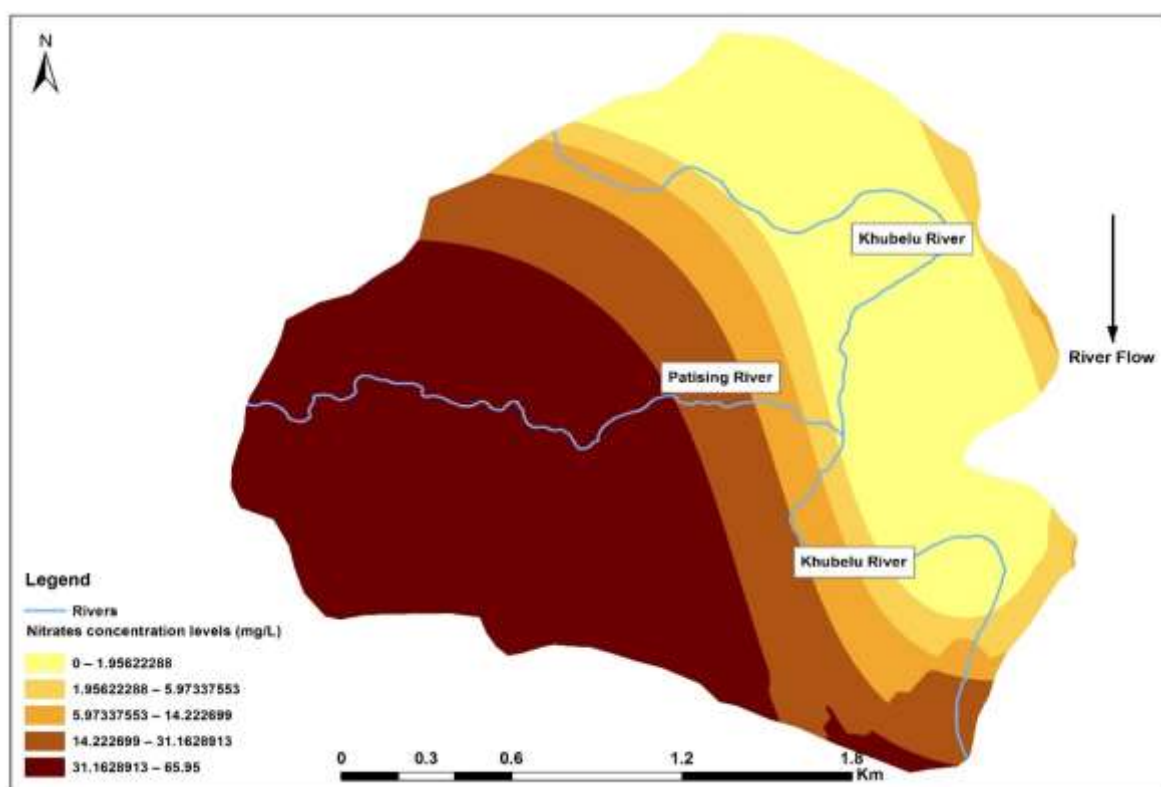


Figure 4.4: Distribution of nitrates/nitrites concentration levels for bottom samples during wet season.

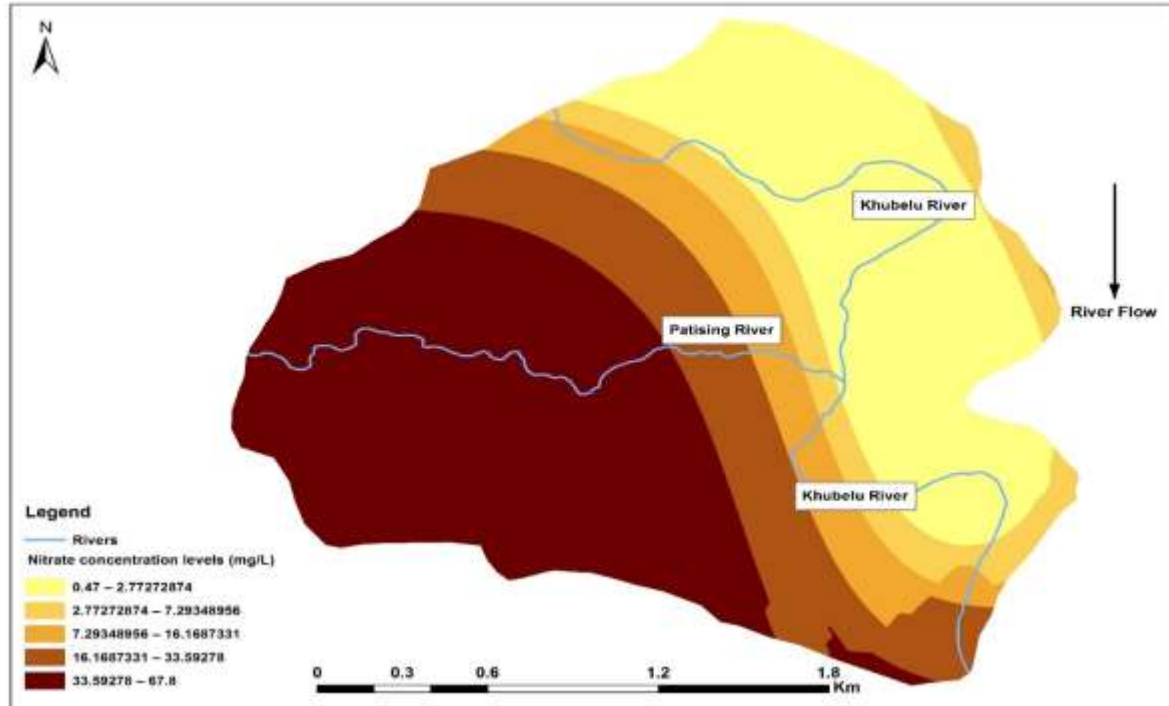


Figure 4.5: Distribution of nitrates/nitrites concentration levels for top samples during wet season.

4.1.2. Ammonia

Comparison of ammonia for both dry and wet season show a decline in concentration levels during wet season as shown in table 4.2 below.

Table 4.2: Comparison of ammonia levels during dry and wet seasons.

Sampling Points	Ammonia as N (mg/L)-dry season	Ammonia as N (mg/L)- wet season
KR DS - Sediment	0.92	0.24
KR DS- Top Sample	0.17	0.16
KR US- Sediment	0.46	0.25
KR US- Top Sample	0.18	0.15
Patising Sediment	1.89	0.68
Patising Top Sample	0.27	0.21

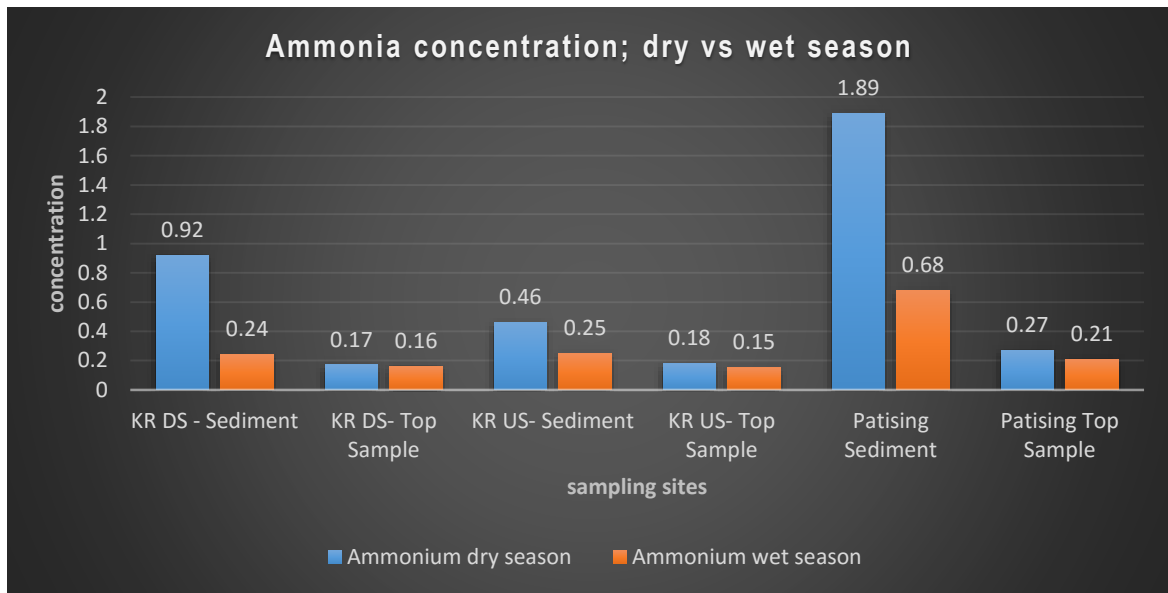


Figure 4.6: Comparison of ammonia levels during dry and wet seasons.

The distribution of ammonia during both dry and wet seasons for top and bottom samples is shown in figure 4.7 to figure 4.10.

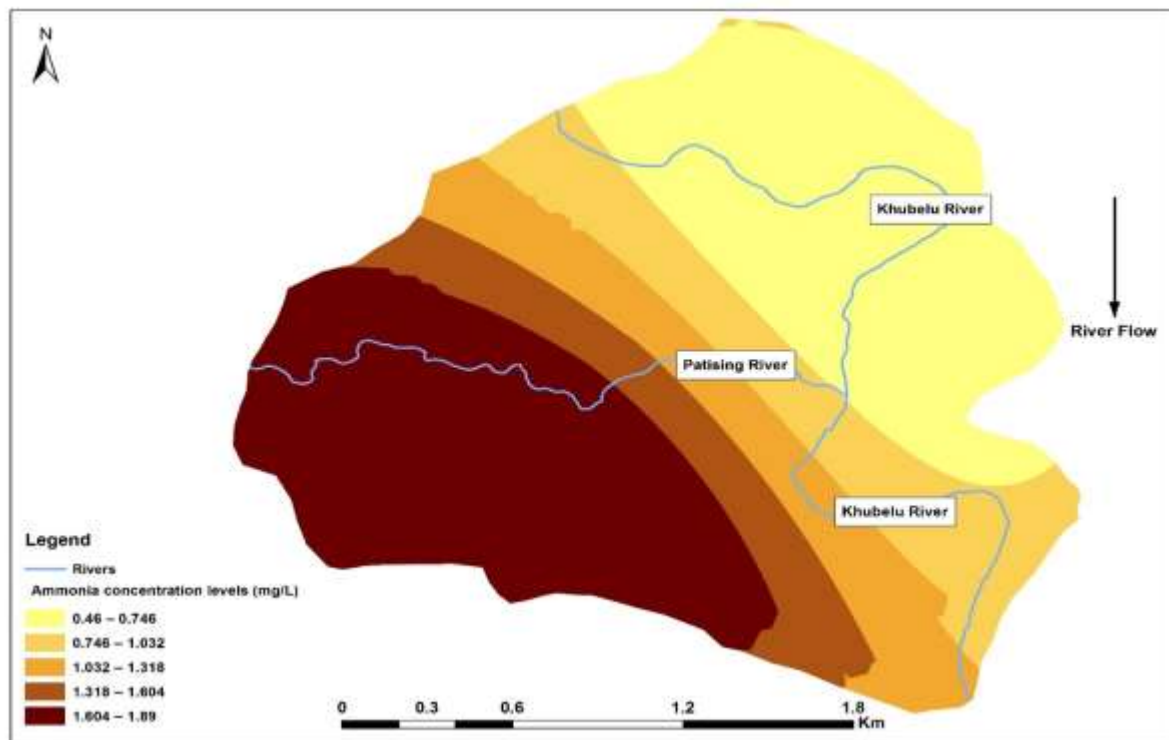


Figure 4.7: Distribution of ammonia concentration levels during dry season for bottom samples.

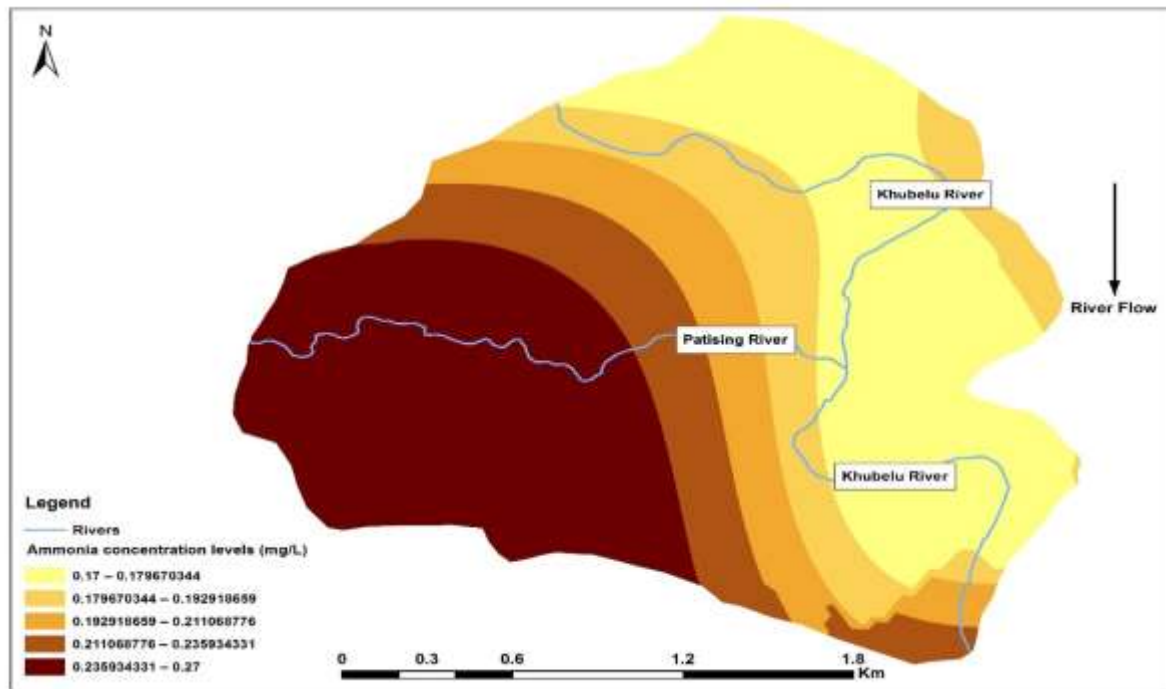


Figure 4.8: Distribution of ammonia concentration levels for top samples during dry season.

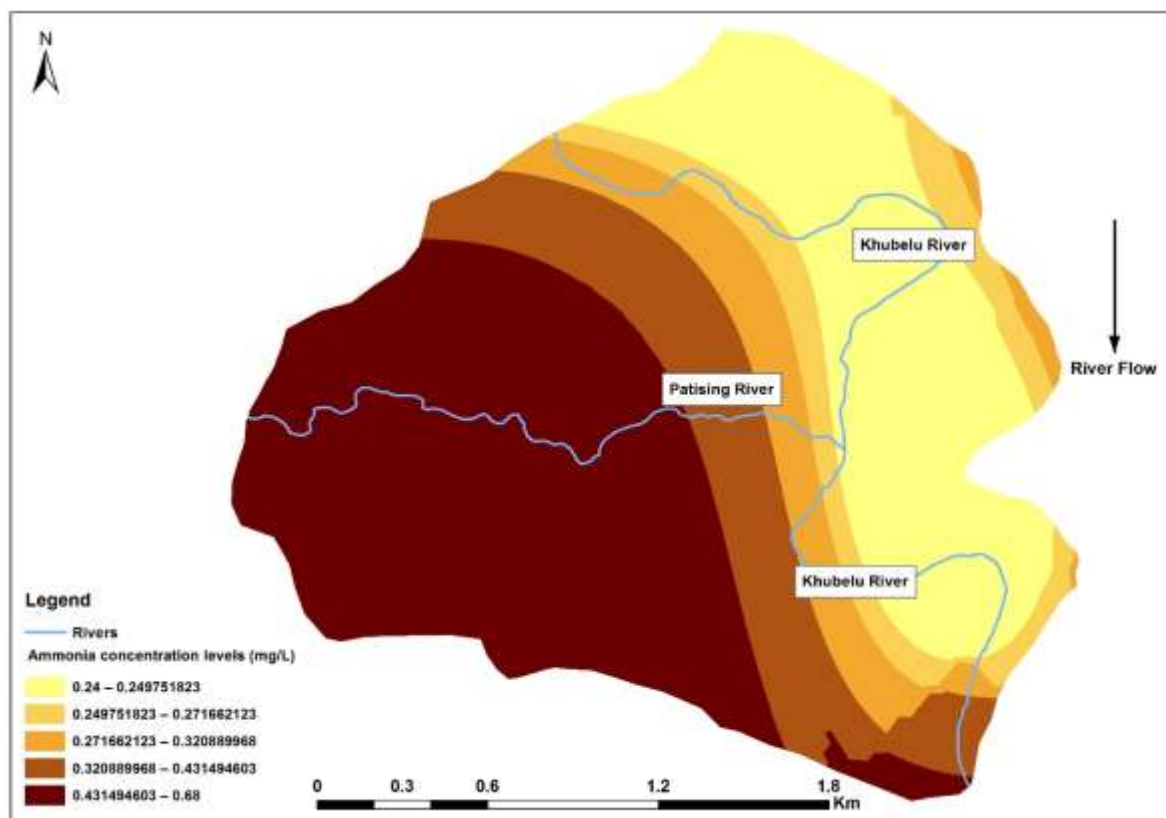


Figure 4.9: Distribution of ammonia concentration levels for bottom samples during wet season.

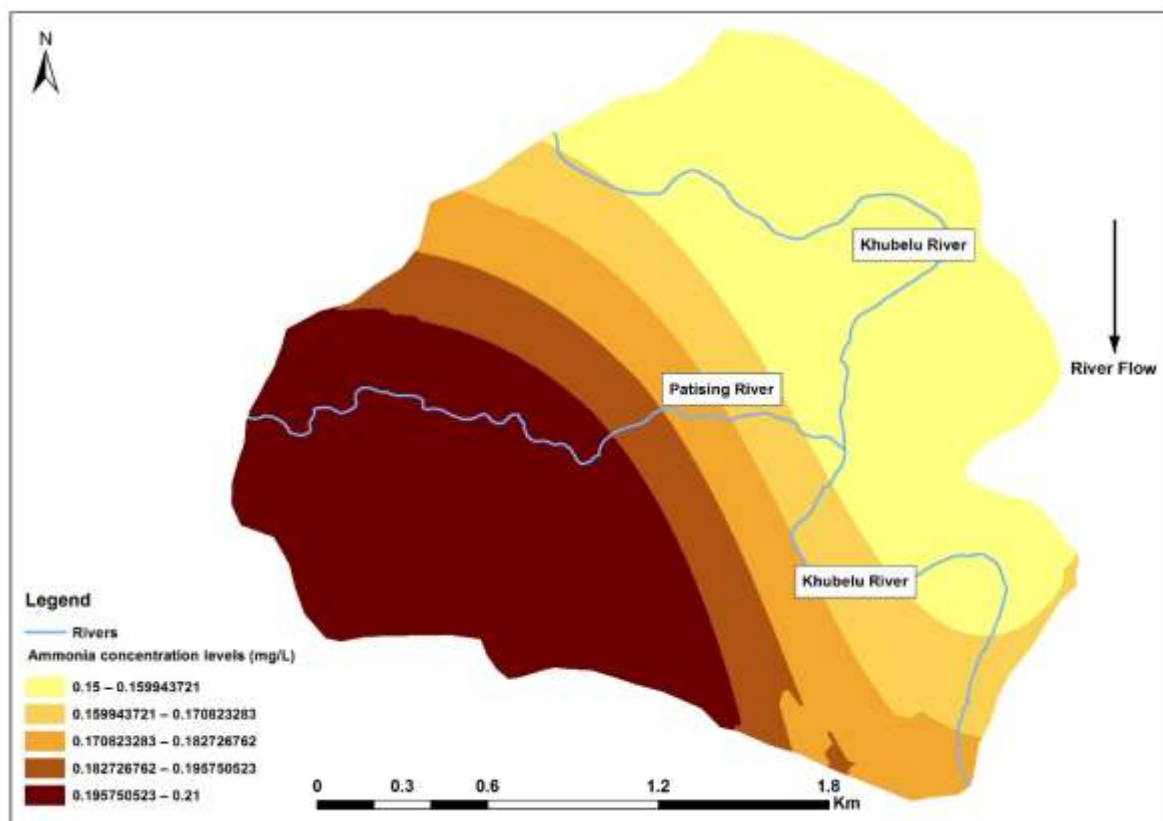


Figure 4.10: Distribution of ammonia concentration levels for top samples during wet season.

4.1.3. Heavy Metals

The concentration levels of the selected heavy metals analysed are as shown in table 4.3 below with graphical representation in figure 4.11 and distribution maps. Of the six heavy metals analysed, only three were detected during dry season; copper, lead and chromium. Amongst these three heavy metals, copper had the highest concentration level and was the only metal detected during wet season.

Table 4.3: Levels of heavy metals in Khubelu River catchment during dry season.

Sampling Points	Arsenic	Cadmium	Chromium	Copper	Mercury	Lead
KR DS - bottom	<0.0200	<0.0030	<0.020	0.146	<0.0001	0.02
KR DS- Top	<0.0200	<0.0030	<0.020	0.045	<0.0001	<0.015
KR US- bottom	<0.0200	<0.0030	0.03	0.175	<0.0001	0.019
KR US- Top	<0.0200	<0.0030	<0.020	0.039	<0.0001	<0.015
Patising bottom	<0.0200	<0.0030	0.046	0.219	<0.0001	0.017
Patising Top	<0.0200	<0.0030	<0.020	0.059	<0.0001	0.016

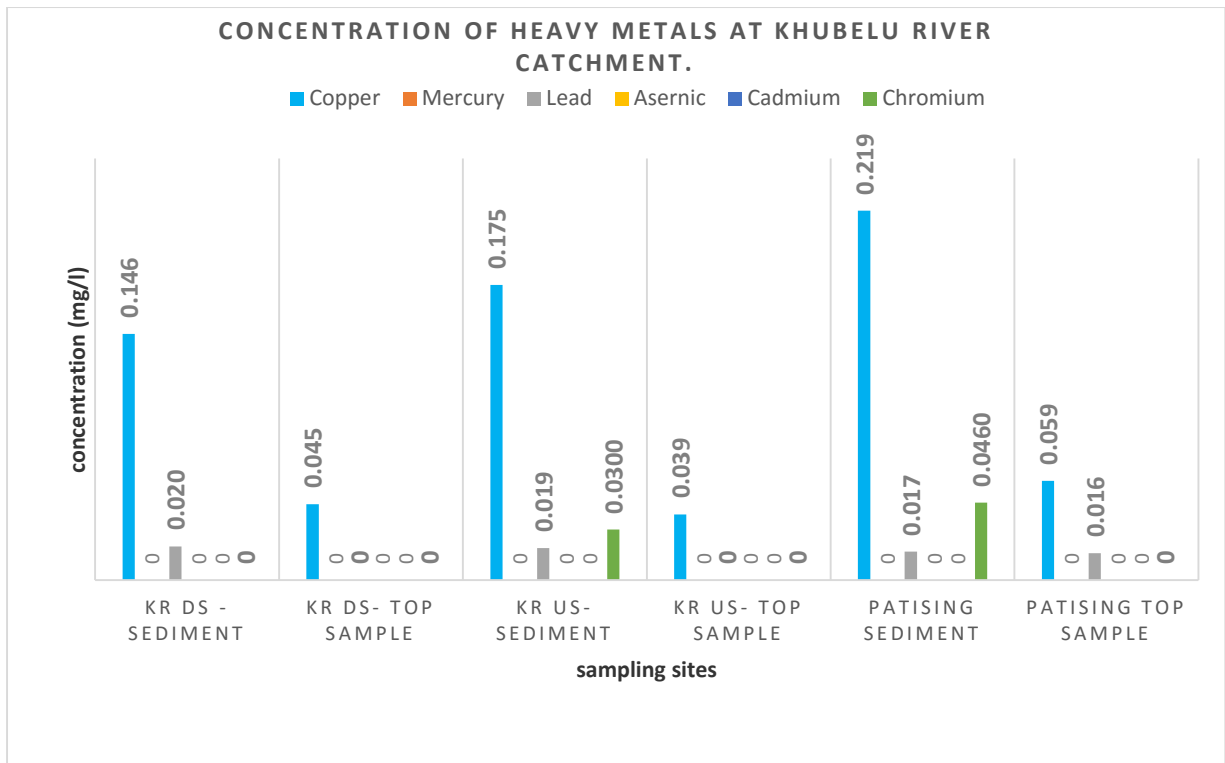


Figure 4.11: Levels of heavy metals in Khubelu River catchment during dry season

The distribution maps of copper, chromium and lead during dry season.

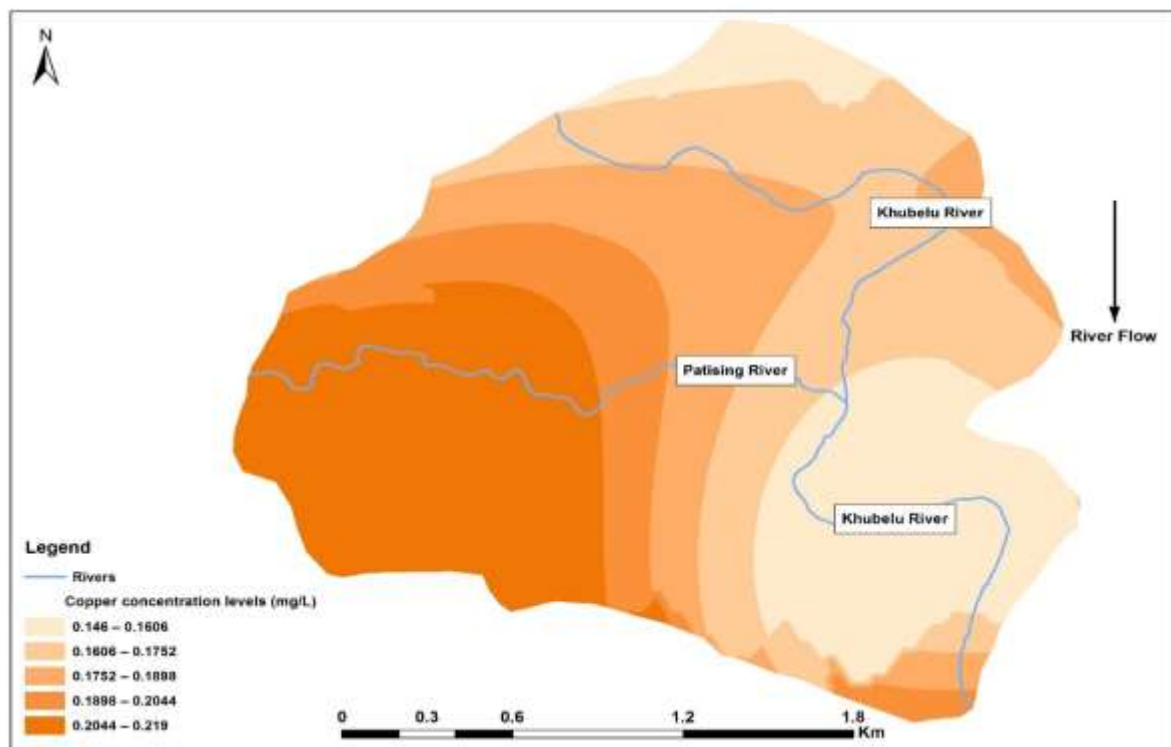


Figure 4.12: Distribution of copper concentration for bottom samples during dry season.

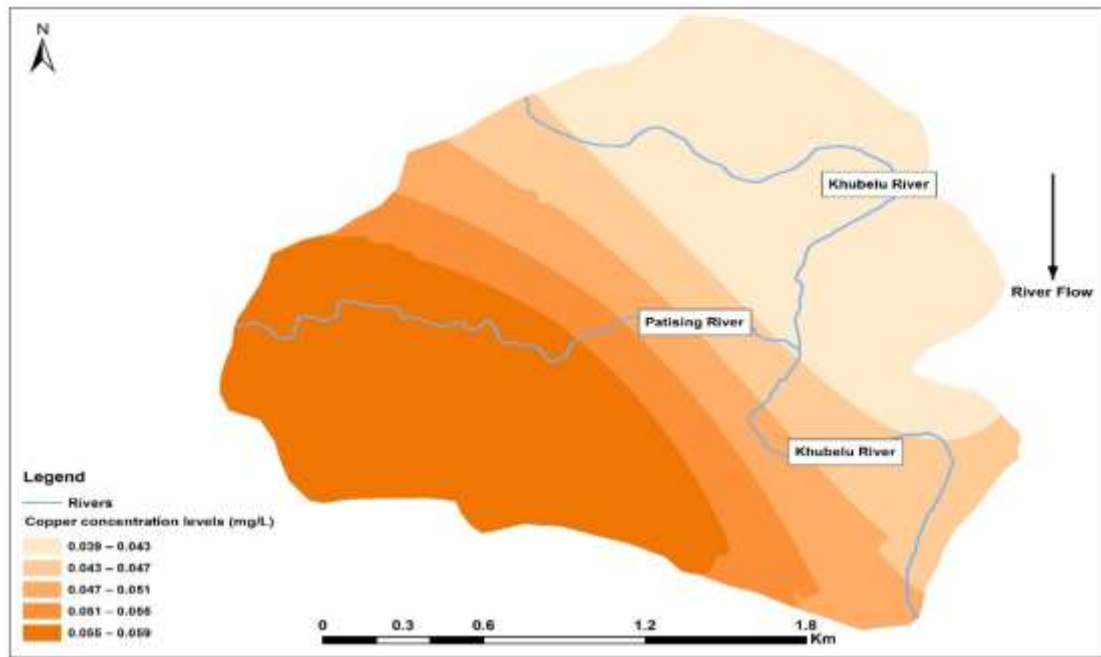


Figure 4.13: Distribution of copper concentration for top samples in dry season.

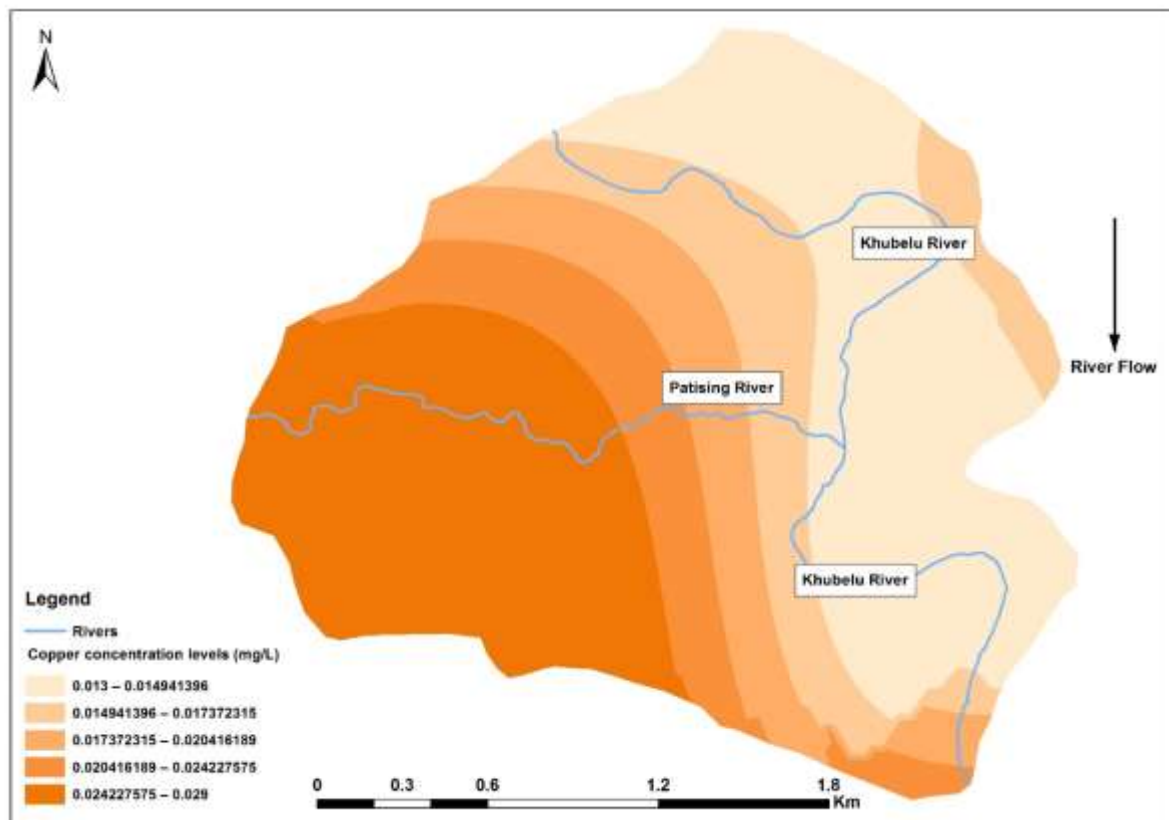


Figure 4.14: Distribution of copper concentration for bottom samples during wet season.

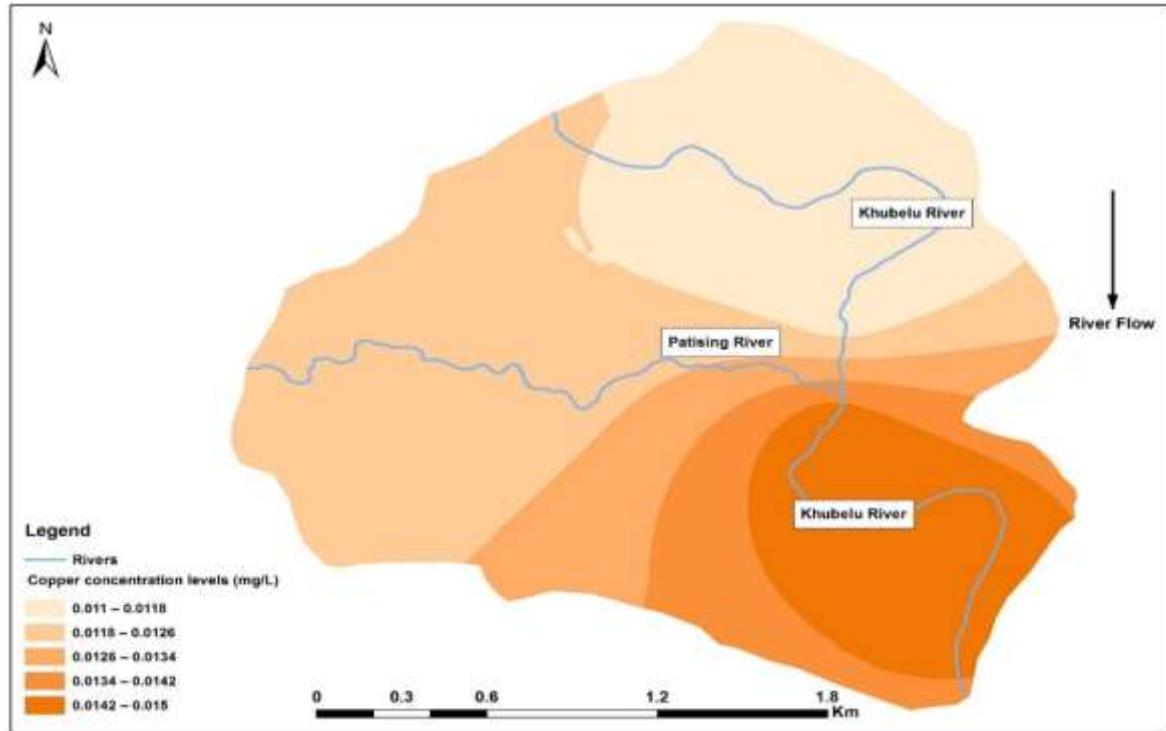


Figure 4.15: Distribution of copper concentration for top samples in the wet season.

Chromium was only detected from bottom samples during dry season. The spatial distribution of chromium was as shown in figure 4.16.

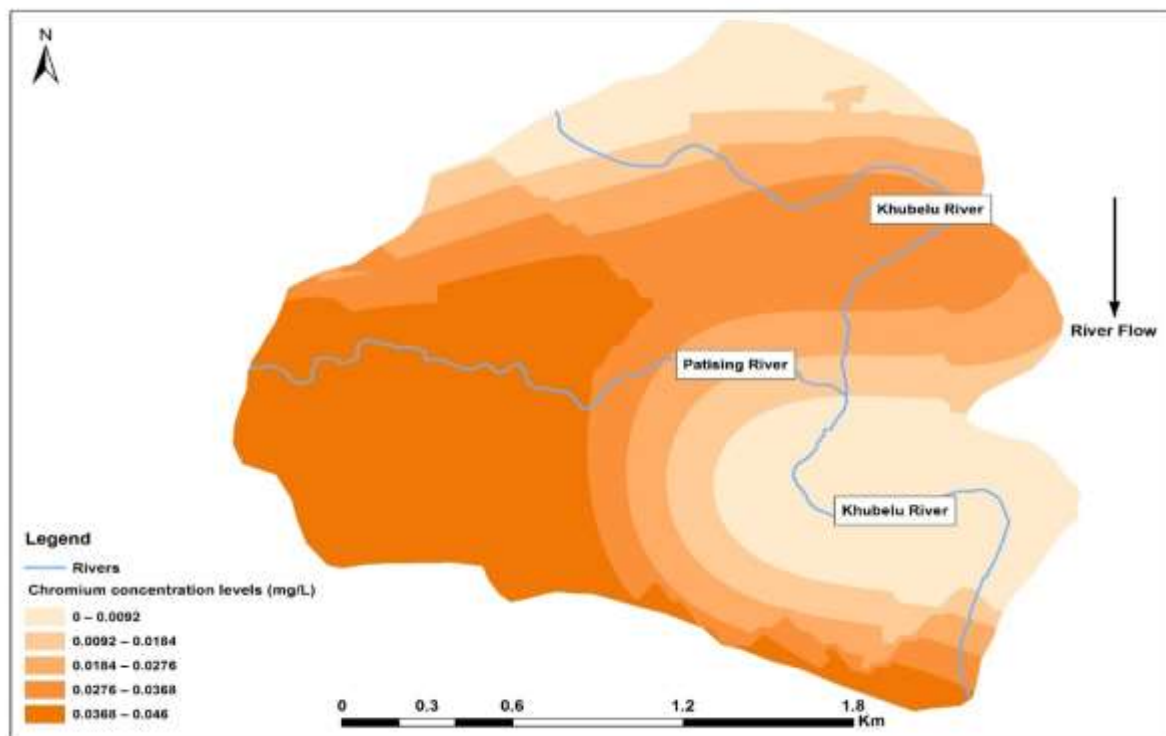


Figure 4.16: Distribution of chromium concentration for bottom samples during dry season.

During the same sampling period, dry season, lead was detected for both top and bottom samples and the concentration distribution is as shown in figure 4.17 and figure 4.18.

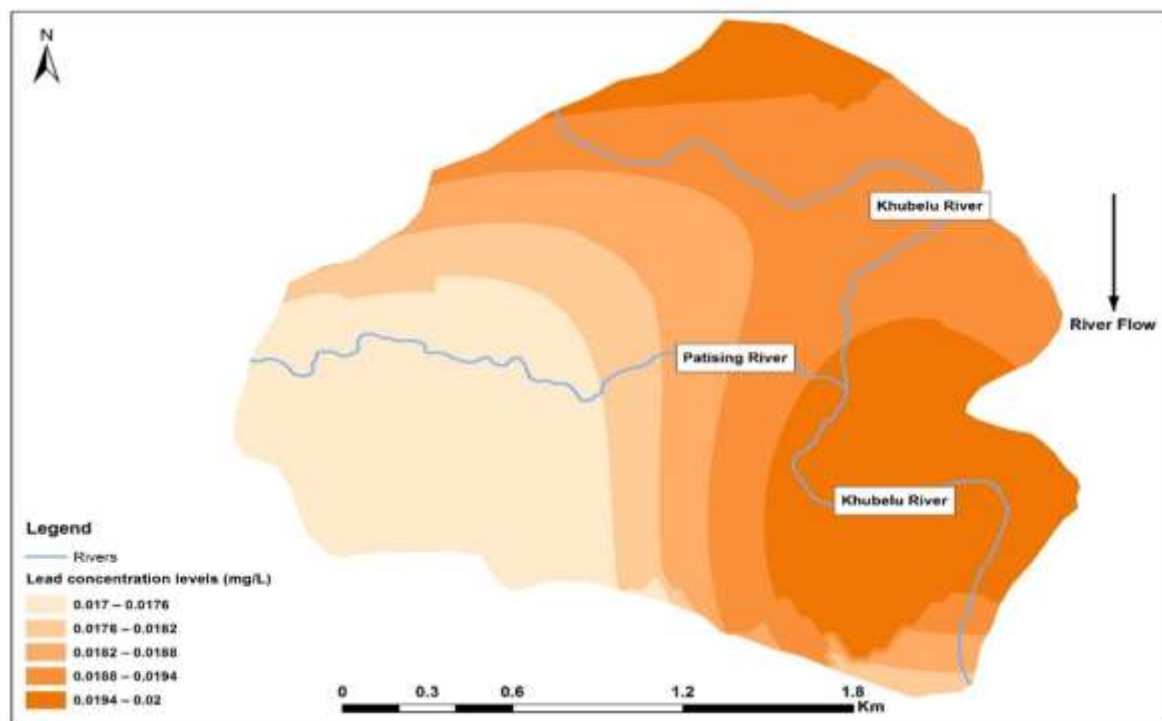


Figure 4.17: Distribution of lead concentration for bottom samples during dry season.

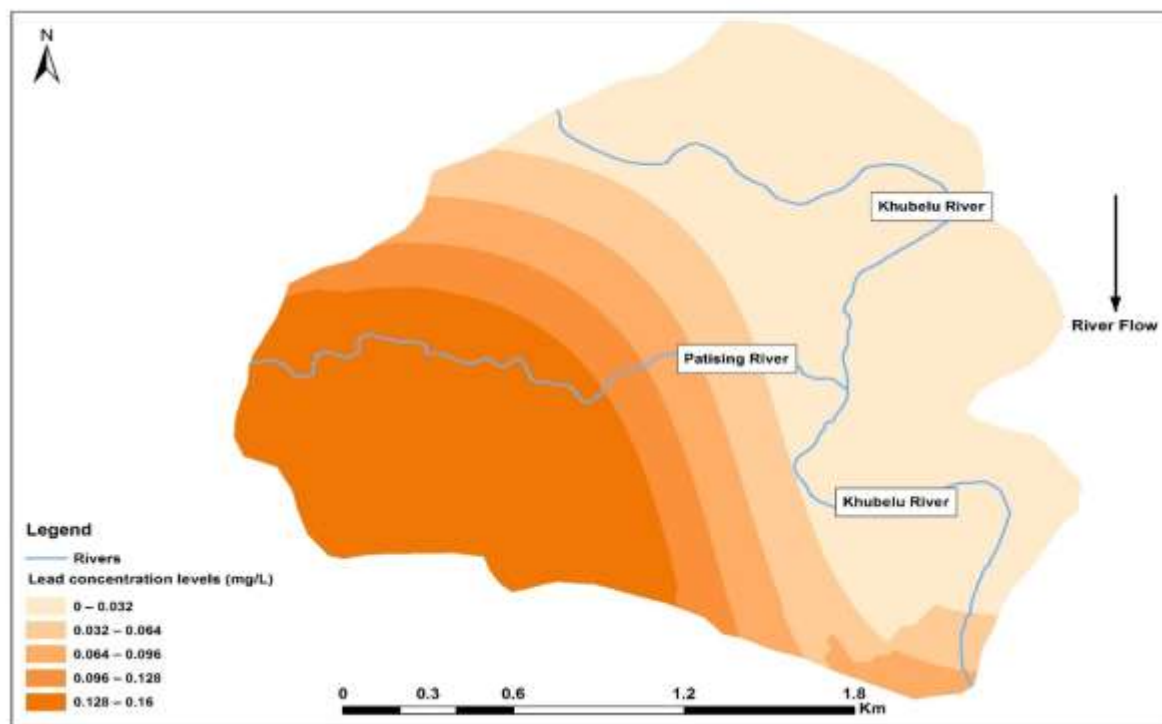


Figure 4.18: Distribution of lead concentration for top samples during dry season.

Table 4.4 represents levels of heavy metals at KRC during wet season, with copper as the only metal that was detected during this sampling season.

Table 4.4: Levels of heavy metals in Khubelu River catchment during wet season.

Sampling Points	Arsenic	Cadmium	Chromium	Copper	Mercury	Lead
KR DS - bottom	<0.020	<0.003	<0.020	0.013	<0.0001	<0.015
KR DS- Top	<0.020	<0.003	<0.020	0.015	<0.0001	<0.015
KR US- bottom	<0.020	<0.003	<0.020	0.015	<0.0001	<0.015
KR US- Top	<0.020	<0.003	<0.020	0.011	<0.0001	<0.015
Patising bottom	<0.020	<0.003	<0.020	0.029	<0.0001	<0.015
Patising Top	<0.020	<0.003	<0.020	0.012	<0.0001	<0.015

The comparison of dry and wet copper concentration levels is presented in figure 4.19. There is an observed decline in concentration levels during wet season.

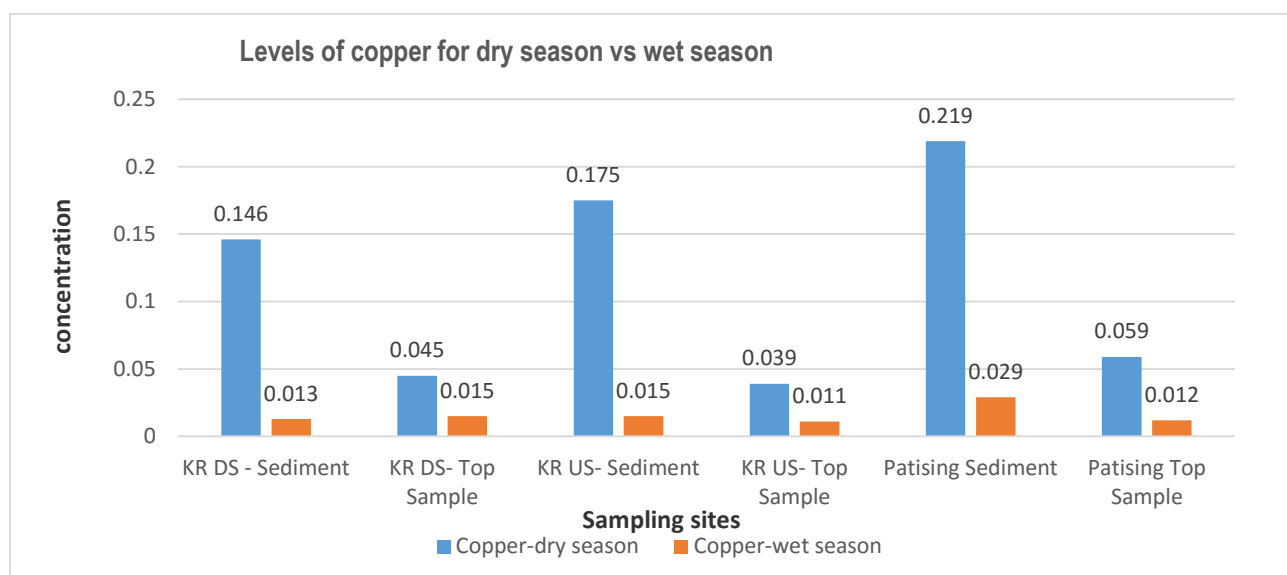


Figure 4.19: Comparison of copper levels for dry and wet season.

4.2 Discussion

4.2.1 Nitrates/Nitrites

The highest concentration levels for nitrates/nitrites were obtained at Patising bottom sample with 167 mg/L during dry season and the top sample at similar site and season with 114.1 mg/L. This was followed by top sample at Khubelu River (KR) downstream of the confluence with Patising River (PR) with 14.98 mg/L and 10.04 mg/L bottom sample. The least were 0.4 mg/L bottom sample and 0.13 mg/L top sample at KR upstream of the confluence, still during dry season. The concentrations trend for the dry season results starting with the highest concentration level as designated by their sampling locations is PR>KR-DS>KR-US. Breaking

them further into top and bottom samples, the trend is $PR_{(bottom)} > PR_{(top)} > KR-DS_{(top)} > KR-DS_{(bottom)} > KR-US_{(bottom)} > KR-US_{(top)}$. In the wet season, the results followed a slightly different trend, where top samples for both PR and KR-US are higher; otherwise, the trend is similar under both dry and wet conditions. Considering top and bottom samples for the wet season, the trend is; $PR_{(top)} > PR_{(bottom)} > KR-DS_{(top)} > KR-DS_{(bottom)} > KR-US_{(top)} > KR-US_{(bottom)}$.

Generally, high concentrations (167 mg/L bottom, 114.1 mg/L top and 65.95 mg/L bottom, 67.8 mg/L top, respectively for dry and wet seasons) of nitrates/nitrites are found at Patising and are far beyond the recommended standards by both WHO (2011) and SANS:241 (2015), which is ≤ 11 mg/L for nitrates and ≤ 0.9 mg/L for nitrites. Downstream of Patising and Khubelu rivers confluence, top sample is slightly above the recommended threshold value and is approximately 15 mg/L indicating a good assimilative capacity of the Khubelu River. For wet season, the assimilative capacity strength was more pronounced giving concentration levels ranging from 2- 2.9 mg/L downstream the confluence. However, in a long run, Patising exhibits a threat to the Khubelu River water quality, especially because it is non-compliant in both dry and wet seasons. Therefore, the stream predisposes surrounding communities to diseases in case they need to use this stream for portable water. These nitrates concentration levels according to WHO (2016) and WHO (2011) can introduce methaemoglobinemia (methHb), particularly to bottle-fed infants less than 6 months old and the general public. The methHb levels $> 10\%$ of the normal haemoglobin (Hb) are known to cause cyanosis and asphyxia (WHO, 2011).

The toxicity of nitrate to humans is basically associated with its reduction potential to nitrite, with an estimated 25% of ingested nitrate being secreted into saliva and 20% being reduced to nitrite and another unaccounted percent is reduced by bacteria in other body parts (WHO, 2011). Over and above this, approximately 5% of the dietary nitrates are converted to nitrites. There is also a cancer risk associated with endogenous nitrosation resulting from high nitrate/nitrite intake (Ward *et al.*, 2018). Another study from Canada indicated gastric cancer development predominantly associated with exogenous nitrite intake (WHO, 2011; Ward *et al.*, 2018). Birth defects are also implicated to result from elevated levels of nitrate in drinking water ingested by women in their first trimester of pregnancy (Sadler *et al.*, 2016).

Nitrates and nitrites are not only toxic to human beings but on aquatic fauna as well. Exogenous nitrate is known to be biologically active and capable of affecting hormonal production, reproduction, secondary sex characteristics, growth, behaviour, and development in aquatic organisms (Moore and Bringolf, 2018). Nitrate has also been implicated as an endocrine disrupting chemical (EDC) in other aquatic species, including fish and amphibians

where concentration levels of approximately 5 mg/L were found to decrease sperm counts and increased testicular weight in male mosquito fish (Kellock *et al.*, 2018). Additionally, Northern Leopard frogs (*Rana pipiens*) subjected to nitrate levels of approximately 2.26 mg/L at their early developmental stages had testicular oocytes, female skewed sex ratios as well as less-developed testicular tissue (Kellock *et al.*, 2018).

The mechanism of nitrate and nitrite toxicity in aquatic animals particularly fish is linked to conversion of oxygen-carrying pigments to forms that are incapable of carrying oxygen, causing hypoxia and ultimately death (Camargo and Alonso, 2006). The entry of nitrate and nitrite into the red blood cells is associated with the oxidation of iron ($\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$), functional haemoglobin being converted into methaemoglobin that is unable to release oxygen to body tissues because of its high stability (Kroupova *et al.*, 2016; Camargo and Alonso, 2006). In addition to these deleterious effects, nitrites are toxic to fish species because they cause severe electrolyte imbalance, are both mutagenic and carcinogenic because of N-nitroso compounds formation, damage mitochondria in liver cells causing tissue oxygen deficiency and lastly causing repression of immune system, thus prone to infectious diseases (Camargo and Alonso, 2006; Kroupova *et al.*, 2016; Jia *et al.*, 2015). Because of the sensitivity of freshwater animals and toxicity these nitrogen ions pose (NO_2^- and NO_3^-) it is suggested that save threshold values that can adequately protect these species be between 0.08–0.35 mg/L nitrite and approximately 2 mg/L for nitrates (Camargo and Alonso, 2006; Camargo *et al.*, 2005).

4.2.2 Ammonia

The levels of ammonia were highest during dry season, particularly in bottom samples. PR was leading followed by KR-DS and the least was KR-US. During wet season, still on bottom samples, PR was leading followed by KR-UP and finally the KR-DS. This second trend gives the implication that there are still some activities happening upstream the confluence that contribute to the overall ammonia concentration in the Khubelu river system. For top samples during dry season, PR was still leading followed by KR-US with KR-DS as the least. During wet season for top samples the trend is; $\text{PR}_{(\text{top})} > \text{KR-DS}_{(\text{top})} > \text{KR-US}_{(\text{top})}$.

The only case when the concentration level of ammonia was found exceeding the recommended standards as stipulated in SANS: 241 (2015) (NH_3 as N ≤ 1.5 mg/L) is at Patising during dry season and the concentration was 1.89 mg/L. This concentration level according to SANS: 241 (2015), does not exhibit any health risk issues but is aesthetic, implying that it is likely to taint taste, colour and odour of the Patising stream. There are no

implicated chronic or acute health impacts at this concentration level. In all other sampled sites whether top or bottom samples, the concentration levels were well below the recommended threshold value.

Despite the low ammonia concentration obtained in this study, ammonia is toxic to both humans and aquatic life, hence its levels in freshwater ecosystems needs to be properly monitored. Elevated ammonia levels in the brain as a result of hyper-ammonemia as viewed by Bosoi and Rose (2009) leads to cerebral dysfunction which involves a spectrum of neuropsychiatric and neurological symptoms (impaired memory, shortened attention span, sleep-wake inversions, brain oedema, intracranial hypertension, seizures, ataxia and coma). Other studies confirm ammonia as a key player in the neuropathophysiology associated with liver failure and inherited urea cycle enzyme disorders (Bosoi and Rose, 2009). Other impacts of elevated ammonia concentration on central nervous system eventually lead to energy deficit, oxidative stress and cell death (Braissant, 2010).

In aquatic life particularly fish, ammonia is known to cause stress and damages gills and other tissues, even in low concentration levels (Floyd *et al.*, 2012). The fish exposed to low concentration levels of ammonia over long periods are more susceptible to bacterial infections and often experience poor growth (Wang *et al.*, 2017; Floyd *et al.*, 2012). Acute toxicity of ammonia according to Randall and Tsui (2002) is mainly due to its effect on the central nervous system of vertebrates, which after acute ammonia intoxication, convulsions and death soon follow. Even though most fish species are intolerant to elevated environmental ammonia levels, some species are ammonia-tolerant and use a variety of strategies to avoid ammonia toxicity (Randall and Tsui, 2002). Figure 4.8 below summarises such strategies.

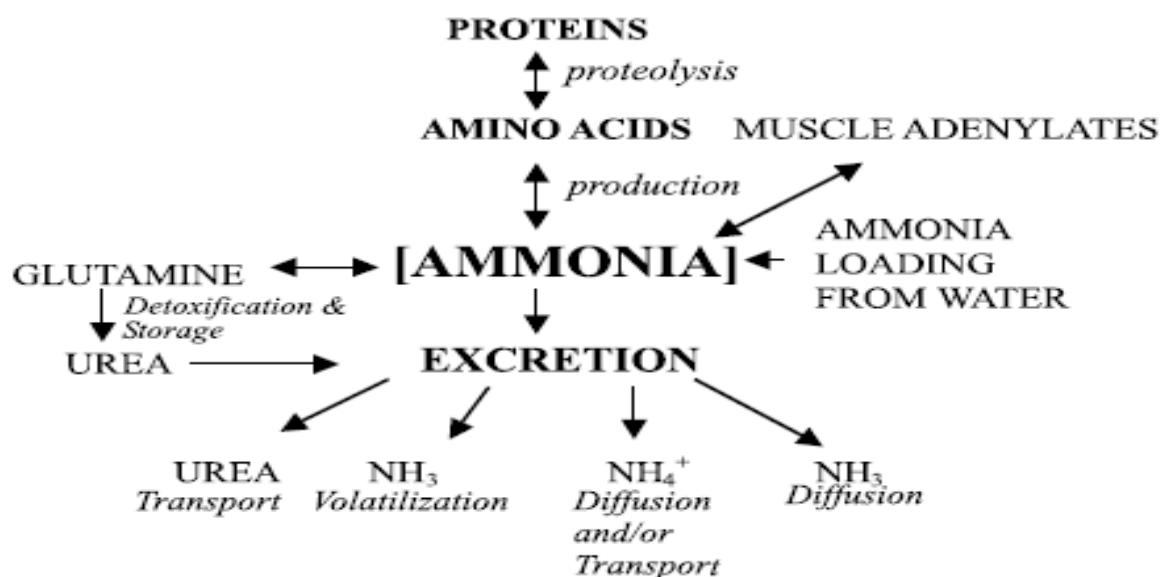


Figure 4.20: Summary of strategies used by some fish species to ameliorate ammonia toxicity (Randall and Tsui, 2002).

Besides fish, there are other aquatic fauna species that include benthic macroinvertebrates, which are equally important. This is due to their susceptibility to long-term exposure to pollution as their mobility in aquatic ecosystems is relatively limited (Alonso and Camargo, 2009). One compound that can alter the behaviour of macroinvertebrates is ammonia. The study on long-term effects of ammonia on the behavioural activity of the aquatic Snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca) by Alonso and Camargo (2009) revealed that ammonia is highly toxic to mollusks, Unionidae family, and *P. antipodarum*. In particular, the study showed that long-term and sub chronic exposures to nonionized ammonia concentrations from 0.05 to 0.13 mg N-NH₃/L affected behavioural activity indicating that the freshwater recommended value for macroinvertebrates must be lower than 0.05 mg N-NH₃/L to avoid behavioural effects. These estimated recommended values are meant to avoid behavioural effects and are relevant from an ecological point of view, as toxicants can alter the behaviour of snails, for example, the feeding activity, time to start normal movement, and ability to avoid polluted conditions, retract their foot, and close their operculum (Alonso and Camargo, 2009). All these factors affect ecological interactions of animals for example; ability to avoid predation and individual fitness-related traits (growth rate and reproduction) (Alonso and Camargo, 2009). It is noted thereof that species richness and a degeneration in biodiversity are the likely consequences and at extreme cases biodiversity loss, species extinction and degradation of ecosystem if ammonia concentration levels are not properly monitored and managed.

4.2.2 Copper

In the analysis of copper in the KRC, the highest concentration levels were during dry season for both top and bottom samples. The concentration levels for bottom samples ranged from 0.175 mg/L to 0.219 mg/L and top samples from 0.039 mg/L to 0.059 mg/L both for dry season period. Under wet season, bottom samples' concentration ranged from 0.013 mg/L to 0.029 mg/L and top samples from 0.011 mg/L to 0.015 mg/L. The concentration trend for dry season was; $PR_{(bottom)} > KR-US_{(bottom)} > KR-DS_{(bottom)} > PR_{(top)} > KR-DS_{(top)} > KR-US_{(top)}$ indicating high copper concentrations at bottom samples. The wet season trend was; $PR_{(bottom)} > KR-DS_{(top)} \geq KR-US_{(bottom)} > KR-DS_{(bottom)} > PR_{(top)} > KR-US_{(top)}$, in this case, there is complete mix up between bottom and top samples implying probable resuspension of benthic copper due to high flows. Looking at the concentration levels for both seasons; top and bottom samples, they all fall below the recommended drinking water standards as stipulated by SANS:241:2015 and WHO 2011 which specify the threshold value of 2.00 mg/L, hence, all the sampling sites do not exhibit any chronic or acute health risks according to SANS:241:2015.

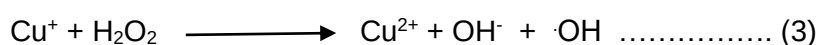
It is noted from these results that copper exists in all three sampling locations which might be attributed to the natural occurrence. Patising exhibited highest concentration levels probably due to mining activities happening in Letšeng which might have exposed more copper particles during excavating and ore crushing operations. The KRC is mainly sedimentary (Van Rooy, 1993) and according to Hitzman *et al.* (2010), the largest known deposits of copper occur within sedimentary basins, generally at the contact between subaerial red-bed sequences and overlying lacustrine shales, siltstones, sandstones or carbonate rocks. Deposits in such settings have been termed sediment-hosted stratiform copper deposits Hitzman *et al.* (2010).

However, having values below the recommended standards does not completely imply no inherent risks because the literature also points otherwise. Georgopoulos *et al.* (2001) is of the view that if only the drinking water can be considered as the sole supplier of human body with copper, then it is likely to be underestimated because other factors such as diet contribute significantly to the total copper intake. It is believed that the usual diet contains between 2 to 5 mg/day and that the richest sources of dietary copper contain from 0.3 to over 2 mg/100g. These include shellfish, nuts, seeds (including cocoa powder), legumes, bran and germ portions of grains, liver and meat organs (Georgopoulos *et al.*, 2001). That is why some states like California, through its California Environmental Protection Agency has published a guideline for copper in drinking water of 0.17 mg/L (Georgopoulos *et al.*, 2001).

There are also some inborn errors with regard to maintaining copper homeostasis and excretion which are at a very high risk of contracting Wilson's disease (Stern, 2010; Angelova *et al.*, 2011). This according to Stern (2010) and Dusek *et al.* (2014) is a rare autosomal

recessive disorder of copper transport, resulting in copper accumulation and toxicity to the liver and brain (neurological/psychiatric). Wilson's disease may become clinically evident at any time from infancy through early adulthood (Stern, 2010). This disease is controlled by Cu chelation therapy and restriction of dietary metal intake as well as limiting copper intake through drinking water with copper or inhaling air contaminated with copper (Bandmann *et al.*, 2014; Georgopoulos *et al.*, 2001; Stern, 2010). It has been suggested that heterozygote carriers of the Wilson's disease gene mutation are potentially more susceptible to elevated Cu intake than any other individual (Stern, 2010; Gupta and Lutsenko, 2009).

Though copper is an essential nutrient that is incorporated into a number of metalloenzymes involved in haemoglobin formation, carbohydrate metabolism, and many more, its ability to cycle between an oxidized state, Cu(II), and reduced state, Cu(I), when used by cuproenzymes involved in redox reactions is the one that makes it potentially toxic (Tchounwou *et al.*, 2014). This is because the transitions between Cu(II) and Cu(I) can result in the generation of superoxide and hydroxyl radicals (Hodgkinson and Petris, 2012; Tchounwou *et al.*, 2014). Hodgkinson and Petris (2012) and Valko *et al.* (2016) also add that under aerobic conditions, this redox property enables copper to catalyse the production of hydroxyl radicals via the Fenton and Haber-Weiss reactions 3 & 4 below;



The hydroxyl radicals are reactive with most types of macromolecules, resulting in damage to lipids, proteins, and nucleic acids (Valko *et al.*, 2016; Hodgkinson and Petris, 2012). A second mechanism of copper toxicity is the disruption of protein structure which occur through interactions with the polypeptide backbone (Hodgkinson and Petris, 2012).

In aquatic life, copper can still induce some deleterious effects even at concentration levels less than the drinking water standards as recommended by WHO (2011) and SANS: 241:2015. Khangarot and Das (2010) have indicated in their study on effects of copper on the egg development and hatching of a freshwater pulmonate snail that at concentration levels of 10- 32 µg/L of Cu, the survival rate of embryos was significantly reduced and at concentration levels of 100- 320 µg/L of Cu, all the embryos died within 168 hours of copper exposure indicating the sensitivity of this species to copper thus are good indicators of environmental toxicity assessments. Fish species on the other hand are negatively impacted by presence of elevated copper concentrations in aquatic environment. Copper concentration as low as 68

µg/ L are capable of inducing mortality, hatching inhibition and impairment of larval development of *Danio rerio* species (Sfakianakis *et al.*, 2015). Copper is also known to affect the olfactory system (chemosensation) of fishes and the consequences of impaired olfactory system include fishes being unable to avoid predation and find food as well as impaired sex cue perceptions that may ultimately affect the reproduction process (Pyle and Mirza, 2007; Sandahl *et al.*, 2006). The effect is more hazardous on the developmental stages of the embryonic olfactory system because that can translate to an irreversible chemosensory impairment at a later stage (Pyle and Mirza, 2007). At elevated copper concentrations, oxidative stress, DNA damage, cell death and ion osmo homeostasis are still experienced in aquatic animals (Eyckmans *et al.*, 2010).

Despite the dangers and toxicity discussed on copper, it stills has some vital importance and key roles in our everyday life other than being an essential nutrient. (Georgopoulos *et al.* (2001) points out some importance of copper as its ability to inactivate bacteria which is quite beneficial in recreational and potable water. It is also observed that copper is probably the only common plumbing material that can be used to suppress bacterial growth, *Legionella pneumophila* (a bacterium that causes the respiratory illness Legionnaire's disease). Another inherent benefit of copper is its ability to reduce dental plaque formation and dental caries if present in drinking water (Georgopoulos *et al.*, 2001).

4.2.3 Chromium

Chromium ions were detectable only during the dry season in the two sampling locations, Patising stream and the upstream of the confluence of Khubelu river and Patising. It was only detected in the bottom samples with Patising the highest with 0.046 mg/L and KR-US with 0.03 mg/ L. The observation deduced from this result is that, Cr occurs naturally in the Khubelu river catchment. Studies have shown that the geological setting of KRC is made up of large shallow basin of mainly sedimentary rocks (Van Rooy, 1993). Mishra and Bharagava (2016) on the other hand complement sedimentary rocks and shales as having very high concentrations of chromium thus justifying the presence of chromium in the KRC. The concentration levels for both sampling locations appear to be less than the recommended standard as stipulated in SANS: 241: 2015 and WHO 2011 of threshold value of ≤50 µg/L above which, chronic health is realised. The Patising concentration level is very close to this recommended value with slight difference of 4 µg/L.

Despite chromium concentration levels being found well below standards, long term exposure to this metal can still exhibit health risks given its bioaccumulation nature and persistence in the environment (Belay, 2010; Velma *et.al.*, 2010). In addition, these concentration levels can

be elevated depending on individual dietary choices because chromium content in foods depend on the processing and preparation method thus the majority of fresh foods contain chromium levels ranging from <10 to 1,300 µg/kg (Tchounwou *et al.*, 2014). There is a very narrow range of concentration levels between beneficial and toxic effects of chromium (Tchounwou *et al.*, 2014). Because of these discussed factors, there is still an inherent health risk of chromium exposure even at low concentration levels.

The most toxic form of chromium is the hexavalent form Cr(VI), of which its mechanism of toxicity is more pronounced once it enters human cells. This is because it enters the cells more readily than does Cr(III) and once in the cells, Cr(VI) gets reduced to Cr(III) via a number of reactions (Jaishankar *et al.*, 2014). During this reduction process, several intermediates, such as pentavalent and tetravalent chromium species, are produced together with reactive oxygen species (super oxide ions, hydrogen peroxide and hydroxyl radicals), which lead to oxidative stress in the cell causing damage to DNA and proteins thus resulting in a number of health abnormalities in humans (Jaishankar *et al.*, 2014; Mishra and Bharagava, 2016; Tchounwou *et al.*, 2014).

However, not only humans are affected by chromium toxicity but freshwater animals as well. The toxicity is more realised in freshwater fauna and flora because of chromium bioaccumulation effect, a study on the rate of accumulation of chromium in four fresh water plant species, clams, crabs, and fishes showed high concentration levels of chromium in all the four fresh water species and animals indicating high accumulation potential of the chromium metal (Belay, 2010). Praveena *et al.* (2013) reported that the exposure of *Labeo rohita* fish to chromium results in the genotoxic effects like increase in the frequency of micronucleus, and Erythrocyte abnormalities as well as cell death. Chromium induced clastogenic effects and damage to the DNA of erythrocytes in the exposed fishes (Praveena *et al.*, 2013). Physiological alterations of Chinook salmon exposed to Cr concentrations of 0-266 µg/ L occurred after exposure to ≥ 120 µg/L, with DNA damage occurring after exposure to a concentration of 24 µg/L (Velma *et al.*, 2010).

In another study, rainbow trout exposed to Cr concentrations of 0.02 to 2 mg/L at different pH values showed greater susceptibility to the metal at lower pH (6.5) than at higher pH (7.8). Further, the lowest concentrations of Cr at different pH values induced the mortality of embryos (0.2 mg/L at pH 6.5 and 2.0 mg/L at pH 7.8) (Velma *et al.*, 2010). Hematologic studies in *T. sparrmanii* chronically exposed to 0.098 mg/L Cr showed a significant decrease in haemoglobin concentrations and alteration of enzymes' activities (Velma *et al.*, 2010). Both

forms of chromium, Cr (III) and Cr(VI) induce oxidative stress in fish species (Kumari *et al.*, 2014; Lushchak, 2011).

Besides fish species, nematode on the other hand are good indicators of environmental relevant concentrations. Wu *et al.* (2012) investigated *Caenorhabditis elegans* on chronic toxicity of chromium (Cr(VI)) at environmental relevant concentrations ranging from 5.2 µg/L to 260 µg/L and apparently found that at concentration levels as low as 13 µg/L, head thrash, body bend, intestinal autofluorescence, and reactive oxygen species production were significantly influenced indicating some chronic toxicity. exposure to 260 µg/L Cr significantly reduced the percentage of survival nematodes in comparison to the control. It is noted therefore that even though the observed concentration levels for chromium in the KRC are well below the recommended standards by SANS and WHO, they may still pose a threat to other sensitive species like nematodes hence are good indicators for biomonitoring exercises especially with regard to chromium.

However, not all is hazardous about chromium, it still has beneficial impacts on organisms other being an essential trace element responsible for multiple regulation of biological processes, in particular glucose metabolism. In experiments with guppies *Poecilia reticulata*, it was found that low concentration levels ($<10^{-4}$ M) of Cr^{6+} increased the maximum lifespan in both male and female species (Lushchak, 2011). Also, fishes have their defensive mechanism in dealing with chromium toxicity, Interestingly, fish mucus can reduce Cr^{6+} and Cr^{3+} and this mechanism is proposed to be involved in Cr^{6+} detoxification (Lushchak, 2011).

4.2.4 Lead

Lead was detected in all bottom samples of the three sampling locations except in Patising where it was only detected in top samples. The highest levels were detected downstream of the confluence. The concentration trend starting with the highest concentration is as follows; $\text{KR-DS}_{(\text{bottom})} > \text{KR-US}_{(\text{bottom})} > \text{PR}_{(\text{bottom})} > \text{PR}_{(\text{top})}$. The concentration levels ranged from 16 µg/L to 20 µg/L and all these values appear to be above the recommended standards as stipulated in SANS: 241:2015 and WHO 2011. It is observed from these results that lead settles at the bottom, the reason it is detected on top sample in Patising is probably due to high flow rates attributed to the steepness of the stream (figure 4.18) which might have led to sediment resuspension. All the sites exhibit concentration levels that differ slightly from one another with downstream being the highest, implying a combined effect from upstream and Patising. Again, the spatial distribution of lead within the three sampling sites does not display an anthropogenic influence. The KRC geological setting is mainly sedimentary rocks (Van Rooy,

1993). Sedimentary rocks are known to contain some amounts of lead at concentration levels ranging from approximately 10 ppm to 100 ppm thus reinforcing natural occurrence of lead in the KRC (Lovering, 1976).



Figure 4.21: A picture indicating the direction of water flow in a sloppy Patising stream.

The high levels of lead above the recommended standards poses threat to both humans and aquatic animals. Lead exposure especially in pregnant women is known to result in reduced birth weight and preterm delivery with neuro-developmental abnormalities in children (Tchounwou *et al.*, 2014). In adults, high levels of lead exposure are associated with reproductive effects, such as decreased sperm count in men and spontaneous abortions in women as well as anaemia and cancer (Mudgal *et al.*, 2010; Tchounwou *et al.*, 2014). Central nervous system, blood pressure, kidneys, and vitamin D metabolism are some of affected organs and or areas due to chronic exposure on lead (Mudgal *et al.*, 2010; Mason *et al.*, 2014; Tchounwou *et al.*, 2014). Studies have revealed that exposure to lead can result in declines in intelligence, memory, processing speed, comprehension and reading, visuospatial skills, motor skills as well as antisocial behaviour which is usually linked to early lead exposure (Mason *et al.*, 2014).

The toxicity mechanism of lead within the cells is linked to its ability to interfere with calcium release from the mitochondria, resulting in the formation of reactive oxygen species, speeding mitochondrial self-destruction through formation of the permeability transition pore, and priming activation of programmed cell death processes (Mason *et al.*, 2014). Also, the lead-induced oxidative stress involves an imbalance between generation and removal of reactive oxygen species in tissues and cellular components which ultimately results in damage to membranes, DNA and proteins (Patra *et al.*, 2011; Gillis *et al.*, 2012). The mechanism

underlying lead-induced oxidative damage to membranes is associated with changes in its fatty acid composition specifically through lipid peroxidation of the membrane (Gillis *et al.*, 2012; Patra *et al.*, 2011).

On aquatic animals, lead is also believed to have some detrimental impacts. Ahmed and Bibi (2010) have indicated in their study that fishes exposed to elevated concentration levels of lead exhibit a wide range of effects which include amongst others the muscular and neurological degeneration and destruction, growth inhibition, mortality, reproductive problems, and paralysis. Authman *et al.* (2015) clarifies further the reproductive problems caused by lead as inducing pathological changes in tissues and organs of fish species which as a result, impairs the embryonic and larval development. Chronic lead exposure on aquatic animals is characterised by changes in the blood parameters with severe damage to erythrocytes and leucocytes as well as damage in the nervous system (Authman *et al.*, 2015).

Lead depletes major antioxidants in the cell, especially thiol-containing antioxidants and enzymes thus causing a significant increase in reactive oxygen species (ROS) production, followed by oxidative stress which then leads to various dysfunctions in lipids, proteins and DNA (Authman *et al.*, 2015; Jastrzębska, 2010). Weakened immune system resulting in increased susceptibility to infections, deformities as body curvatures, reduced locomotion and foraging by deformed juveniles were some of other impacts observed due to chronic exposure to lead (Authman *et al.*, 2015). Not only the fish species are affected by lead exposure, but frogs also do experience similar problems such as oxidative stress, DNA, protein and lipids damage as well as reduced reproductive capacity at lead concentration levels of approximately 0.08 mg/L (Wang and Jia, 2009).

Chapter 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Protection of water resources is not only crucial to human health but on aquatic biota as well as terrestrial. It is well said by Selinus *et al.* (2013) in his essential to medicinal geology book that the geology and the environment we live in says a lot about us and as a result, we are what we eat, drink and breathe. This is not only applicable to us homosapiens but to biotic factors as well. The global community is well considerate about the protection of water resources and the environment as a whole (SDG 6: clean water & sanitation), Lesotho is not an exemption given the economic value inherent in this natural capital and ecosystem services that flow from a healthy environment.

This study therefore carried out an investigation to identify and determine the concentration levels of selected nutrients and heavy metals in the KRC with the aim of evaluating the impacts of these contaminants' accumulation in the environment, identifying point and non-point sources of nutrient and metal contamination as well as mapping and modelling the levels of these selected nutrients and heavy metals within the KRC. The levels of nitrates/nitrites as N are in the range 0.13 – 167 mg/L in dry season and 0 – 67.8 mg/L during wet season, which pose health risks to human and are toxic to aquatic fauna. Ammonia as N ranged from 0.17 – 1.89 mg/L during dry period and 0.15 – 0.68 mg/L in the wet season. Copper levels ranged from 0.039 – 0.219 mg/L during dry season and 0.011 – 0.029 in the wet season. Cadmium, arsenic and mercury were not detected in both sampling seasons whereas lead and chromium were only detected during dry season. The concentration levels for lead ranged from 0 – 0.020 mg/L and that of chromium were found to lie between 0 and 0.046 mg/L.

The trends and spatial distribution of nutrients and heavy metals understudy give information on point and non-point sources of pollution. All the heavy metals detected were non-point and the geology of the area supported their natural occurrence. The nitrates and nitrites on the hand were mainly point sources because they were highest at Patising river which carries some effluent from Letseng diamond slime dams. The downstream concentration levels confirm this as they are also higher than the upstream levels. The presence of these nutrients upstream also confirms some contribution by land use activities such as livestock and crop farming, thus minor component can be attributed to non-point sources. Ammonia is also distributed across all the sampling locations but highest at Patising still confirming the influence by the mine. Its presence in the upstream also confirms some contribution from non-point sources and natural processes of ammonia cycle.

The results obtained in the present study indicated that there was a definite pollution in the Khubelu River Catchment with respect to HMs and nutrients studied. Land use activities around the catchment were the root source of this pollution particularly nutrients because heavy metals proved to occur naturally within the KRC and the geology of the area supported that. These HMs (Pb, Cu & Cr) and nutrients (NO_3^- , NO_2^- & NH_3) can have serious health implications for both human beings and aquatic biota.

5.2 Recommendations

- Appropriate joint monitoring programme by the Department of Environment and Department of Water Affairs is needed in order to minimise the impacts.
- Detailed river health study that will investigate all drivers and response factors is needed so as to evaluate all possible migration routes of these HMs and their impacts
- Another study on determination of HMs on fish tissues, liver and kidney might be helpful to investigate the extend of HMs and their bioaccumulation effects
- Public health study on Patising community to investigate residents' health status with respect to the pollution in this catchment might be of importance.
- It might also worth investigating how the Patising stream has impacted on the livestock of the community residing in the area, my observation and through communication with the area chief is that, the stream is no longer used for portable water instead water from Khubelu river is used.

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Appendices

Appendix 1A: Dry season laboratory results as analysed by IGS, University of the Free State.

		Institute for Groundwater Studies University of the Free State IGS Laboratory Services, Dekaan Street (Campus) 339, BLOEMFONTEIN, 9300 +27-(0)51 - 401 2317 +27-(0)51 - 401 3005 E-mail: igslab@ufs.ac.za		Sec 5.10 F1 Revision 9
		Test Report Case no: 2018 - 915		Page 1 of 2
Client/Company name: Bokang Shakhane Contact person: Bokang Shakhane Contact number: +266 589 89097 shakhanebokang@gmail.com Postal address: PO Box 180, Roma, Maseru, 100 Order number:				
Sample information Sample type: 6 water samples (chem) Delivered by: Bokang Shakhane - 12:35 Date received: 19/10/2018 Reporting date: 05/11/2018 Final report: 05/11/2018				
Subcontracted analysis are indicated by * and non-accredited analysis by # Methods are available on request of client. Statement (HPC) - All counts below 30 and above 300 per plate will also be reported. Counts above 300 are estimated Disclaimer: For HPC - zero or no counts means ≤ 10 in 1ml. Results marked with # in this report, are not included in the SANAS Schedule of Accreditation for this laboratory. Results marked "Subcontracted Test" in this report are not included in the SANAS Schedule of Accreditation for this laboratory, unless indicated that it was done by a SANAS Accredited laboratory.				
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Compiled by: Accreditation Officer	Approved by: Director	Effective date: 03/01/2018	Controlled document	

Institute for Groundwater Studies

University of the Free State

IGS Laboratory Services, Dekkan Street (Campus)

336, BLOEMFONTEIN, 9300

Tel: +27-(0)51 - 401 2317

Fax: +27-(0)51 - 401 3005

E-mail: igslab@ufs.ac.za

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Test Report Case no: 2018 - 915

18/10/2018

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Determinant	Units	Method used	South African National Standard (SANS) 241:2005/2011 for drinking water (partial)	Client sample name:								
				Kruehse River Downstream - Sediment	Kruehse River Downstream	Kruehse River Upstream - Sediment	Kruehse River Upstream	Feddersburg above the village - sediment	Feddersburg above the village			
				Lab number:								
				Class 1 (Recommended for use)	Class 2 (Maximum allowable for limited use) ** EU standard	Class 1 (Recommended for use)	Class 2 (Maximum allowable for limited use) ** EU standard	Class 1 (Recommended for use)	Class 2 (Maximum allowable for limited use) ** EU standard			
Value				Value	Value	Value	Value	Value	Value			
Chemical report												
Nitrate/Nitrite as N #	mg/L	Chem-Tell	< 11	10.04	14.55	0.4	0.13	167.0	114.1			
Ammonia as N #	mg/L	Chem-Tell	< 1.0	0.82	0.17	0.46	0.16	1.66	0.27			
Calcium as Ca #	mg/L	Chem-TM22	< 180 - 340	367.5	24.0	225.5	7.35	464.1	65.4			
Magnesium as Mg #	mg/L	Chem-TM22	< 70 - 120	52.8	4.9	42.5	3.09	98.2	7.0			
Sodium as Na #	mg/L	Chem-TM22	< 200	100.4	59.4	11.5	2.59	661.7	395.8			
Potassium as K #	mg/L	Chem-TM22	< 50 - 100	12.6	4.6	4.9	0.39	97.1	21.9			
Aluminium as Al #	mg/L	Chem-TM22	< 0.300	48.27	0.066	73.151	0.065	49.16	0.475			
Arsenic as As #	mg/L	Chem-TM22	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001			
Barium as Ba #	mg/L	Chem-TM22	< 0.700	0.004	< 0.001	0.009	< 0.001	0.077	0.029			
Cadmium as Cd #	mg/L	Chem-TM22	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001			
Cobalt as Co #	mg/L	Chem-TM22	< 0.001	0.009	< 0.001	0.166	< 0.001	0.002	< 0.001			
Chromium as Cr #	mg/L	Chem-TM22	< 0.001	< 0.001	< 0.001	0.001	< 0.001	0.046	< 0.001			
Copper as Cu #	mg/L	Chem-TM22	< 0.001	0.146	0.049	0.175	0.019	0.219	0.099			
Iron as Fe #	mg/L	Chem-TM22	< 2.000 (drinking health) < 0.300 (aesthetics)	15.348	0.121	63.816	0.066	11.663	0.632			
Manganese as Mn #	mg/L	Chem-TM22	< 0.400 (drinking health) < 0.100 (aesthetics)	12.368	< 0.001	16.940	< 0.001	25.566	0.029			
Mercury as Hg #	mg/L	Chem-TM14	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001			
Nickel as Ni #	mg/L	Chem-TM22	< 0.010	< 0.001	< 0.001	0.157	< 0.001	0.487	< 0.001			
Lead as Pb #	mg/L	Chem-TM22	< 0.010	0.025	< 0.001	0.019	< 0.001	0.017	0.016			
Selenium as Se #	mg/L	Chem-TM22	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.060	0.026			
Vanadium as V #	mg/L	Chem-TM22	< 0.001	0.004	0.013	0.111	0.013	0.242	0.022			
Zinc as Zn #	mg/L	Chem-TM22	< 0.001	0.071	< 0.001	0.110	< 0.001	0.229	< 0.001			

Note:

Results marked with (R) in this report, are not included in the SANS 241 Schedule of Accreditation for this laboratory.

Parameters, higher than the specifications, are clearly marked. Tables with specifications included for comparison.

Bacteriological results obtained from samples older than the prescribed 24 hours may be inaccurate.

Signature:

Dr L. Dippen (Technical signatory / Technical manager)

END OF REPORT

Appendix 1B: Wet season laboratory results as analysed by IGS, University of the Free State



Institute for Groundwater Studies

University of the Free State

IGS Laboratory Services, Dekaam Street (Campus)

339, BLOEMFONTEIN, 9300

+27-(0)51 - 401 2317

+27-(0)51 - 401 3005

E-mail: igslab@ufs.ac.za

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Revision 9

Test Report

Case no: 2018 - 1033

Page 1 of 2

Client/Company name: Bokang Shakhane
Contact person: Bokang Shakhane
Contact number: +266 589 89097 shakhanebokang@gmail.com
Postal address: PO Box 180, Roma, Maseru, 100
Order number:

Sample information

Sample type: 8 water samples (chem)
Delivered by: Bokang Shakhane - 12:38
Date received: 28/11/2018
Reporting date: 19/12/2018
Final report: 19/12/2018

Subcontracted analysis are indicated by * and non-accredited analysis by #

Methods are available on request of client.

Statement (HPC) - All counts below 30 and above 300 per plate will also be reported. Counts above 300 are estimated

Disclaimer: For HPC - zero or no counts means ≤ 10 in 1ml.

Results marked with # in this report, are not included in the SANAS Schedule of Accreditation for this laboratory.

Results marked "Subcontracted Test" in this report are not included in the SANAS Schedule of Accreditation for this laboratory, unless indicated that it was done by a SANAS Accredited laboratory.

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- The IGS Laboratory has a strict confidentiality policy and the policy may be obtained from the Laboratory on client request.
- Amended reports replace all previous reports and will be marked accordingly.
- Calculated values according to Standard Methods for the examination of water and waste water - 2005.
- The client will be afforded the right to monitor the performance of the laboratory in relation to the work performed, provided that the laboratory ensures confidentiality to other customers.

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University of the Free State

IGS Laboratory Services, Dekaam Street (Campus)

00 339, BLOEMFONTEIN, 9300

☎: +27-(0)51 - 401 2317

☎: +27-(0)51 - 401 3005

E-mail: igslab@ufs.ac.za

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Test Report Case no: 2018 - 1033

26/11/2018

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Determinand	Units	Methods used	South African National Standard (SANS) 241:2008/2016 for drinking water (partial)	Client sample name:								
				Potlising Upstream Village - Top	Potlising Upstream Village - Bottom	Potlising Down Village Top	Potlising Down Village Bottom	Upstream Confluence with Khubela - Top	Upstream Confluence with Khubela - Bottom	Downstream Confluence Top	Potlising above the village	
				Lab number:								
				1033-1	1033-2	1033-3	1033-4	1033-5	1033-6	1033-7	1033-8	
Value				Value	Value	Value	Value	Value	Value	Value	Value	Value
Chemical report												
Electrical conductivity	mS/m	Chem-TM06	≤ 170	190	191	190	190	7.96	8.07	17.7	20.2	
Nitrate/nitro as N #	mg/L	Chem-TM18	≤ 11	87.8	88.05	87.2	86.93	0.47	<0.1	2.9	2.9	
Ammonia as N #	mg/L	Chem-TM18	≤ 1.5	0.21	0.69	0.2	0.32	0.15	0.25	0.16	0.24	
Orthophosphate as P #	mg/L	Chem-TM18	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	
Calcium as Ca #	mg/L	Chem-TM02	≤ 150 - 300	70.8	73.2	76.0	75.7	9.4	11.85	12.1	8.7	
Magnesium as Mg #	mg/L	Chem-TM02	≤ 70 - 100	6.0	8.8	8.0	8.5	2.7	3.44	3.0	2.2	
Sodium as Na #	mg/L	Chem-TM02	≤ 200	357.1	366.2	368.5	365.8	3.8	4.23	19.1	30.9	
Potassium as K #	mg/L	Chem-TM02	≤ 50 - 100	21.0	20.6	21.4	22.2	0.7	0.91	1.6	2.9	
Aluminium as Al #	mg/L	Chem-TM02	≤ 0.300	0.026	0.427	0.042	<0.020	0.028	0.038	0.044	0.431	
Arsenic as As #	mg/L	Chem-TM02	≤ 0.010	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	
Barium as Ba #	mg/L	Chem-TM02	≤ 0.700	0.029	0.029	0.029	0.032	<0.020	<0.020	<0.020	<0.020	
Cadmium as Cd #	mg/L	Chem-TM02	≤ 0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	
Cobalt as Co #	mg/L	Chem-TM02	≤ 0.000	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	
Chromium as Cr #	mg/L	Chem-TM02	≤ 0.050	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	
Copper as Cu #	mg/L	Chem-TM02	≤ 2.000	0.012	0.029	0.009	0.021	0.011	0.015	0.013	0.015	
Iron as Fe #	mg/L	Chem-TM02	≤ 2.000 (chronic health) ≤ 0.300 (aesthetic)	0.029	0.396	0.034	0.025	0.029	0.403	0.035	0.355	
Manganese as Mn #	mg/L	Chem-TM02	≤ 0.400 (chronic health) ≤ 0.100 (aesthetic)	<0.020	0.032	<0.020	<0.020	<0.020	0.336	<0.020	0.055	
Mercury as Hg #	mg/L	Chem-TM14	≤ 0.006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
Nickel as Ni #	mg/L	Chem-TM02	≤ 0.070	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	
Lead as Pb #	mg/L	Chem-TM02	≤ 0.010	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015	
Selenium as Se #	mg/L	Chem-TM02	≤ 0.040	0.030	0.049	0.029	0.036	<0.020	<0.020	<0.020	<0.020	
Vanadium as V #	mg/L	Chem-TM02	≤ 0.300	0.014	<0.010	0.011	0.011	<0.010	0.012	<0.010	<0.010	
Zinc as Zn #	mg/L	Chem-TM02	≤ 5.000	<0.020	0.021	<0.020	<0.020	<0.020	0.021	<0.020	0.023	

Note:
Results marked with (R) in this report, are not included in the SANS Schedule of Accreditation for this laboratory.
Parameters higher than the specifications, are clearly **marked**. Table with specifications included for comparison.

Bacteriological results obtained from samples older than the prescribed 24 hours may be inaccurate.

Signature:
Dr L. Deyzel (Technical signatory / Technical manager)

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IGS Laboratory Services, Dekaan Street (Campus)

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+27-(0)51 - 401 2317

+27-(0)51 - 401 3005

E-mail: igslab@ufs.ac.za

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Test Report

Case no: 2018 - 1033

Page 1 of 2

Client/Company name: Bokang Shakhane
Contact person: Bokang Shakhane
Contact number: +266 589 89097 shakhanebokang@gmail.com
Postal address: PO Box 180, Roma, Maseru, 100
Order number:

Sample Information

Sample type: 8 water samples (chem)
Delivered by: Bokang Shakhane - 12:38
Date received: 28/11/2018
Reporting date: 19/12/2018
Final report: 19/12/2018

Subcontracted analysis are indicated by * and non-accredited analysis by #

Methods are available on request of client.

Statement (HPC) - All counts below 30 and above 300 per plate will also be reported. Counts above 300 are estimated

Disclaimer: For HPC - zero or no counts means ≤ 10 in 1ml.

Results marked with # In this report, are not included in the SANAS Schedule of Accreditation for this laboratory.

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28/11/2018

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Determinand	Units	Method used	South African National Standard (SANS) 241:2006 & 2415 for drinking water (partial)	Client sample name:								
				Potting Upstream Village - Top	Potting Upstream Village - Bottom	Potting Down Village Top	Potting Down Village Bottom	Upstream Confluence with Khubela - Top	Upstream Confluence with Khubela - Bottom	Downstream Confluence Top	Potting above the village	
				Lab number:								
				1033-1	1033-2	1033-3	1033-4	1033-5	1033-6	1033-7	1033-8	
				Value	Value	Value	Value	Value	Value	Value	Value	
Chemical report												
Electrical conductivity	mS/m	Chem-TM08	≤ 170	190	191	189	190	7.96	8.87	17.7	20.2	
Nitrate/Nitrite as N #	mg/L	Chem-TM18	≤ 11	67.8	65.95	67.2	66.93	0.47	<0.1	2.9	2.0	
Ammonia as N #	mg/L	Chem-TM18	≤ 1.8	0.21	0.86	0.2	0.30	0.15	0.25	0.16	0.24	
Orthophosphate as P #	mg/L	Chem-TM18		<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	
Calcium as Ca #	mg/L	Chem-TM02	<150 - 300	70.8	73.2	78.0	75.7	9.4	11.85	12.1	9.7	
Magnesium as Mg #	mg/L	Chem-TM02	<70 - 100	8.0	8.8	8.0	8.5	2.7	3.44	3.0	2.2	
Sodium as Na #	mg/L	Chem-TM02	≤ 200	287.1	286.2	286.5	285.6	3.8	4.23	19.1	30.9	
Potassium as K #	mg/L	Chem-TM02	<50 - 100	21.0	20.8	21.4	22.2	0.7	0.91	1.8	2.9	
Aluminium as Al #	mg/L	Chem-TM02	≤ 0.300	0.036	0.427	0.042	<0.020	0.038	0.036	0.044	0.401	
Arsenic as As #	mg/L	Chem-TM02	≤ 0.010	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	
Barium as Ba #	mg/L	Chem-TM02	≤ 0.700	0.026	0.026	0.026	0.032	<0.020	<0.020	<0.020	<0.020	
Cadmium as Cd #	mg/L	Chem-TM02	≤ 0.003	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	
Cobalt as Co #	mg/L	Chem-TM02	≤ 0.030	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	
Chromium as Cr #	mg/L	Chem-TM02	≤ 0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	
Copper as Cu #	mg/L	Chem-TM02	≤ 2.000	0.012	0.029	0.009	0.021	0.011	0.015	0.013	0.015	
Iron as Fe #	mg/L	Chem-TM02	≤ 2.000 (chronic health)	0.029	0.398	0.034	0.025	0.029	0.483	0.035	0.365	
Manganese as Mn #	mg/L	Chem-TM02	≤ 0.300 (aesthetic)									
	mg/L	Chem-TM02	≤ 0.400 (Chronic health)	<0.020	0.020	<0.020	<0.020	<0.020	0.336	<0.020	0.025	
Mercury as Hg #	mg/L	Chem-TM14	≤ 0.006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
	mg/L	Chem-TM02	≤ 0.070	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	
Lead as Pb #	mg/L	Chem-TM02	≤ 0.010	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015	
Selenium as Se #	mg/L	Chem-TM02	≤ 0.040	0.030	0.040	0.029	0.028	<0.020	<0.020	<0.020	<0.020	
Vanadium as V #	mg/L	Chem-TM02	≤ 0.200	0.014	<0.010	0.011	0.011	<0.010	0.012	<0.010	<0.010	
Zinc as Zn #	mg/L	Chem-TM02	≤ 5.000	<0.020	0.021	<0.020	<0.020	<0.020	0.021	<0.020	0.023	

Notes:

Results marked with (N) in this report, are not included in the SANS Schedule of Accreditation for this laboratory.

Parameters higher than the specifications, are clearly marked. Table with specifications included for comparison.

Bacteriological results obtained from samples older than the prescribed 24 hours may be inaccurate.

Signature: _____

Dr L. Deyzel (Technical signatory / Technical manager)

END OF REPORT

Appendix 2A: Coordinates of the sampling sites and insitu parameters for dry season

Sample ID	Longitude	Latitude	Temp. (°C)	DO	EC (µS/cm)	PH
Patising- 01	28.9210152	-28.993031	5.3	12.15	1423	12.15
Patising-02	28.9161846	-28.9914784	5.8	11.9	1401	13.74
Patising-03	28.9125797	-28.9922808	7.4	12.6	1437	11.89
KR US- 04	28.9307696	-28.9903995	11.5	7.99	64.7	7.99
KR US-05	28.9342761	-28.9869316	11.9	8.12	53.0	8.01
KR US-06	28.9286756	-28.9865128	12.6	8.30	62.3	7.78
KR DS - 08	28.9322215	-28.99755355	13.2	9.17	325.2	8.12
KR DS-07	28.93040299	-28.99437002	13.7	10.32	318.1	7.65
KR DS-09	28.935467	-28.99683333	14.1	9.54	343.7	7.61

Appendix 2B: Insitu parameters for wet season

Sample ID	Longitude	Latitude	Temp. (°C)	EC (µS/cm)	PH
Patising- 01	28.9210152	-28.993031	16.5	1900	7.33
Patising-02	28.9161846	-28.9914784	16.8	1890	7.65
Patising-03	28.9125797	-28.9922808	17.0	1910	7.23
KR US- 04	28.9307696	-28.9903995	17.5	79.8	7.46
KR US-05	28.9342761	-28.9869316	18.1	98.7	7.66
KR US-06	28.9286756	-28.9865128	18.4	83.4	7.92
KR DS - 08	28.9322215	-28.99755355	19.0	177	8.02
KR DS-07	28.93040299	-28.99437002	19.2	202	7.42
KR DS-09	28.935467	-28.99683333	21.3	192	7.87

Appendix 3: Letter of approval to sample Khubelu River by the Department of Water Affairs.



**Department of Water Affairs
P.O. Box 772
Maseru, Lesotho**

Fax: 22310437

Tel: 22317516

W/WA/A/7.8

March 28, 2018

Head of Programme
Faculty of Applied Sciences
Programme of Environmental Management
P.O. Box 652
Cape Town, 8000

Dear Dr. Malaza,

Permission for Mr. Bokang Shakhane (217137326) to Take Samples from Khubelu River

Reference is made to your letter on the subject, dated 13th March 2018.

Permission is hereby granted for the above-mentioned Student to undertake the above-mentioned sampling, in collaboration with the Department of Water Affairs.

We look forward to outcomes of the analysis and the study.

Yours sincerely,



**Motoho Maseatile (Mr.)
Director**

cc: Mr. Bokang Shakhane (217137326)