The ecological assessment of the influence of anthropogenic activities on Palala River, Limpopo, South Africa

by

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Declaration

I Mr Sifundile Sibiya declare that the dissertation submitted for the Master of Science degree in Environmental Science at UNISA (University of South Africa) is my own work and has not been submitted for a degree in any other institution of higher learning. The work presented here is that of the author unless otherwise stated. I declare that I adhered to the Research Ethics Policy of UNISA and I received ethics approval before commencing with field work.

Student's signature

Date

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Abstract

Water quality integrity deterioration is a severe global issue due to urbanisation, population growth, pollution and other anthropogenic activities threatening freshwater integrity. Anthropogenic activities alter freshwater integrity which leads to negative impacts on general ecological functioning of rivers. The current study was conducted along the Palala River in the Waterberg district in Limpopo, South Africa. The study was designed to employ multivariate and multimeric methods to determine relationships between environmental variables and macroinvertebrate communities. Macroinvertebrates were used to assess spatial and temporal changes in water quality using SASS5 (South African Scoring System version 5). The ecological status of the Palala River was clearly revealed through measuring of nutrient concentrations and environmental variables that influence water quality, and the macroinvertebrate communities that are found within the river. The results indicated the water quality was significantly impacted by changes in chloride concentrations at the site which had the most human interactions. The multivariate analysis revealed that macroinvertebrate communities were impacted by changes in the concentrations of chlorides (Cl^-), total dissolved solids (TDS), as well as electrical conductivity (EC). Additionally, simple linear regression indicated that the abovementioned environmental variables had an impact on Taxa richness, total abundance and taxa diversity. The river was revealed to be in a natural state as it drains through upstream (P1 and P2) and midstream sampling sites (P3 and P4). Interestingly, there was a massive improvement in water quality as the river drains within Lapalala Wilderness Reserve (sites P3 and P4). Unfortunately, there was a dramatic decrease in water quality as the river exited the reserve draining through downstream sites P5 and P6. The sampling site P5 was highly impacted by human settlements and domesticated livestock increasing nutrient concentrations in the river. The results revealed that an increase in chloride concentrations affected macroinvertebrate abundances at sampling site P5. Sampling site P5 was dominated by highly tolerant taxa at 75%, intermediate 25% and 0% sensitive taxa. The tolerant animals such as Chironomidae, Hydracarina and Ceratopogonidae were found in abundance at this site. The highest recorded SASS5 score was 165 at sampling site P4 and lowest was 80 at sampling site P5. The highest ASPT score was 8,5 at sampling site P3 and lowest was 3.8 at sampling site P6. The scores indicated that the river was severely impaired at sites P5 and P6.

Keywords: Anthropogenic activities, ecological status, macroinvertebrates, water quality, diversity

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List of Symbols and Acronyms

Abbreviation	Designation
ANOVA	Analysis of Variance
ASPT	Average Score per Taxon
dbRDA	distance based Redundancy Analysis
DO	Dissolved Oxygen
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitation
Cl^-	Chloride
EC	Electrical Conductivity
GSM	Gravel Sand and Mud
IHI	Index of Habitat Integrity
K	Potassium
KZN	KwaZulu Natal
Mg	Magnesium
Na	Sodium
NO_3^-	Nitrate
NWA	National Water Act
PERMANOVA	Permutational analysis of Variance
PET	Potential Evapotranspiration
PRIMER	Plymouth Routines in Multivariate Ecological Research
RHP	River Health Programme
SASS	South African Scoring System
SO_4^{2-}	Sulphate
TDS	Total Dissolved Solids
TVHR	Transparent Velocity Head Rod

List of Definitions

Term	Definition
Anthropogenic	- influenced by human activities in nature
Biotope	- the habitat together with its recurring associated community of species, operating together at a particular scale
BVSTEP	- a function within BEST routine used to select environmental variables that were closely correlated with patterns in species data
Canonical analysis of principal	- an ordination procedure that uses a resemblance matrix to analyse (dis)similarities between environmental variables
Correlation	- interdependence of variable quantities
DistLM	- distanced based linear model used to explain species-environmental relationships
Euclidean distance model	- the ordinary distance between two points determining similarities and relationships in data sets
Habitat modification	- changes in ecological processes and species composition due to human activities
PERMANOVA	- is a non-parametric test alternative multivariate ANOVA test
RELATE	- a procedure that performs non-parametric correlations between environmental and biological data
Regression	- a measure of the relationship between the mean value of one variable
Riparian	- pertaining to the banks of a river
River continuum	- the entire catchment system as the river flows from source to mouth

CHAPTER 1
GENERAL INTRODUCTION: LITERATURE REVIEW AND THESIS OUTLINE

1.1 Introduction and background

Rivers are an important part of life on earth in many ways. They host a lot of organisms and provide freshwater that is vital for human and animal survival. Humans have lived along rivers throughout history, using them for the provision of drinking water, fishing, irrigation and sanitation. Governments of countries across the world have taken lead responsibilities to control the sustainable use of water extracted from rivers. As the demand for water increases above the available water resources, rivers are faced with multiple threats resulting from anthropogenic activities (Skoulikidis *et al.*, 2022). The quality of water in rivers is usually altered as the river flows through multiple land use practices. Such changes have measurable impacts on biota, water chemistry, levels of turbidity and the structural make up of a river (Mantel *et al.*, 2010). The degree of impact is largely dependent on the duration of dominant influential activities occurring along the river. In ecosystem studies, land use practices that affect the overall integrity of the habitat have resulted in riverbank modifications, alteration of channel flow, increased turbidity and a decrease in biological diversity (Kleynhans and Louw, 2007).

The term "water quality" is generally used by ecologists to describe the physical, chemical, biological, and aesthetic properties of water, which determines its fitness for use and its ability to maintain the health of aquatic ecosystems (Kempster *et al.*, 2012). Among other methods, macroinvertebrates have been widely used as indicators to determine the changes in water quality in the field of aquatic science. The need to constantly study the status of water in rivers have gained global popularity, owing to the importance of this natural resource to humans.

In global terms the geographic distribution of water resources is scarce and limited (Liu *et al.*, 2017). This is due to the imbalance between available freshwater for aquatic life and human consumption, representing 0.26% of the world's resources (Shiklomanov, 1998). Freshwater demands, as the human population grows each day, outweighs available and accessible water for usage (Shiklomanov, 1998), and it functions as a required natural resource for household usage, crop production (irrigation), waste disposal (mines and domestic) and also used for recreational purposes (Malmqvist and Rundle, 2002).

Although water is generally regarded as a renewable resource, it is vital to carefully manage and protect it due to its vulnerability to exploitation and susceptibility to pollution (Schwabe *et al.*, 2013). In terms of the United Nations definition, South Africa is regarded as a semi-arid water stressed country (Schwabe *et al.*, 2013). Therefore, it is vital that rivers should be prioritised as areas of conservation value. Current conservation efforts have emphasised the protection of terrestrial ecosystems with little attention focused to river protection (Nel *et al.*, 2007). Therefore, it is essential that terrestrial protected areas are strategically placed such that there is a natural water body that they can protect. Most river channels have been affected by the erection of dam walls for water storage in non-protected areas (Davies and Day, 1998). These problems will continue if the causes and forms of degradation are not removed leading to further habitat destructions.

Land degradation caused by previous land-use practices along the Palala River has been observed to cause water quality problems associated with wildlife trampling. Such problems limit easy access to usable clean water as water is turbid containing a lot of silt (Muller, 2020, pers. comm.). Water scarcity is already a problem and the number of people facing lack of access to usable freshwater might increase in the near future. Water degradation becomes a looming issue following population growth and urbanisation, usually in poor developing countries (Dudgeon, 1992) including South Africa (Oberholster and Ashton, 2008; Simaika and Samways, 2012), due to the fact that developing countries lack sufficient freshwater treatment facilities (Dudgeon, 1992; Malmqvist and Rundle, 2002). The use of water resources within a protected area is commonly driven by wildlife distribution and by human demands (Owen-Smith, 1996).

1.2 Water pollution in rivers

Agriculture and urban or rural activities are major causes of excessive phosphorus and nitrogen in aquatic ecosystems leading to anthropogenic influences of water quality alterations (Carpenter *et al.*, 1998). In addition, the presence of natural and anthropogenic sources for heavy metals in the riverbed sediments further exacerbates the quality of water in rivers. Furthermore, atmospheric depositions significantly contribute as a source of nitrogen in rivers (Carpenter *et al.*, 1998; Jarvie *et al.*, 1998). The inputs of nutrients are challenging to quantify because they are driven from activities over large areas and often vary due to the perturbations and non-static weather conditions (Jarvie *et al.*, 1998). In aquatic ecosystems, the nutrients affect aquatic species in a diversely causing problems such as toxic algal blooms, reduced levels, fish mortality, loss of biodiversity and other aquatic problems

(Durand *et al.*, 2011). Nutrient enrichment generally degrades aquatic ecosystems and significantly impairs the use of water for drinking, agriculture, recreation, and other common water usage purposes (Carpenter *et al.*, 1998). Influxes of nutrients such as nitrogen (N) and phosphorus (P) are responsible for eutrophication and water quality degradation in many rivers (Longley *et al.*, 2019).

1.3 Anthropogenic influences on rivers

For decades, pressures from human activities have impacted the ecological status of rivers all over the world. A lot of work has been done to understand, quantify and account for the collective influences of climate and human activities on many rivers worldwide (Allan *et al.*, 2021). Studies have further indicated that the indirect influences of climate change on river ecosystems are, in some degree also human induced (Jiang *et al.*, 2021; Grizzetti *et al.*, 2017). In a comprehensive study to quantify the effects of climate change and human activities on river hydrological health variations, Jiang *et al.* (2021) reported that human activities were the main driving factors for the hydrological health degradation during the whole human influenced period, contributing more than 80% in three studied catchments. The dependence of human development activities to constant water supply has pushed the levels of available freshwater in major rivers to a point of depletion (Connor, 2015). The most dominant and well-studied human activities with devastating impacts on water resources have been identified as agriculture and urbanisation or human settlements (Lovelock *et al.*, 2019). To effectively assess such impacts, researchers have studied the responses of aquatic biota to disturbances in their habitat (Bilotta and Brazier, 2008).

Macroinvertebrates have gained a lot of attention as biological indicators of habitat change in rivers (Oeding, 2019). They are known to be perfect bio indicators since they are extremely sensitive to sudden changes in water quality, they are widely distributed and are cost effective to use for rapid monitoring assessments (Anyanwu *et al.*, 2019). In the current study, the influences of activities such as agriculture, domestic water use and nature conservation were investigated to determine the ecological status of the Palala River using macroinvertebrates as bio indicators.

1.3.1 Agricultural influences on rivers

Agriculture has been, and remains important for food security globally, and rural communities rely on local farms for survival (Mănescu *et al.*, 2016). Agriculturally developed regions have been reshaped, altering hydrology and ecology in general. A study

conducted by Strungaru *et al.* (2021) suggested significant longitudinal changes in physical and chemical parameters observed in the heavily agriculturally developed areas of the Bahlui River Basin. Furthermore, Shabalala *et al.* (2013) suggested that agriculture is one of the major causes of surface water quality degradation mainly as a result of the excessive use of agrochemicals. The primary means by which ecological water quality can be impacted by agriculture is through the introduction of nutrients such as nitrogen (N) and phosphorus (P) to water, which can lead to eutrophication of waterbodies (O'Donoghue *et al.*, 2021). The combination of livestock manure and mineral fertilisers lead to significant enrichments of surface soils with nutrients.

During rainfall events, overload flow from agricultural areas, containing high levels of nutrients, organic matter and suspended particles, is transported into streams. Stable isotopic studies have shown the significant contribution of animal manure and fertilisers in river water, for example (Torres-Martínez *et al.*, 2021). Damming and the construction of water diversions aimed for irrigation have been reported to impede fish migration, alter stream flow, water temperatures and trap sediments (Malmqvist and Rundle, 2002). According to Ekka *et al.* (2020), dam construction results in the development of stagnant waters and reduced water velocity, which creates habitat conditions that disturbs the life cycle and growth rate of aquatic species. As a result, species diversity is also altered.

1.3.2 Influence of rural settlements

Human settlement, economic development, and population growth, all have some level of contribution to the pollution of streams to some extent. River pollution can be caused by (1) high sediment content derived from erosion, mining, construction, land clearing and other activities; (2) organic waste from human, animal and plant activities; or (3) the rate of addition of chemical compounds originating from industrial activities that dispose of their waste into the water (Pongoh *et al.*, 2021). These three mechanisms are all end products of human civilisation and industrialisation. Studies associated with human welfare and environmental impacts have reported that globally, 80% of municipal wastewater is discharged into the environment untreated, and industry is responsible for dumping millions of tonnes of heavy metals, solvents, toxic sludge and other wastes into water bodies every year (Sato *et al.*, 2013; Mateo-Sagasta *et al.*, 2015). This have long term effects on the quantity and quality of freshwater resources that are available for rural and underdeveloped areas. Access to clean and safe drinking water is still a problem in developing countries

especially in rural areas (Edokpayi *et al.*, 2018). Due to shortage of supply, rural communities often resort to alternative sources of water to meet their basic needs.

1.3.3 Influence of protected areas

Protected areas are one of the main instruments in conserving biological diversity and ecological integrity (Keppeler *et al.*, 2017). The process of incorporating all ecosystems when proclaiming protected areas is becoming increasingly sophisticated (Roux *et al.*, 2008). Influences of protected areas on the conservation of surface waters were studied by Mancini *et al.* (2005). A total of 19 protected areas were studied in relation to the biological quality of 32 streams running within those protected areas. Their findings indicated that the biological quality of streams was higher inside the protected areas when compared to the same streams in the surrounding areas. Although there are limited studies in the importance of nature reserves in river conservation (Gaston *et al.*, 2008), available literature strongly emphasises the need for the creation of reserves or landscape designations specifically for aquatic conservation (Keppeler *et al.*, 2017; Brashares *et al.*, 2001). More so because it has been evident that nature reserves shelter river systems from unauthorised anthropogenic disturbances, especially since most are usually privately owned. Regardless of their geographic location, the most common feature of Southern African rivers is the extent to which they have suffered anthropogenic disturbances, including organic enrichment, salinisation, acid pollution, over-utilisation and accidental and deliberate introduction of exotic, invasive plant and animal species (Davies and Day, 1998). As such, more research needs to be done to rapidly assess their ecological status and sustainable use.

1.4 The national river health programme (RHP)

Comprehensive monitoring programmes have long been established for chemical and physical characteristics of the water and are conducted regularly at numerous sites on the country's rivers. Biological monitoring, defined here as the utilisation of biota to provide an indication of the quality of the riverine environment, has only recently become a point of focus of organisations interested in ascertaining the biological characteristics and status of rivers in South Africa (Culp *et al.*, 2011). In addition, biomonitoring has become a very important tool in Europe and many other regions as a result of strong anthropogenic pressures affecting the health of lakes, rivers, oceans and groundwater (Leese *et al.*, 2018). Biomonitoring utilises one or more components of the biota such as fish, macroinvertebrates,

diatoms, etc., to provide an integrated full assessment of the catchment system (Lowe *et al.*, 2013).

Biomonitoring has been acclaimed as a reliable measure of environmental conditions, either physical or chemical. The use of both biomonitoring and physicochemical monitoring should actually be viewed as complementary. The ultimate goal of biomonitoring, therefore, is to evaluate the effect of human activities on biological resources (Fore *et al.*, 1996). In 1994, the country's first national biomonitoring programme for river ecosystems (i.e., the RHP) was initiated by the Department of Water Affairs and Forestry (DWAF, 2006). In South Africa, as in other countries such as the United States, Chile and Australia, the method of biomonitoring has gained much attention (Dallas and Rivers-Moore, 2012).

1.5 General state of water resources in South Africa

Management of water resources, i.e., provision of safe and reliable supplies for drinking water and irrigation, adequate sanitation, protection of aquatic ecosystems and flood protection, poses huge challenges in many parts of the world (Hering and Ingold, 2012). The Department of Water and Sanitation (DWS) is the branch of the South African government that is responsible for water management and monitoring in the country (DWAF, 1996). In South Africa, the issue of over-exploitation of freshwater resources is a huge challenge (Hedden and Cilliers, 2014). Throughout the history, the supply of quality water supply has been one of the key limiting factors in the economic development of this country (Lester *et al.*, 2000). Water scarcity is a significant constraint for South Africa and is acknowledged by the Department of Water Affairs and has projected that water the demand will exceed supply by 2025, unless significant attention given to managing water demand (Steele and Schulz, 2012).

Agriculture, industrial and urban or rural expansion combined require significant quality water supply (Swatuk, 2010). Supplying water involves the apportionment of water from common sources to a wide range of people to fulfil their needs and specific uses (Nakayama, 2003). It is, however, very challenging to meet the requirements making it a huge constraint to the improvement of access to adequate water sources (Nakayama, 2003). Therefore, it is essential to ensure that no harm is done to the water sources and to facilitate the sustainable management of water including all other related natural resources interconnected with water sources (Nakayama, 2003).

1.6 Biological assessment and monitoring of rivers

A common problem in community ecology is to determine how different species respond to external factors such as environmental variables, pollution and other anthropogenic factors (ter Braak, 1988). According to (Lowe *et al.*, 2013) 'biomonitoring is the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in a quality-control programme'. It is designed to be a more sensitive and reliable evaluation of environmental conditions than either physical or chemical measurement approaches (Lowe *et al.*, 2013). Biomonitoring techniques have been used successfully for several decades to determine water pollution (Debén *et al.*, 2016). Generally, biological monitoring can be done with any living organisms, but benthic macroinvertebrate, fish and periphyton (algal) assemblages are used most often, in that order (Engel and Voshell, 2002). Traditionally, physical and chemical assessments formed the backbone of most water quality assessment programmes, but there were limitations identified (Lowe *et al.*, 2013).

Most aquatic species spend part or all of their lives in rivers or water, experiencing various physical and chemical changes that occur inside the water bodies over time (Briere *et al.*, 1999). They are therefore highly considered as great indicators of stream ecological health (Dallas and Rivers-Moore, 2012). The ultimate goal of biological monitoring is to assess the anthropogenic activities on biological resources (Fore *et al.*, 1996). In the past, water quality monitoring was only based on physical and chemical assessments (Ouyang *et al.*, 2006). However, the challenge with measuring only chemical and physical variables is that no information is provided on the ecological effects or impacts on aquatic biota (de la Rey *et al.*, 2008).

1.6.1 Using macroinvertebrates in biomonitoring

In biomonitoring, benthic macroinvertebrates are the most commonly recommended group of organisms for freshwater monitoring and have been researched extensively (Stein *et al.*, 2008). Biomonitoring uses concepts of biological integrity and is made of biological indicators and indices (e.g., diatoms. macroinvertebrates, fish, riparian vegetation), as well as indices for assessing instream and river ecosystems (Lowe *et al.*, 2013). Mostly, lotic systems are characterised by macroinvertebrates whose presence generally indicates the condition of the aquatic habitat (Sheldon and Walker, 1998). Macroinvertebrates play a very important role as source of food they acquired from primary producers to a number of fish and also general instream food source providing linkages with upper-level organisms within the food web (Wallace and Webster, 1996). Macroinvertebrates also play a role of being

indicators of stream health or degradation (Wallace and Webster, 1996). In addition, macroinvertebrates respond spontaneously to water alterations and respond differently to various types of pollution (Ollis *et al.*, 2006). Macroinvertebrates are extremely popular in South Africa and are mostly applied and are reliable method of stream assessments (Dickens and Graham, 2002).

1.6.2 Using biological indices for ecological assessment of rivers

One of the best practical and easily applied methods to assess the ecological status of a river and to determine human impacts and anthropogenic activities reducing water quality is using macroinvertebrates (Sharifinia *et al.*, 2012). The measurements are taken using biotic index measuring the quality of an environment by the types of species that are found in it (Lenat, 1993). The collected and measured biotic indices are for species to be given scores based on their abundance based on their sensitivity or tolerance to pollution (Ollis *et al.*, 2006). The scores are then given for each taxa and then combined and averaged to provide values to measure and interpret the integrity of water per site (Ollis *et al.*, 2006). The South African Scoring System (SASS) is the preferred method among several techniques validated tested and modified (Dickens and Graham, 2002).

1.6.3 The South African Scoring System Version 5 (SASS5)

The South African Scoring System (SASS) is a macroinvertebrate based biotic index that studies the ecological condition of rivers assessing any potential or occurring anthropogenic influence (Dickens and Graham, 2002). The rapid bio assessment method, SASS was developed to assess water quality in river ecosystems (Dallas, 1997). The scoring system is based on the availability or absence of certain macroinvertebrates groups for the purposes of calculating the SASS score, number of taxa and Average Score Per Taxon (ASPT) (Dallas, 1997). The scores are then interpreted to determine the status of the river. The method was designed and aimed for a rapid and cost-effective evaluation of the South African rivers (Chutter, 1972). After collection, macroinvertebrate families are given sensitivity scores ranging from 1 to 15 in increasing order of their sensitivity to water quality changes, and the results are expressed as an index score (SASS score) and an average score per recorded taxon (ASPT) value (Dickens and Graham, 2002). The SASS5 is the approach which is currently used and have been recommended by environmental practitioners as the preferred version that supports the RHP (Dickens and Graham, 2002).

1.6.3.1 Presenting and comparing the SA approach

In many countries, the use of macroinvertebrates in ecological monitoring of aquatic ecosystems that are impacted by anthropogenic influences is common when assessing water quality integrity (Hering *et al.*, 2006). The use of macroinvertebrates is widespread and considered as a reliable method, and is normally applied in water quality assessments and its reliability has been tested for the assessment of water quality in South Africa (Dickens and Graham, 2002).

The method widely used in many areas across the world is the Biological Monitoring Working Party (BMWP) scoring index that was designed by Armitage *et al.* (1983) and Hawkes (1979) to assess and classify water quality in British rivers. The BMWP score system was initially introduced in 1980 to provide an index of water quality in rivers of England and Wales based on macroinvertebrates communities (Paisley *et al.*, 2014). The BMWP was later modified to accommodate various areas including South Africa (Dickens and Graham, 2002). The method was later adapted after being evaluated and tested in South African rivers. The BMWP was further modified to form the SASS in the 1990s (Dickens and Graham, 2002). The use of SASS method is not only successful and restricted to South Africa (Dallas, 2004), but also been utilised in other countries in southern Africa (Bere and Nyamupingidza 2014). Interestingly, SASS5 has been systematically modified into the NASS (Namibian Scoring System) to include certain species that occur in some parts of Namibia (Palmer and Taylor, 2004).

1.7 The Index of Habitat Integrity (IHI)

Alteration of natural landscapes into agricultural land-uses causes major threats to global biodiversity (Brasil *et al.*, 2020). In river ecosystems, macroinvertebrates are affected and are most vulnerable to anthropogenic activities, therefore, it is very important to study and assess how habitat integrity is affected by anthropogenic activities (Brasil *et al.*, 2020). The Index of Habitat Integrity (IHI) assesses the anthropogenic activities and damage they cause in a river ecosystem (Ollis *et al.*, 2006). In addition, the assessment includes identifying in-stream and riparian zone integrity by collecting information about water clarity, nutrients, bed modification, algal growth, floods and general in-stream physical and chemical factors (Dallas, 2021). Habitat integrity is determined by classifying IHI scores into percentage classes from A to F, where class A indicates unmodified habitat (Dallas, 2005).

1.8 Problem statement

Agriculture does not only reduce the quality of water in adjacent rivers, but also affects the quantity of clean water especially since most rivers get diverted to cater for agricultural fields (Dahal *et al.*, 2007). The only natural way to replace the water lost through agriculture is through significant rainfall. However, high rainfall comes with high amounts of agricultural run-off that brings several residual fertilisers back into rivers (Mcdowell and Smith, 2012). Chemicals such as phosphates and nitrates leached from agricultural soils are a central factor in poor surface water quality. Much loss of phosphates and nitrates takes place immediately after the application of highly water-soluble fertilisers in crop producing farms (Mcdowell and Smith, 2012). Numerous natural aquatic ecosystems are driven by specific quantities of phosphorus, which plays a huge role in determining high levels of biodiversity (Kumar, 2011). The anthropogenic increase of phosphorus in rivers have the potential to produce negative effects on natural aquatic ecosystems (Kumar, 2011).

On the other hand, channel modifications also contribute to alterations of aquatic ecosystems. Natural channels have been straightened and deepened for surface water drainage ditches with significant effects on channel morphology, instream habitats for aquatic organisms, floodplain and riparian connectivity, sediment dynamics and nutrient cycling (Blann *et al.*, 2009; Bunting *et al.*, 2021). Cumulatively, these changes have profound implications for aquatic ecosystems and the biodiversity they support. Structurally, channelisation can affect the environment by cutting off oxbows and meanders, lowering ground water levels, reducing ground water recharge from stream flow, and increasing downstream flooding (Saad and Habib, 2021). Biologically, channelisation reduces the size, number, and species diversity of fish and other aquatic animals in rivers (Saad and Habib, 2021). River channels are also altered by sewage and other domestic waste disposal (Dragon *et al.*, 2016). Sewage disposal areas are called point sources of pollution, and the major point sources of organic pollution are septic tanks (Lasagna *et al.*, 2016). These tanks are usually located in rural settlements where no proper sewer systems exist, as such, there is easy contamination of both surface and groundwater, eventually changing the natural state of channel hydrology (Lasagna *et al.*, 2016).

Some altered natural landscapes have been deserted and neglected after extensive anthropogenic activities such as agriculture (Sultana, 2020) . Based on the location and state of conservation importance of some of these affected landscapes, conservation efforts tend to work towards proclaiming them to protect and restore. Lapalala Wilderness Reserve (Lapalala) is one of the conservation organisations that supports the restoration of degraded

lands. A portion of the Reserve has severely degraded soils that were previously used for tobacco farming. Unfortunately, these tobacco fields occurred immediately adjacent to the Palala River, directly affecting the quality of water in the river, especially during rainy seasons. Soon after the soil lost its fertility and ability to produce good yields, the tobacco fields were left denuded and barren. This is not surprising because for decades, tobacco production has moved from one location to another due to the loss of soil fertility (Sultana, 2020). As tobacco farming reduces soil fertility, other crops do not grow well (Motaleb and Irfanullah, 2011; Sultana, 2020).

When a nature reserve attempts to restore previous tobacco fields, they are faced with the great challenge of trying to evaluate which restoration methods will be effective, considering the natural impacts of wildlife on the already affected area. The current study addresses the ecological effects of the activities that occur along the old tobacco fields on the water quality of the Palala River within Lapalala. Furthermore, the effects of agricultural and domestic activities that occur outside the nature reserve will also be addressed.

1.9 Justification

The assessment of health status of water resources today is extremely relevant due to the increased pressure posed by humans to the environment. The main river ecosystems in South Africa are in a dire state: 84% of the ecosystems are threatened, with a disturbing 54% critically endangered, 18% endangered and 12% vulnerable (Nel *et al.*, 2007). It is clear that water resources are among the most vulnerable objects to this pressure. There has been rising concerns in the state of water quality in the Palala River especially during rainy seasons. High levels of turbidity have been reported in some areas within Lapalala causing a need for study. The water quality status of Palala River is therefore highly uncertain due to the prevailing past land use practices such as tobacco and cattle farming, agricultural fields and domestic water usage outside the reserve. These problems will continue if the causes and forms of degradation are not studied and remain unknown leading to further habitat destructions. This study will provide enough information that is needed by decision makers to take informed decisions regarding their impact on the river and how they could mitigate their impact going forward.

1.10 Approach to the study

This research was designed to employ multivariate and multimeric methods to determine relationships between environmental characteristics and biological communities.

Descriptive approaches were used to analyse habitat conditions and anthropogenic modifications using methods that involved recording of measurements and observations on standardised field-data collection sheets. The aim was to examine whether pollutants originating from activities that occur in the catchment areas influence the river ecosystem and ecological functions in general. Macroinvertebrates were collected at different locations in the Palala River, with each location having a specific land use (farming, nature reserve and rural settlements). It was predicted that there would be different levels of disturbances and pollution in the form of nutrients at each site, and such changes would result in variations in water chemistry and macroinvertebrate species composition and community structure. Regression methods were used to further test the predictions.

1.11 Research aims, objectives and hypotheses

The study aims to determine the ecological status of the Palala River following the river health program as stated by (Dallas, 2005).

The objectives are to:

- assess the variability of chemical and environmental variables such as total ammonia, dissolved potassium, total phosphorus, nitrates, sulphate, sodium, chloride, total dissolved solids, electrical conductivity, dissolved magnesium, dissolved oxygen, water depth, flow velocity, water temperature and pH of the water from source to mouth under various land use practices;
- study the longitudinal trends in macroinvertebrate communities along the Palala River as it drains through different land use practices;
- assess the influence of chemical and environmental variables such as total ammonia, dissolved potassium, total phosphorus, nitrates, sulphate, sodium, chloride, total dissolved solids, electrical conductivity, dissolved magnesium, dissolved oxygen, water depth, flow velocity, water temperature and pH on macroinvertebrate diversity, abundance and richness. The influence of these variables on water quality was also assessed using the ASPT, SASS5 scoring techniques.

The following hypotheses were tested:

Hypothesis 1: the concentration of total ammonia, dissolved potassium, total phosphorus, nitrates, sulphate, sodium, chloride, total dissolved solids, electrical conductivity and dissolved magnesium will be lower at the sites located at the source and those located within Lapalala, and higher, at sections located downstream of the reserve;

Hypothesis 2: the sites located upstream of Lapalala, and those that are located within the reserve will be characterised by high ASPT, SASS scores, macroinvertebrates abundance, high taxa richness and taxa diversity when compared to those located downstream of the reserve;

Hypothesis 3: the concentration of total ammonia, dissolved potassium, total phosphorus, nitrates, sulphate, sodium, chloride, total dissolved solids, electrical conductivity and dissolved magnesium will have an influence on macroinvertebrate community structure, such that areas with high levels of nutrients will have low ASPT, SASS score, macroinvertebrate abundance, low taxa richness and low diversity when compared to sites that have high concentrations of the above nutrients.

1.12 Thesis structure

Chapter 1 is the general introduction on the global and national water crisis issues. The problem statement, justification, objectives and hypotheses of the study are presented in this chapter. This chapter also outlines the literature review. The possible impacts of agriculture, conservation and rural settlement and human impact is explained in detail. **Chapter 2** is the description of the study site, physico-chemical parameters measured, macroinvertebrate sampling procedures, macroinvertebrate identification protocols and the methods to be employed for statistical analyses is given in great detail in this chapter. **Chapter 3** contains the results on the effects of various anthropogenic activities on the macroinvertebrate community structure and water quality. Relationships between physico-chemical parameters and the macroinvertebrate communities are described in this chapter. **Chapter 4** is the discussion of the results in relation to current literature. The description of patterns and trends are detailed in this chapter, considering all factors that could have possibly caused the identified patterns and trends. **Chapter 5** outlines the general recommendations made according to the research findings.

1.13 Ethical considerations and limitations of the study

The collection of macroinvertebrates was done such that all collected macroinvertebrates were sampled, identified and released immediately onsite after being identified and recorded for analysis. Additionally, the method employed is a non-destructive method which does not kill or remove macroinvertebrates from their natural habitat. Overall, collection methods for both macroinvertebrates and water samples, does not disturb the natural functioning of the ecosystem.

Despite being reliable and able to determine the ecological integrity of the rivers, SASS5 can be limited by damming, flooding, heavy rainfall and habitat disturbances that can alter the natural functioning of the ecosystems (Dickens and Graham, 2002). In addition, the limitations include lack of water during dry seasons which can affect the results interpretation for comparison purposes. This can have a negative implication when comparing seasons and sampling sites.

CHAPTER 2
STUDY AREA, METHODS AND MATERIALS

2.1 Study area

The study area is at Lapalala Wilderness Reserve (Lapalala), a 48 000 ha nature reserve located in the Waterberg district within the Limpopo Province 23°52'33"S 28°18'20"E (Fig. 2.1). The site is located on the Waterberg plateau approximately 50 km north of Vaalwater, 60 km south east of Lephalale and 100 km west of Polokwane (Coetzee, 2016). The altitude of the reserve is at an average of 1175 m above sea level (Walker, 2014).

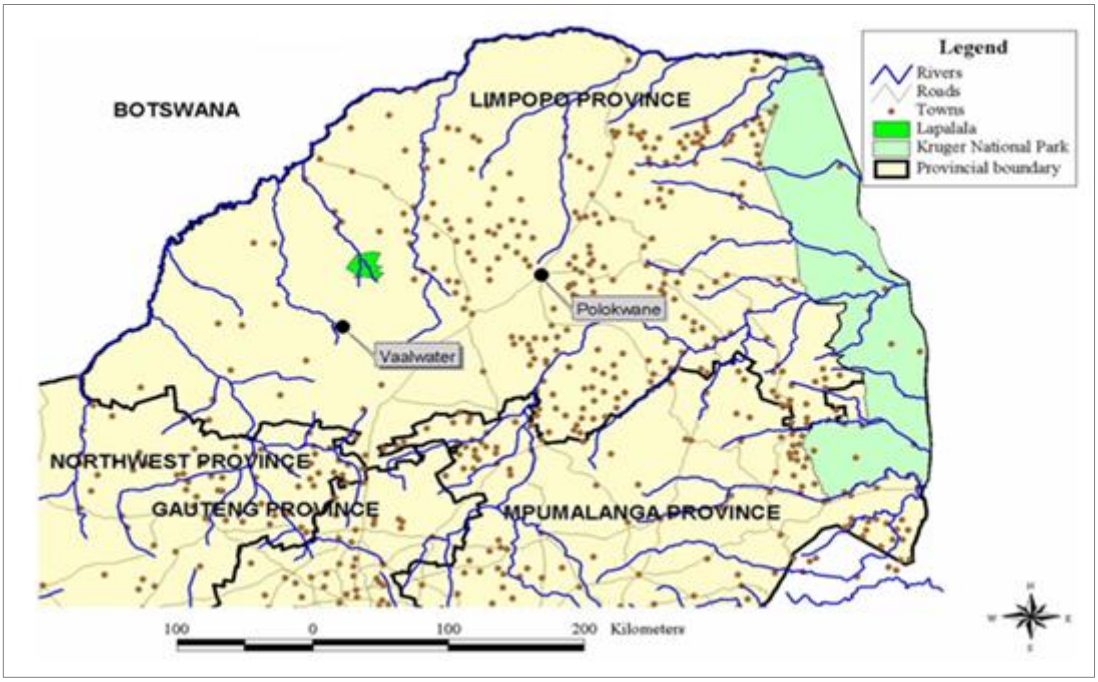

Figure 2.1: The location of Lapalala in relation to its surrounding areas in the Limpopo Province (Lapalala Wilderness, n.d.)

The area is classified as mixed bushveld which is a subdivision of a Savanna biome (Low and Rebelo, 1998; Rutherford *et al.*, 2006). Lapalala is bisected by the perennial Palala River that drains the area from south to north (Fig. 2.2). The river flows through Lapalala for approximately 55 km and is merged with the most important tributary, Bloklandspruit river (Angliss *et al.*, 2007). The area has a network of smaller streams that drains and forms part of the entire catchment system. Several dams have been erected on Bloklandspruit and minor streams for water provision for animals. Although accessibility is not easy especially within the reserve because of steep topography, the area is considered to be well watered. Most parts of the reserve are located within 4 km of the two main rivers and the furthest

distance is just above 8 km from the rivers (ICS, 2004). The distances have important implications in terms of water provision to wildlife within Lapalala. The area has seeps and wetlands that occur in and out of the reserve.

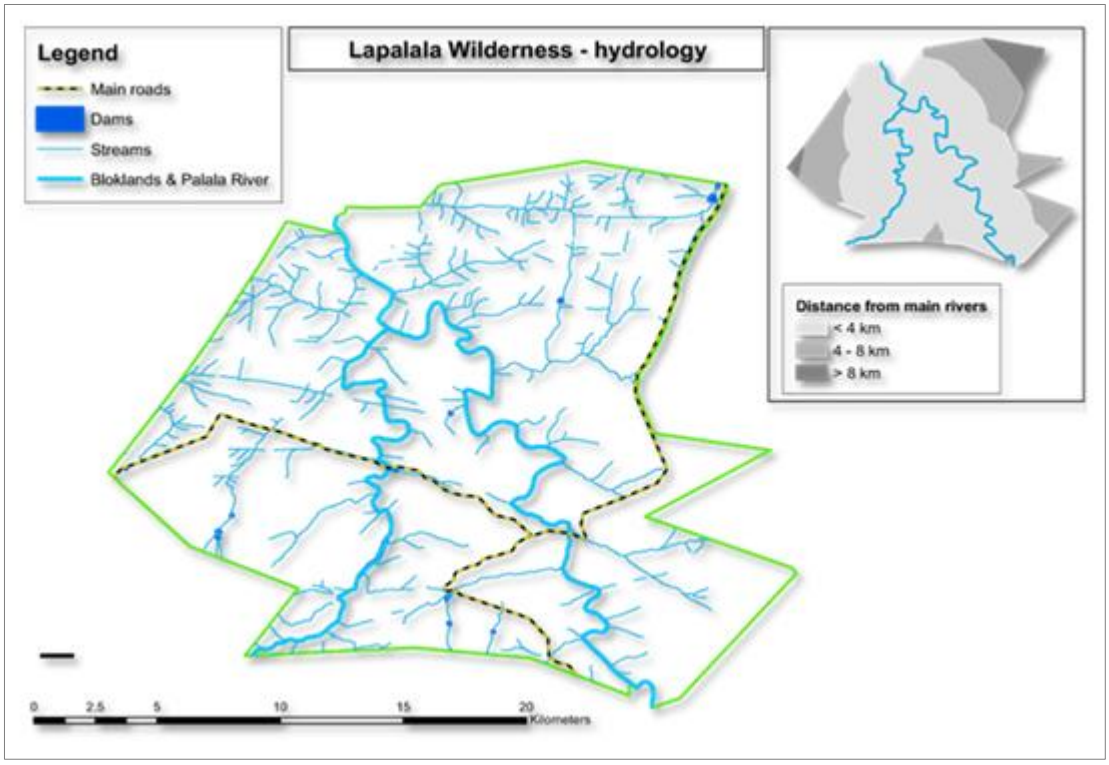

Figure 2.2: Map with Palala and Bloklandspruit Rivers meandering through Lapalala (Lapalala Wilderness, n.d.)

The area is dominated by sandstone sedimentary rocks (Fig. 2.3) which are porous and acts as good aquifers that hold water like sponges, slowly releasing it throughout the rainy season (Olivier *et al.*, 2008). River water levels fluctuates based on current rainy season and amount of rainfall received as well as previous seasons rain (Schulze, 1997). The area is covered with sandy dystrophic soils that are derived from sandstone and rich red soils that are derived from quartzite and dolerite.

Figure 2.3: Showing sedimentary rocks along the Palala River near sample site P4 within Lapalala

The Waterberg receives summer rainfall with a mid-summer seasonality (Adeola *et al.*, 2019). The overall mean annual rainfall for Lapalala is estimated at 600 mm but ranges from 400 mm to 600 mm (Wadley *et al.*, 2021) (Fig. 2.4). The average temperatures range between 30°C in January and 14°C in June (Ruwanza and Mulaudzi, 2018) (Fig. 2.5).

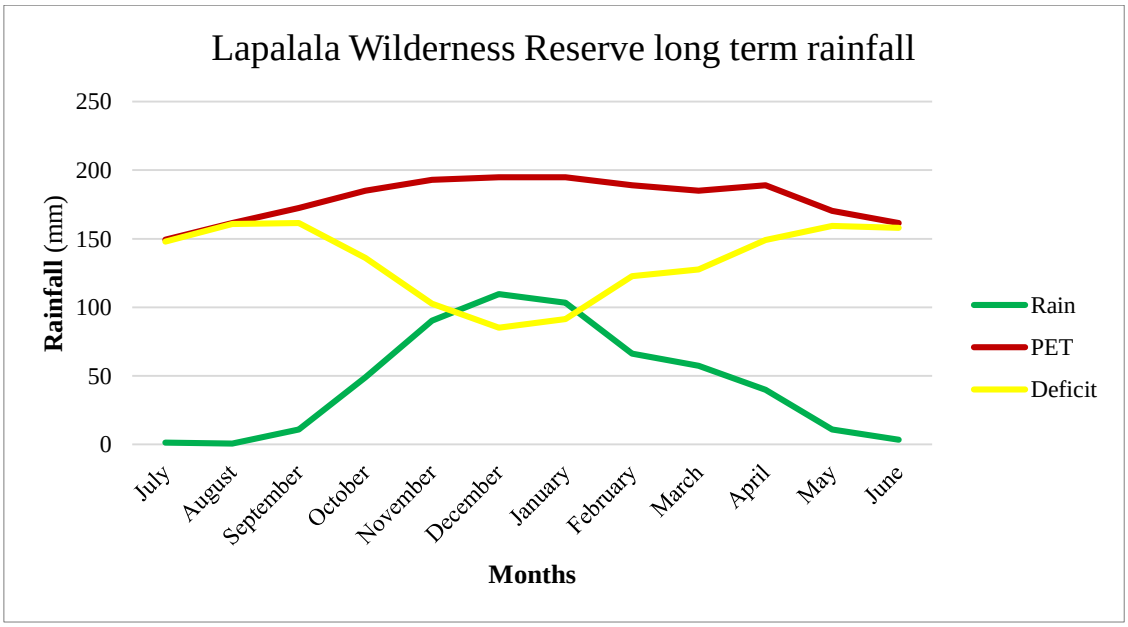

Figure 2.4: Long-term average rainfall, PET and water deficit over 5 years (2016-2021)

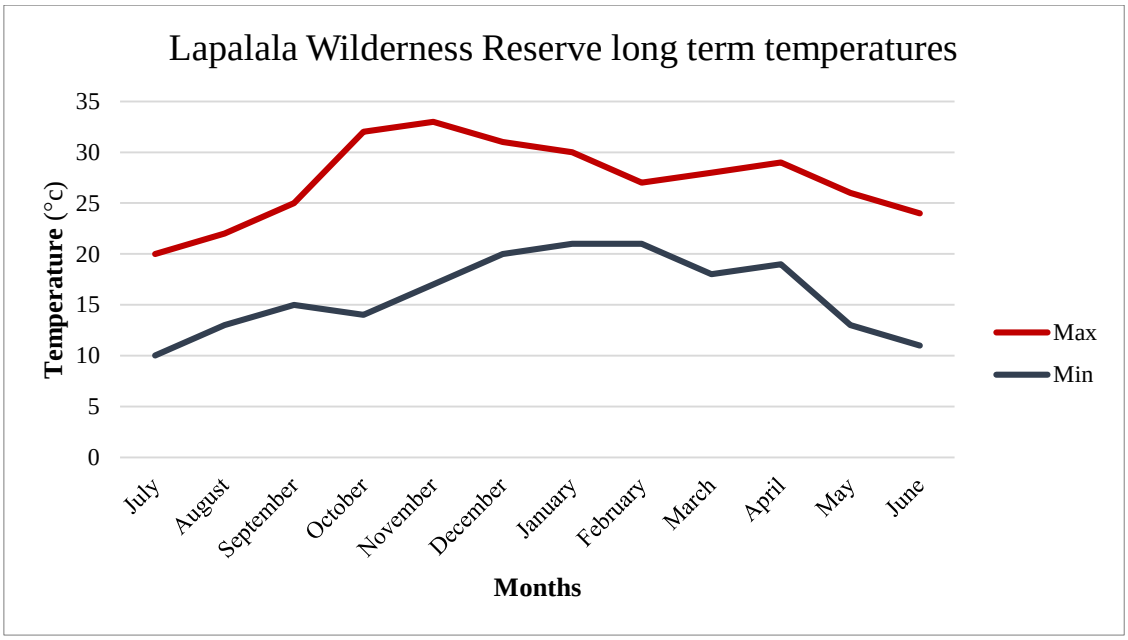

Figure 2.5: Average monthly temperatures of Lapalala over 5 years (2016-2021)

2.2 Study design

The sampling sites were chosen based on various activities occurring along the Palala River from site closest to the source to the site closest to the mouth where it pours into the Limpopo River (Figs. 2.6, 2.7 and 2.8).

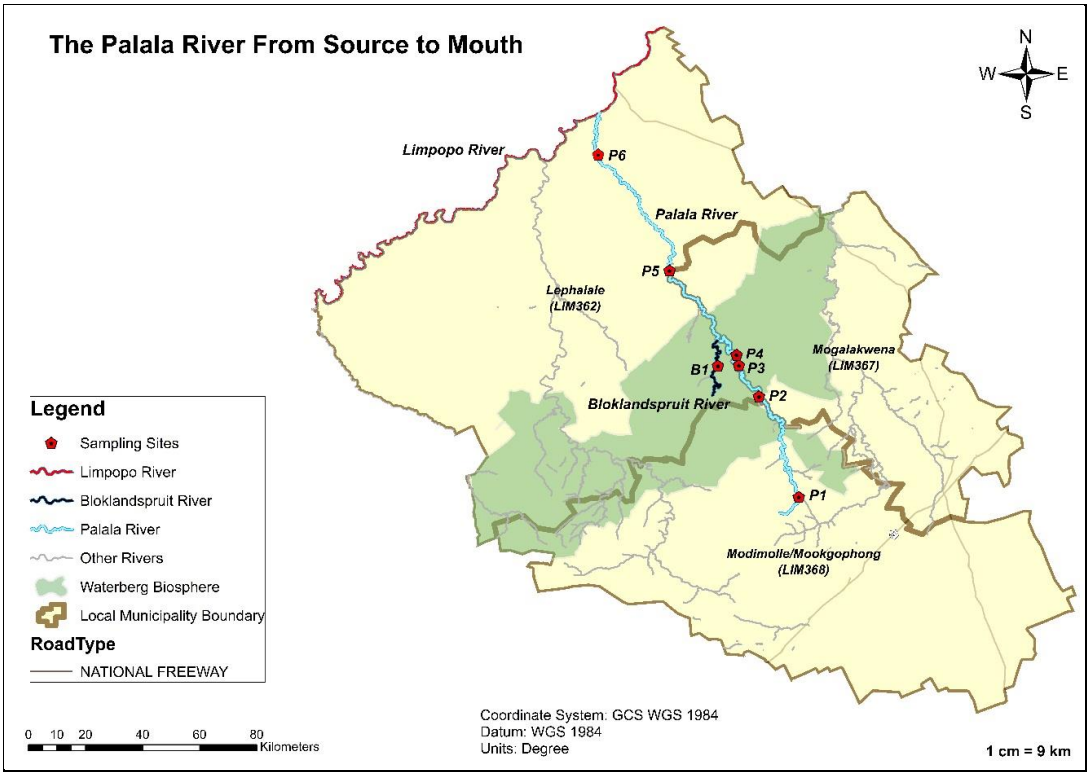

Figure 2.6: Shows the Palala River from source to mouth cutting through Lapalala, agricultural fields and rural communities

Figure 2.7: Sample sites from P1 to P6 (Palala River)

Figure 2.8: Sample site B1 (Bloklandspruit river - tributary)

Refer to sites named P1 to 6, where P represents sample sites along the Palala River and B as the main tributary Bloklandspruit River. Below is the description of each site as depicted on the map (Fig. 2.6):

- Palala (P1)1: represents the first sample site, located approximately 12 km below the source of Palala River;
- Palala (P2): is the second site located approximately 57 km downstream of site 1 before the river enters Lapalala;
- Palala (P3): the third site inside Lapalala (next to Doreenlegte) located approximately 21 km of site 2;
- Palala (P4): is the fourth inside the reserve (next to Mudumela), located 5 km of site 3;
- Bloklandspruit (B1): representing the main tributary river called Bloklandspruit;
- Palala (P5): the fifth site 54 km of site 4 located outside the Reserve after confluence between Palala and Bloklandspruit Rivers representing the influence of agricultural fields as the river exits the reserve and rural settlement as it enters Shongoane Village;
- Palala (P6): the sixth site approximately 59 km of site 5 is located downstream of all the rural settlements to Shongoane to Ga-Seleka (Table 2.1).

Table 2.1: The study sampling sites, and their location along the Palala River

Site No.	Sampling Site Code	Site Name	River	Coordinates
1	P1	Source (± 12 km downstream)	Palala	24˚17'46.81"S 28˚ 31'27.73"E
2	P2	Bridge next to Melkrivier (± 57 km downstream site 1)	Palala	23˚14'21.25"S 27˚54'4.31"E
3	P3	Doreenlegte (± 21 km downstream of site 2)	Palala	23˚52'51.43"S 28˚20'8.81"E
4	P4	Mudumela (± 5 km downstream of site 3)	Palala	23˚50'52.19"S 28˚19'42"E
5	B1	Hippo Dam	Bloklandspruit	23˚52'56.91"S 28˚16'10.04"E
6	P5	Shongoane (bridge R518 ± 54 km downstream of site 4)	Palala	23˚34'56.80"S 28˚07'02"E
7	P6	Ga-Seleka (Bridge R572 ± 59 km downstream of site 5)	Palala	23˚13'01.56"S 27˚53'32.61"E

2.3 Data collection

2.3.1 Collection of physico-chemical parameters

Physico-chemical parameters were measured at each site per sampling period. The temperature and pH readings were measured on site using a portable pH meter which uses an automatic calibration technique. The results are instantly displayed on a dual LCD screen when the meter is in contact with water. The flow velocity and water depth were measured using a portable velocity plank (Fig. 2.9). The velocity plank is a Transparent Velocity Head Rod (TVHR) which originated in the United States of America (USA). The plank is a transparent plastic board with a measuring ruler that is used to record measurements to determine flow velocity and water depth. The board was placed vertically on each biotope on the riverbed. The level of water on both sides of the plank were recorded and the difference between water levels were used to estimate the flow velocity in meters per second (ms^{-1}).

Figure 2.9: Measuring water depth and flow velocity using a TVHR

Collection of water parameters or samples were done before macroinvertebrates sampling to avoid disturbing the water. Water quality testing was completed at three biotopes per site for instream environmental data (i.e., the left riverbank zone, the open river zone and

the right riverbank zone), providing three local biotopes per site per sampling period. The biotope was left undisturbed for a standard collection of macroinvertebrates data. The water samples were then kept in a site specific labelled 250 ml plastic containers. The water samples were stored in a cooler box at 4°C and were then urgently sent to Talbot laboratory in Pietermaritzburg, KZN (Figs C.1, C.2, C.3 and C.4, Appendix C). Standard periodical sampling was employed for each sample site to represent every sampling period of the year. The following parameters were measured per sample site and period for water quality assessment: chloride (Cl^-), total ammonia (NH_3), dissolved potassium (K), sodium (Na), sulphate (SO_4^{2-}), nitrate (NO_3^-), dissolved magnesium (Mg), total dissolved solids (TDS), oxygen absorbed (O_2), electrical conductivity and phosphorus (P), as part of the study. Results were analysed separately for each respective site to critically determine trends in water chemistry composition. A Canon EOS 70D camera was used to capture pictures depicting sampling sessions and other interesting events.

2.3.2 Collection of macroinvertebrate communities

The macroinvertebrates were collected at the same biotopes where water samples were sampled following a standardised method that involves a 30 cm x 30 cm square shaped kick sampler SASS5 net of 1 mm pore/mesh size. Kicking and sweeping was done for approximately 5 minutes in three different biotopes including stone, vegetation, and sand (Dickens and Graham, 2002). A stopwatch was used during the river health assessment, and the assessment was timed per biotope as according to Dickens and Graham (2002). Among the sampled biotopes were; stones in current and out of current, vegetation, gravel, sand and mud, as well as hand picking (Dickens and Graham, 2002). The SASS net was pulled through the vegetation and rocks against the water flow, for the duration of the sampling time. On a rocky biotope, hand picking was done where animals were seen clinging on rocks. The entire procedure included collection of three macroinvertebrate samples collection per site on each sampling period (i.e., from gravel sand and mud, marginal vegetation and stone biotopes). Approximately 2 m of marginal vegetation were sampled at each site. After sampling, macroinvertebrates were transferred into three separate sorting trays for identification on site. Invertebrates were then counted individually and identified at a family level to estimate total abundance (Gerber and Gabriel, 2002). To determine the South African Scoring System (SASS) scores, macroinvertebrates were allocated sensitivity scores between 1 and 15, with the most sensitive taxa scoring the highest (15) and the most tolerant taxa the lowest scores

(Dickens and Graham, 2002; Simaika and Samways, 2012). Three principal indices were determined:

- the SASS Score – which was generated by adding all the sensitivity scores for each taxon present;
- the Number of Taxa – which is the total number of taxa present in the sample, and
- the Average Score Per Taxon (ASPT) was calculated by dividing the SASS Scores with the Number of Taxa in the sample (Dickens and Graham, 2002).

The SASS Score and ASPT values were used for the analysis and interpretation of SASS data (Chutter, 1994). In general, higher SASS and ASPT values indicate less impact on water quality (Dickens & Graham, 2002). However, Chutter (1998) emphasised that the true quality of water is most accurately reflected by the ASPT rather than the SASS score, as the former accounts for taxa richness.

2.3.3 Determination of channel condition (site characterisation)

Site characterisation was completed according to Dallas (2005). The main areas of concern in the catchment were assessed and the occurrences of anthropogenic activities such as sand harvesting, bank erosion, land degradation, agricultural activities and game related trampling were identified. The channel condition of each section was evaluated by observing the presence or absence of any channel and bank modifications. Furthermore, recording of features such as flood alleviations were recorded with specific interest in the presence of sand, gravel, cobble and boulder. Substrate sizes were recorded for silt/clay/mud, sand, gravel, pebble, cobble, boulder and bedrock. The abundance and dominance of each identified substrate instream and on the riverbank were estimated based on the scale: 0 = absent; 1 = rare; 2 = sparse; 3 = common; 4 = abundant; 5 = entire (Dallas, 2005).

2.3.4 Analysis of habitat integrity

At each site, an analysis was done to determine Instream Habitat Index (IHI) scores according to a method that critically evaluate alterations of instream and riparian habitat (Dallas, 2005). Instream habitat refers to the area of water within the demarcated area of 50 m^2 per site, and riparian habitat is the land area covering a 10 m buffer zone away from the water. The analysis of IHI was conducted in the three following steps as listed below:

STEP 1: the presence of instream or riparian zone disturbances were recorded. Impact classes were chosen based on the extent and severity of the impact, ranging from 'no impact' to 'critically impacted' and scored between 0 to 25 (Table 2.2).

Table 2.2: The scoring guidelines used to determine the degree of impact for the instream and riparian zones at each site in the Palala and Bloklandspruit Rivers

Impact Class	Description	Score (%)
None	Any modifications are not located in such a way that they have an impact on habitat quality, diversity, size and variability.	0
Small	The modification is limited to very few localities and the impact on habitat quality, diversity, size and variability is limited.	1-5
Moderate	The modifications occur at a small number of localities and the impact on habitat quality, diversity, size and variability are fairly limited.	6-10
Large	The modification is present with a clearly detrimental impact on habitat quality, diversity, size and variability; however, large areas are not affected.	11-15
Serious	The modification is frequently present and the habitat quality, diversity, size and variability in the whole of the defined area are affected; only small areas are not influenced.	16-20
Critical	The modification is present overall with a high intensity; the habitat quality, diversity, size and variability in the whole of the defined section are influenced detrimentally.	21-25

STEP 2: the impacts were described according to specific criteria on water quality, water abstraction and solid waste disposal. Each criterion was weighed and allocated a standardised rating value (Dallas, 2005) (Table 2.3).

Table 2.3: Weightings (Wgt) for instream and riparian zone criteria used to develop the IHI for the Palala and Bloklandspruit River

Instream criteria	Wgt	Wgt	Riparian zone criteria
Water abstraction	14	13	Water abstraction
Extent of inundation	10	11	Extent of inundation
Water quality	14	13	Water quality
Flow modification	7	7	Flow modification
Bed modification	13		
Channel modification	13	12	Channel modification
Presence of exotic macrophytes	9		
Presence of exotic fauna	8		
Solid waste disposal	6		
		13	Decrease of indigenous vegetation from the riparian zone
		12	Exotic vegetation encroachment
		14	Bank erosion

STEP 3: the impact value of each site was be obtained by dividing the values of impact classes by 25 (maximum impact score) and multiplying by the criterion weight. The IHI scores for both instream and riparian zones was calculated for each site using the equation below:

$$IHI = 100 - [\left(\frac{\Sigma\left(\frac{Mg}{MV}\right) \times Wt}{MV}\right) \times 100]$$

where: IHI = index of habitat integrity (%), Mg = criterion rating value, Wt = criterion weight and MV = the maximum value per criterion based on criterion weights (Dallas, 2005; Kleynhans, 2007). The resulting instream and riparian IHI values were interpreted as habitat integrity classes ranging from A to F (Table 2.4), where class A represented unmodified habitat (Dallas, 2005).

Table 2.4: Habitat Integrity classes used to characterize instream and riparian zones for each study site in the Palala and Bloklandspruit Rivers

Class	Description	Score (%)
A	Unmodified; natural	90-100
B	Largely natural with few modifications; a small change in natural habitats and biota may have taken place, but the assumption is that ecosystem functioning is essentially unchanged.	80-89
C	Moderately modified; a loss or change in natural habitat and biota has occurred, but basic ecosystem functioning appears predominantly unchanged.	60-79
D	Largely modified; a loss of natural habitat and biota and a reduction in basic ecosystem functioning are assumed to have occurred.	40-59
E	Seriously modified; the loss of natural habitat, biota and ecosystem functioning are extensive.	20-39
F	Modifications have reached a critical level and there has been an almost complete loss of natural habitat and biota; in the worst cases, the basic ecosystem functioning has been destroyed.	0-19

2.4 Statistical analyses

Data analysis was handled in three different ways. Firstly, environmental and biological data was grouped according to sites to evaluate special changes during the study. Secondly, the environmental and biological data was grouped according to the four sampling

periods at which data collection was done during this study. The arrangement of data according to sampling periods was done to evaluate any temporal changes that may have occurred during this study. Lastly, the relationship between macroinvertebrate communities and environmental parameters was analysed to determine if environmental parameters had any influences in the macroinvertebrate communities.

2.4.1 Analysing environmental data

A One Way ANOVA was performed to analyse spatial and temporal variations in environmental and chemical parameters across sites and sampling periods at $p \leq 0.05$ level of significance respectively. A two-tailed post hoc student t-test was used to identify any significant changes in environmental and chemical parameters between any two sites and any two sampling periods.

2.4.2 Analysing macroinvertebrate community data

To study longitudinal trends in macroinvertebrate community structure, the following biological matrices were calculated: South African Scoring System (SASS), Average Score Per Taxon (ASPT), Taxa richness, Shannon Diversity Index ($H^{'}$) and Total abundance. A One Way ANOVA was performed to analyse spatial and temporal variations in the calculated biological matrices across sites and sampling periods at $p \leq 0.05$ level of significance respectively. A two-tailed post hoc student t-test was used to identify any significant changes in macroinvertebrate community matrices between any two sites and any two sampling periods.

Water quality was assessed following the SASS 5 method by Dickens and Graham (2002). Furthermore, macroinvertebrate taxa were grouped into three groups according to their levels of tolerance to pollution. Group 1 was named 'Tolerant' and consisted of all tolerant taxa with scores between 1 and 5. Group 2 was named 'Intermediate' and was made of taxa with scores from 6 to 10. Group 3 was named 'Sensitive' and included all the sensitive taxa with scores ranging from 11 to 15. The dominance of each group was set as a benchmark to the entire community across sites and seasons.

2.4.3 Multivariate species-environment analysis

The relationship between environmental variables and macroinvertebrates were assessed using Plymouth Routines In Multivariate Ecological Research Version 7 (PRIMER V7) statistical package for visualisation of the distribution patterns (Clarke and Gorley,

2015). Environmental variables intended for multivariate analyses were normalised in PRIMER V7 to improve normal distribution of data (Clarke and Ainsworth, 1993). Resemblance matrices were calculated for each variable using Euclidean distance similarity measures (Clarke, 1993).

Spatiotemporal changes in environmental variables were explored using canonical analysis of principal (CAP) coordinates, an ordination procedure that uses a resemblance matrix to analyse (dis)similarities between environmental variables in PRIMER V7 (Clarke and Gorley, 2015). To determine any relationship between biological and environmental data, a RELATE routine was employed as a procedure that performs non-parametric correlations between environmental and biological data. This method uses the Spearman rank correlation coefficient where values closest to +1 indicates a strong similarity in the two groups (Clarke and Gorley, 2015). The BVSTEP function within BEST routine in PRIMER V7 was used to select environmental variables that were closely correlated with patterns in species data. The process eliminates variables that are less significant within the model performing a backward and forward selections (Clarke and Gorley, 2015). The environmental variables were then used in the distLM model to explain species-environmental relationships.

2.4.4 Analysing the influence of Environmental parameters on macroinvertebrates

After assessing the possible influences of environmental parameters on the distribution of macroinvertebrates using PRIMER V7, the influential environmental parameters were then used to determine whether they had any significant correlations with macroinvertebrates diversity, richness, abundance and the water quality matrices ASPT and SASS5 scores. Simple linear regression models were conducted to determine the effects of the selected environmental parameters, and the relationships were found to be significant at $p \leq 0.05$.

CHAPTER 3
RESEARCH RESULTS

3.1 Impacts of anthropogenic activities on water quality and macroinvertebrates

3.1.1 Habitat characterisation and modification

River characterisation is the process of determining the biophysical characteristics of a river using methods that will identify anthropogenic features and disturbances (Thoms *et al.*, 2018). The concept of habitat modification refers to anthropogenic activities that have dramatically or generally altered the habitat (Pollock *et al.*, 2014). High levels of habitat modifications can dramatically alter the structure of ecosystems (Moore *et al.*, 2004). For the purposes of this study, habitat modification was conducted in the year 2022. The assessment was conducted during four seasons of the year to represent different sampling periods of the year. An overall assessment indicated changes between sampling periods and sites as the river drains through different land use practices. The variation was significantly observed on P 5 and P 6 sampling sites. The six river sample sites represented 3 sections based on land use practices. The upstream sites (P1 and P2) are located outside the Lapalala Wilderness Reserve (Lapalala), middle stream sites (P3 and P4) are located inside the Reserve and the downstream sites (P5 and P6) are located outside Lapalala at Shongoane and Ga-Seleka respectively (Fig. 3.1).

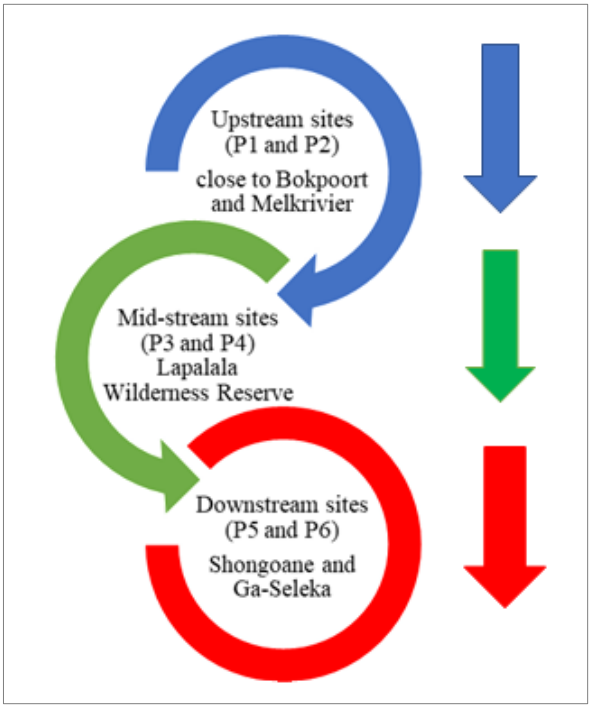

Figure 3.1: Showing a schematic presentation of the sampling sites along the Palala River

During all sampling periods, the Index of Habitat Integrity (IHI) for upstream sites (P1 and P2) were in categories between A and C indicating largely natural with few modifications to unmodified natural conditions. The IHI for the middle stream (P3, P4 and B1) fell between category A and B representing largely natural with few modifications and unmodified, natural conditions. However, there were clear indications of heavy modifications along the rural communities as the river drains through Shongoane and Ga-Seleka where downstream sites (P5 and P6) are located. Habitat alterations increased with seasons mostly around the rural communities indicating serious modifications. For example, in January and March, downstream sites (P5 and P6) were in category C representing a moderately modified status declining to category D during the June/winter period. The downstream sites (P4 and P5) declined dramatically to category E and F during the October/spring sampling period indicating a largely modified condition.

In terms of the riparian zones, the sites also varied throughout the sampling periods with upstream sites (P1 and P2) and middle stream sites (P3 and P4) ranging from category A to category C while downstream sites (P5 and P6) represented categories C to F. The January/summer sampling period maintained mostly category B between upstream and middle stream (P1 to P4) representing a largely natural with few modifications. It was also noted that the upstream sites (P1 to P4) improved to category A during the March/autumn sampling period. During the June/winter sampling period, P1 was in category B, P2 category C, P3 and P4 category B while P5 and P6 were in category D. It was however very different during the October/spring sampling season particularly at downstream sites (P5 and P6). In October/spring, upstream sites (P1 and P2), middle stream sites (P3 and P4) were on category C and downstream sites (P5 and P6) were on category E and F, respectively.

The overall integrity assessment of the Palala River suggests that it is in natural condition with little modifications as it drains through upstream and middle stream (P1, P2, P3 and P4) with natural conditions mostly within Lapalala. The scores in Table 3.1 suggest whether the habitat is largely modified (D), moderately modified (C), largely natural with few modifications (B), or unmodified, natural (A) (Kleynhans *et al.*, 2008). Large modifications were noted at downstream sites (P5 and P6) as the river drains through rural communities. Therefore, the quality of the Palala River deteriorates longitudinally from source to mouth. Throughout the sampling seasons, the assessment indicated low modifications with very little impact within Lapalala.

Table 3.1: The integrity of instream and riparian habitat based on the IHI scores calculated for each site between January and October 2022, at the Palala and Bloklandspruit Rivers

		Sites						
		P1	**P2**	**P3**	**P4**	**P5**	**P6**	**B1**
		0km	57 km	78 km	83 km	137 km	196 km	Tributary
		12 km from source	Melkrivier	Reserve	Reserve	Shongoane	Ga-Seleka	Reserve
Period	**Habitat**	**IHI Score: Category**						
January (summer)	Instream	80.28: B	87.2: B	84.3: B	86.32: B	55: D	56.34: D	80: B
	Riparian	79: C	82.50: B	81: B	83: B	48.4: D	50.06: D	82.4: B
March (autumn)	Instream	97.2: A	94.08: A	98.8: A	98: A	63.6: C	71.16: C	97.2: A
	Riparian	95: A	90.56: A	98.8: A	97: A	52: D	70.08: C	97.4: A
June (winter)	Instream	85: B	72.7: C	92: A	96.3: A	58: D	43: D	83: B
	Riparian	83.07: B	69: C	94: A	94: A	47: D	41.56: D	80: B
October (spring)	Instream	78: C	67.8: C	81: B	87.5: B	32.2: E	15.3: F	63: C
	Riparian	74.1: C	62: C	75.3: C	73: C	25: E	13.5: F	76.6: C

3.2 Spatio-temporal changes in physical and chemical variables

A total of 15 physical and chemical environmental variables were measured between January and October 2022 (Table D.1, Appendix D). The measurement of variation was conducted using CAP (canonical analysis of principal) analysis that indicated significant variations between sampling periods and sampling sites (A and B). Similar sites or periods were grouped closer together based on Euclidean distances. After applying PERMANOVA, there was significant variation in sampling periods and sites (Fig. 3.2). The Euclidean distance model (A) revealed that winter period was significantly different from other sampling periods. Furthermore, the summer period differed from other periods. The model also revealed that the spring and autumn were closely grouped together. The Euclidean distance model (B) revealed no significant variation across sites. The effects of sampling season had an impact on all sampling sites.

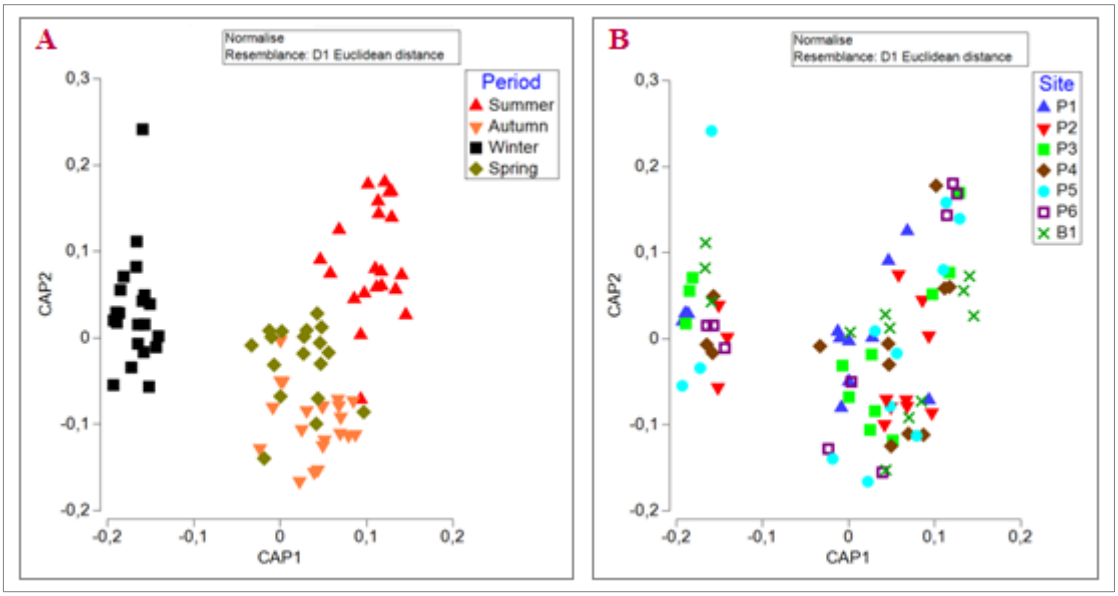

Figure 3.2: The first and second axes of a CAP coordinates showing the separation of sampling period (A) and of sites based on measured environmental variables per site (B)

3.2.1 Potassium

A One Way ANOVA indicated that potassium concentrations varied significantly across sites during the study (F = 5,19, p = 0,0001). A post hoc test indicated that potassium concentrations (Fig. 3.3) were significantly higher at site P5 when compared to all other sites (P5 vs P1; t_{21} = 2.07, p = 0.02: P5 vs P2; t_{21} = 2.07, p = 0.03: P5 vs P3; t_{21} = 2.07, p = 0.03: P5 vs P4; t_{21} = 2.07, p = 0.03: P5 vs P6; t_{21} = 2.07, p = 0.03 and P5 vs B1; t_{21} = 2.07, p = 0.03).

Results from One Way ANOVA indicated significant spatial variations in potassium concentrations across sampling periods (F= 2,694329, p = 0.05). A post hoc test indicated significant variation when comparing summer and spring (t_{21} = 2.02, p = 0.05) and varied significantly when comparing autumn and winter seasons (t_{21} = 2.02, p = 0.03).

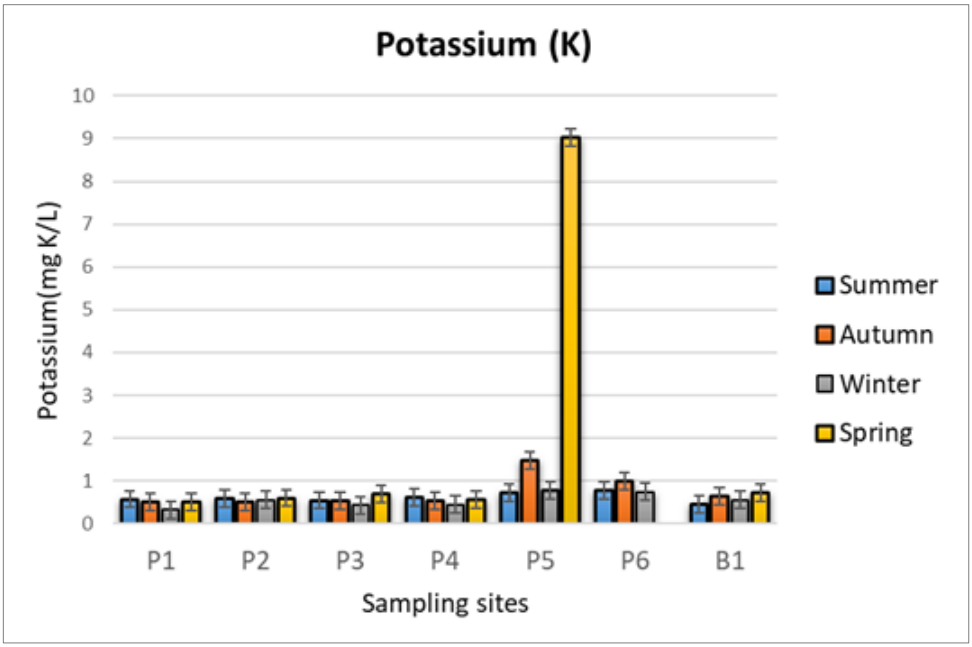

Figure 3.3: Potassium concentration across sites and sampling periods

3.2.2 Magnesium

Magnesium concentrations were higher at the two downstream sites i.e., P5 and P6, and a post hoc test showed no significant differences (t_{21} = 2.07, p = 0.3) in magnesium concentrations (Fig. 3.4) when the two sites were compared to each other. Although all the upstream sites had lower magnesium, its concentrations varied across sites. A One Way ANOVA indicated no significant variation in terms of magnesium (F = 1.28, p = 0.28) across sampling periods.

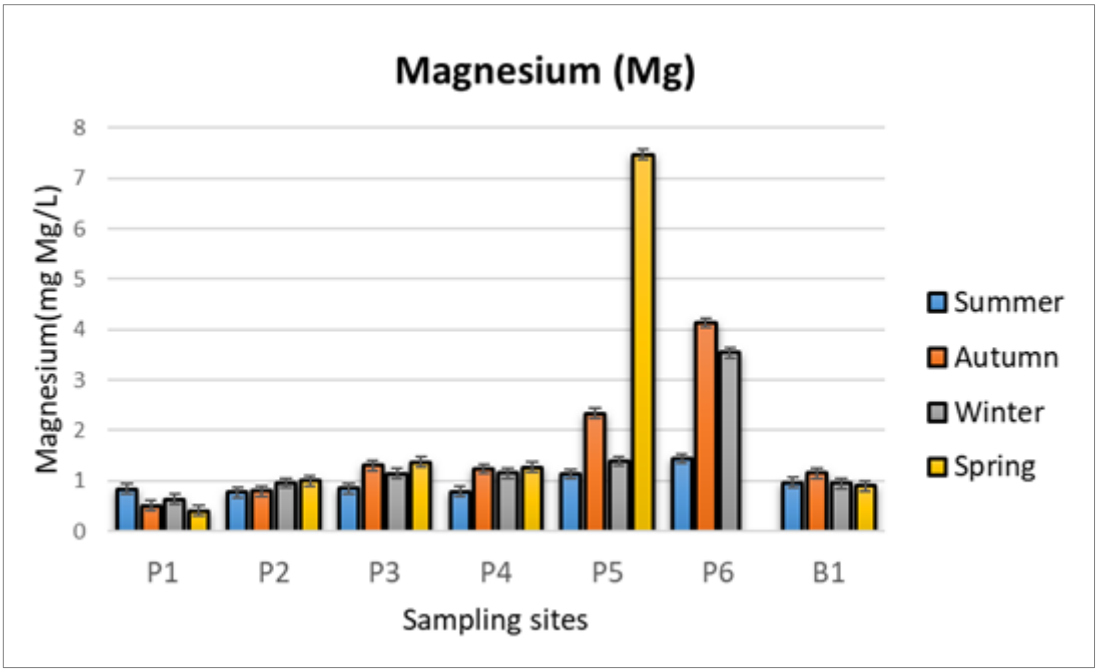

Figure 3.4: Magnesium concentrations across sites and sampling periods

3.2.3 Sodium

Sodium concentrations (Fig. 3.5) were relatively lower upstream and midstream at sites P1, P2, P3, P4 and B1 when compared to both downstream sites. A One Way ANOVA revealed that there were significant variations in sodium concentrations across sites (F = 12.2, p = 0.0001). Higher sodium concentrations were observed from sites P5 and P6 (Fig. 3.9.), with site P5 having significantly higher concentrations than site P6 (t_{21} = 2.07, p = 0.0001). A One Way ANOVA indicated no significant variation in terms sodium (F = 1.89, p = 0.13) across sampling periods.

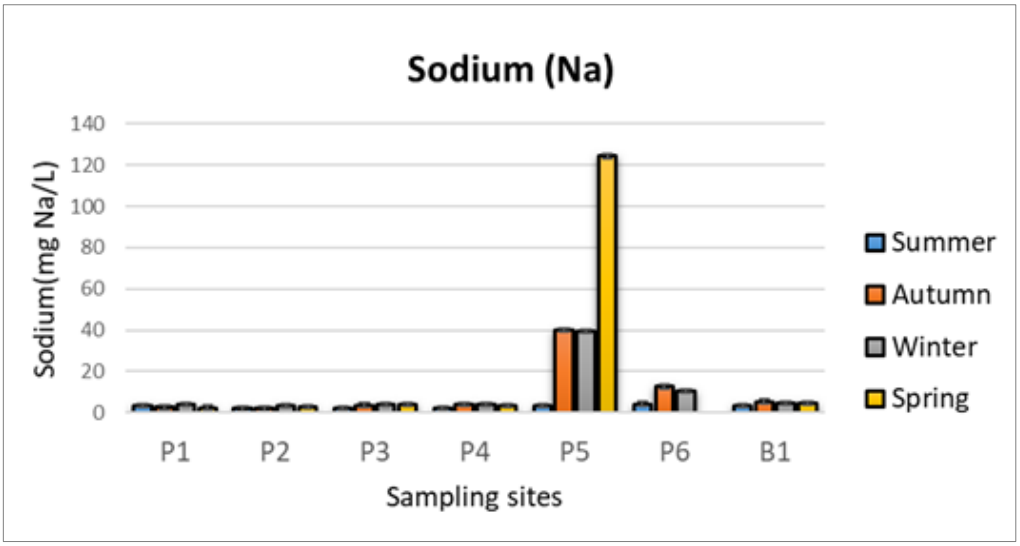

Figure 3.5: Sodium concentrations across sites and sampling periods

3.2.4 Phosphorus

A One Way ANOVA indicated significant variations in phosphorus (F = 3.37, p = 0.02) (Fig. 3.6). A post hoc test showed significant differences when comparing sampling periods: summer and winter (t_{21} = 2.02, p = 0.05), summer and spring (t_{21} = 2.02, p = 0.05), autumn and winter (t_{21} = 2.02, p = 0.003), winter and spring (t_{21} = 2.02, p = 0.009).

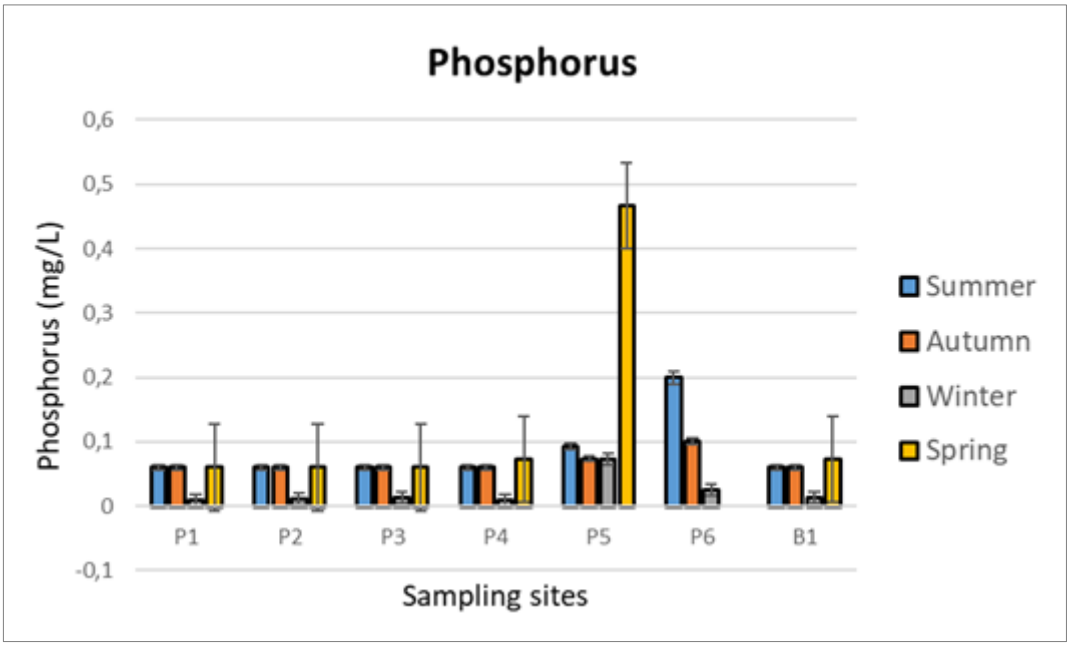

Figure 3.6: Phosphorus concentrations across sites and sampling periods

3.2.5 Chloride

A One Way ANOVA indicated that chloride varied across sites (F = 4.67, p = 0.0004). A post hoc test indicated that chloride concentrations (Fig. 3.7) were significantly higher at site P5 when compared to all other sites (P5 vs P2; t_{21} = 2.07, p = 0.03: P5 vs P1; t_{21} = 2.07, p = 0.03: P5 vs P3; t_{21} = 2.07, p = 0.03: P5 vs P4; t_{21} = 2.07, p = 0.03: P5 vs P6; t_{21} = 2.07, p = 0.05 and P5 vs B1; t_{21} = 2.07, p = 0.04). There were high levels of chloride noticed during the spring period particularly at site P5. A One Way ANOVA indicated significant concentrations of chloride (F = 2.92, p = 0.03). A post hoc test revealed that spring period varied significantly when compared to other sampling periods: spring vs summer; (t_{21} = 2.02, p = 0.03): spring vs autumn; (t_{21} = 2.02, p = 0.02): spring vs winter; t_{21} = 2.02, p = 0.04).

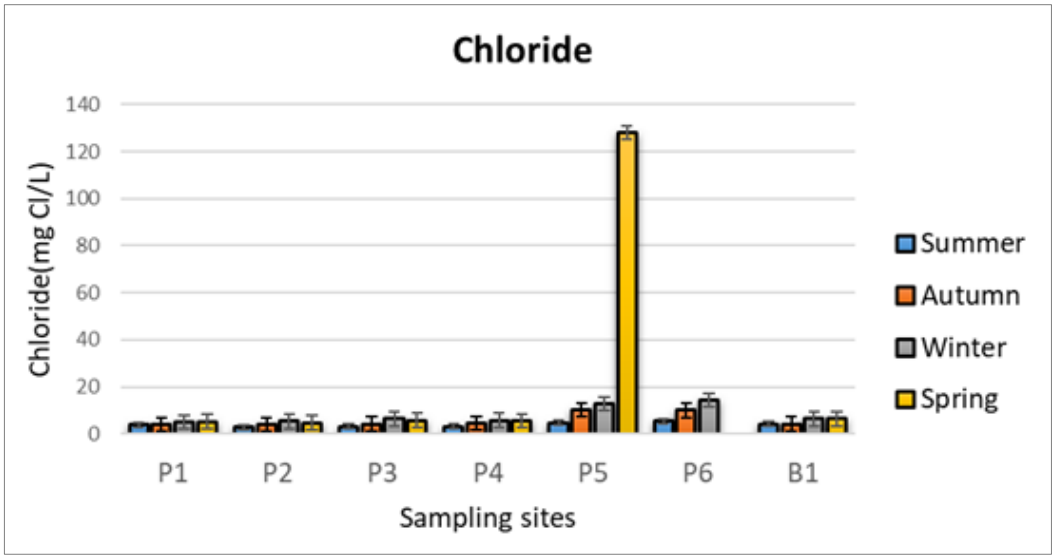

Figure 3.7: Chloride concentrations across all sites and sampling periods

3.2.6 Electrical conductivity

A One Way ANOVA indicated that electrical conductivity varied significantly across sites (F = 15.90, p = 0.0008). Electrical conductivity (Fig. 3.8) was higher at the two downstream sites i.e., P5 and P6, and higher levels were noted at site P5. A post hoc test showed a significant difference when comparing site P5 with other sites (P5 vs P2; t_{21} = 2.07, p = 0.0004: P5 vs P1; t_{21} = 2.07, p = 0.0003: P5 vs P3; t_{21} = 2.07, p = 0.0005: P5 vs P4; t_{21} = 2.07, p = 0.0005: P5 vs P6; t_{21} = 2.07, p = 0.0002 and P5 vs B1; t_{21} = 2.07, p = 0.0005). A One Way ANOVA indicated no significant variation in terms electrical conductivity (F = 1.28, p = 0.28) across sampling periods.

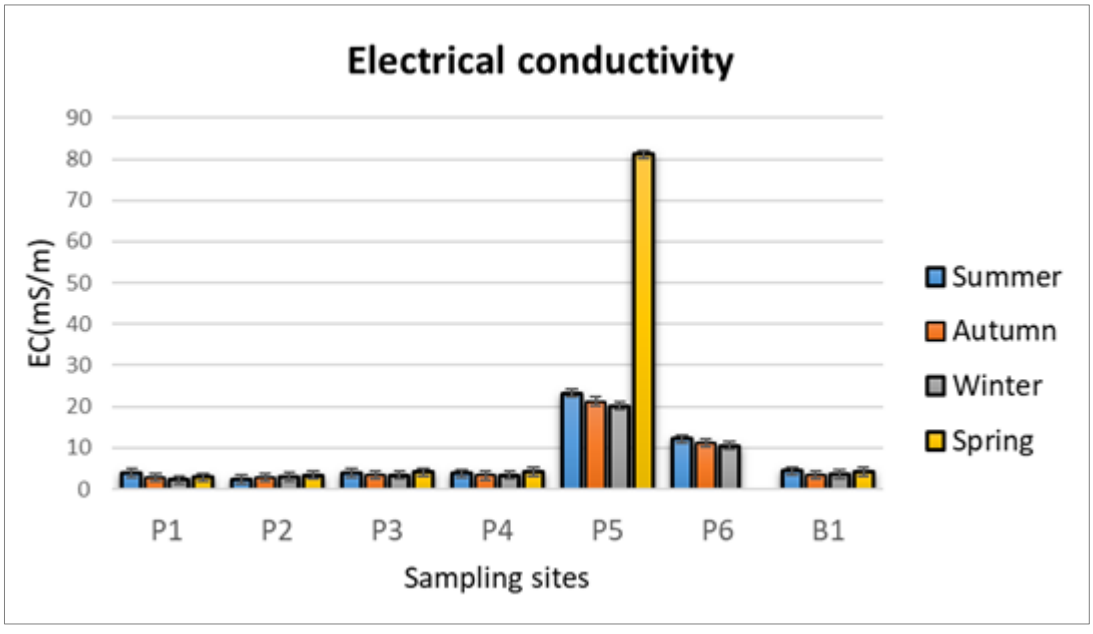

Figure 3.8: Electrical conductivity across all sites and sampling periods

3.2.7 Ammonia

There were no significant differences in concentrations of ammonia (Fig. 3.9) across all sites and sampling periods.

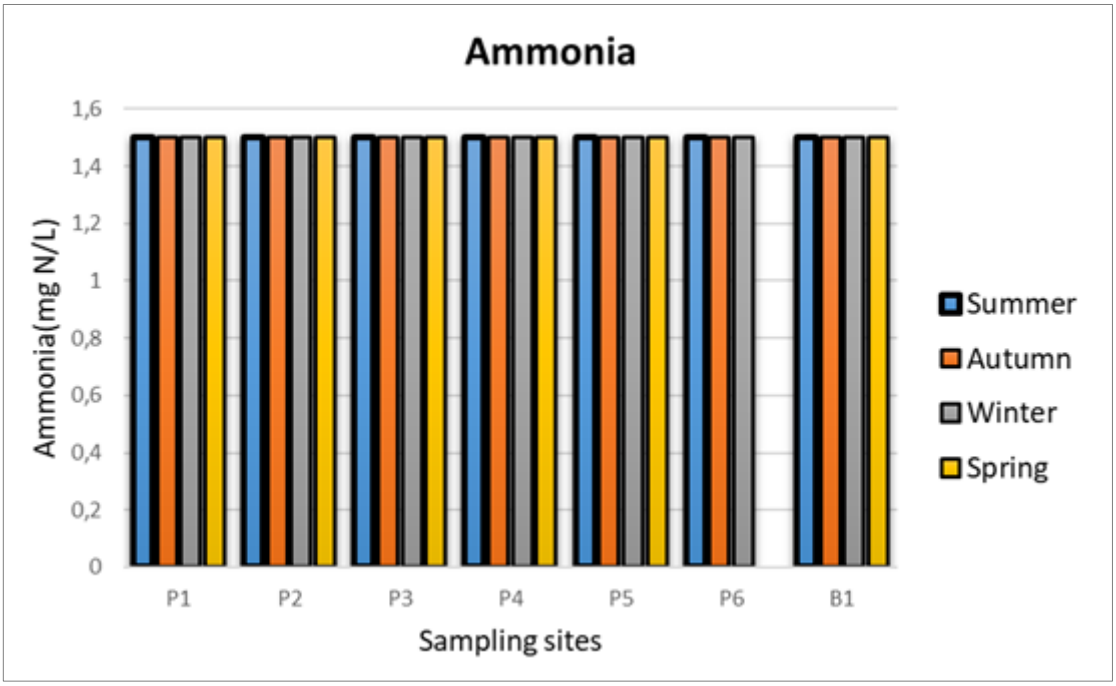

Figure 3.9: Ammonia concentrations across sites and sampling periods

3.2.8 Nitrates

One Way ANOVA indicated that there was a significant difference in nitrate concentrations (Fig. 3.10) across sites (F = 3.05, p = 0.009) and a post hoc test indicated a significant difference when comparing site P2 with all other sites (P1 vs P2; t_{21} = 2.07, p = 0.05: P3 vs P2; t_{21} = 2.07, p = 0.04: P4 vs P2; t_{21} = 2.07, p = 0.03: P5 vs P2; t_{21} = 2.07, p = 0.04: P6 vs P2; t_{21} = 2.07, p = 0.03: B1 vs P2; t_{21} = 2.07, p = 0.03). A One Way ANOVA indicated no significant variation in terms of nitrates (F = 1.89, p = 0.13) across periods.

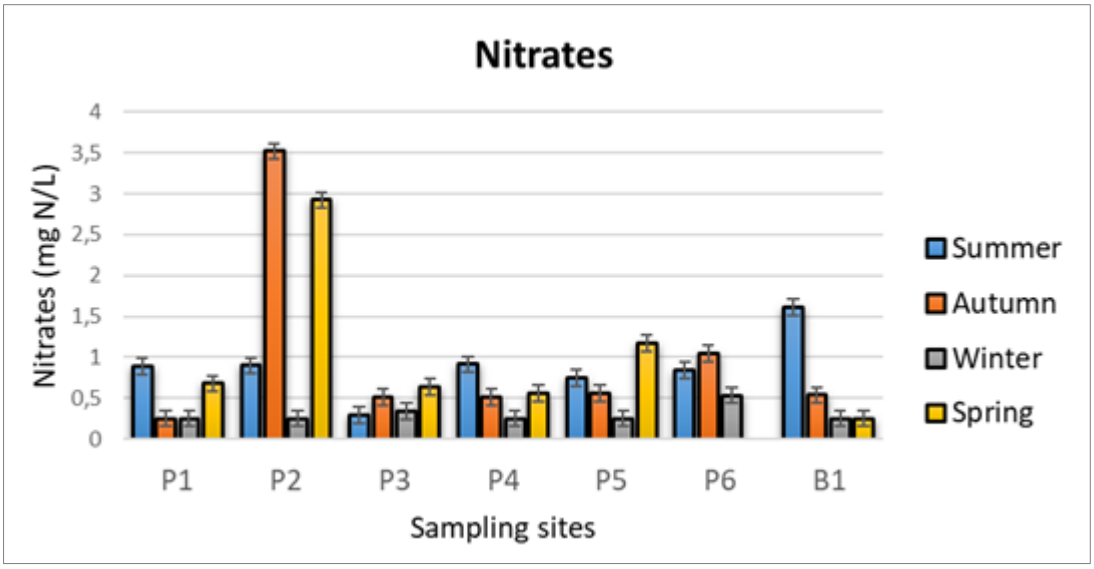

Figure 3.10: Nitrates concentrations across sites and sampling periods

3.2.9 Dissolved oxygen

A One Way ANOVA indicated no significant difference in dissolved oxygen (DO) (Fig. 3.11) concentrations (F = 1.92, p = 0.08).

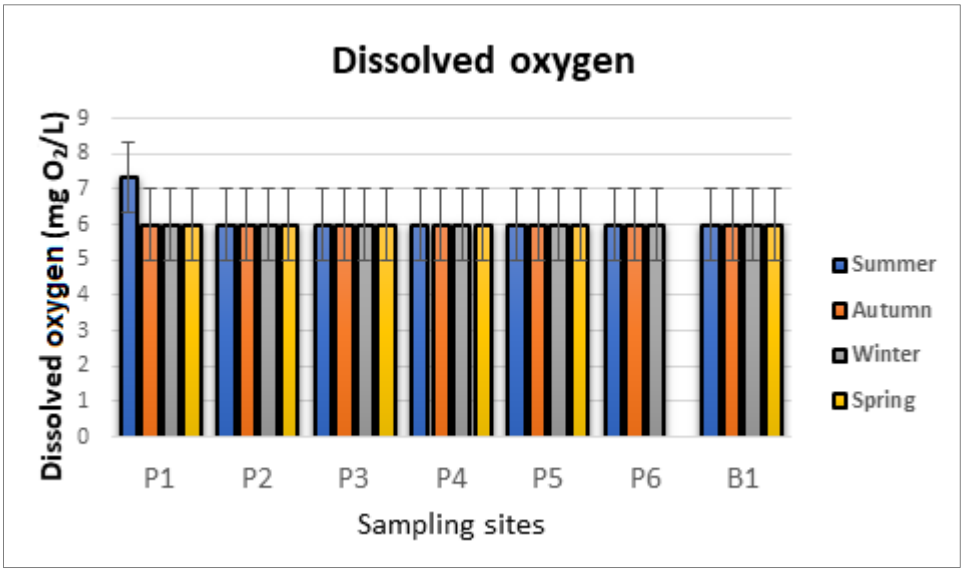

Figure 3.11: Dissolved oxygen levels across sites and sampling periods

3.2.10 Sulphates

The sulphate concentrations (Fig. 3.12) were higher at downstream site P5. A One Way ANOVA indicated a significant variance in sulphates across all sites. A post hoc test further indicated a significant difference when comparing site P5 with all other sites (P1 vs P5; t_{21} = 2.07, p = 0.0004: P2 vs P5; t_{21} = 2.07, p = 0.0004: P4 vs P5; t_{21} = 2.07, p = 0.03: P3 vs P5; t_{21} = 2.07, p = 0.0004: P4 vs P5; t_{21} = 2.07, p = 0.0004: P6 vs P5; t_{21} = 2.07, p = 0.0004:

B1 vs P5; t_{21} = 2.07, p = 0.0004). A One Way ANOVA indicated no significant variation in terms of sulphates (F = 1.50, p = 0.21) across sampling periods.

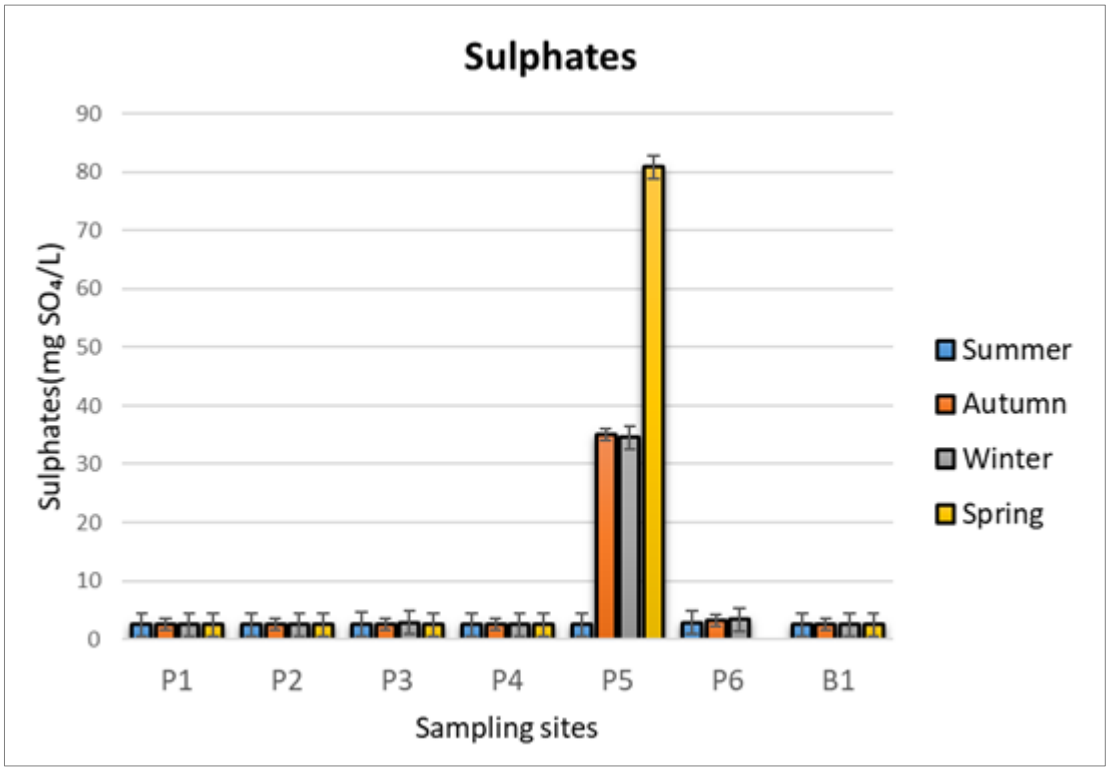

Figure 3.12: Sulphate concentrations across sites and sampling periods

3.2.11 Total dissolved solids

Total dissolved solids (TDS) were higher at the two downstream sites P5 and P6 (Fig. 3.13) when compared to all the upstream sites. Although a One Way ANOVA showed significant differences in TDS across sites, a post hoc test clearly revealed that TDS concentrations were significantly high at site P5 when compared to all the other sites (P5 vs P1; t_{21} = 2.07, p = 0.006: P5 vs P2; t_{21} = 2.07, p = 0.006: P5 vs P3; t_{21} = 2.07, p = 0.007: P5 vs P4; t_{21} = 2.07, p = 0.007: P5 vs P6; t_{21} = 2.07, p = 0.001 and P5 vs B1; t_{21} = 2.07, p = 0.007). A One Way ANOVA indicated no significant variation in terms of total dissolved solids (F = 1.80, p = 0.15) across periods.

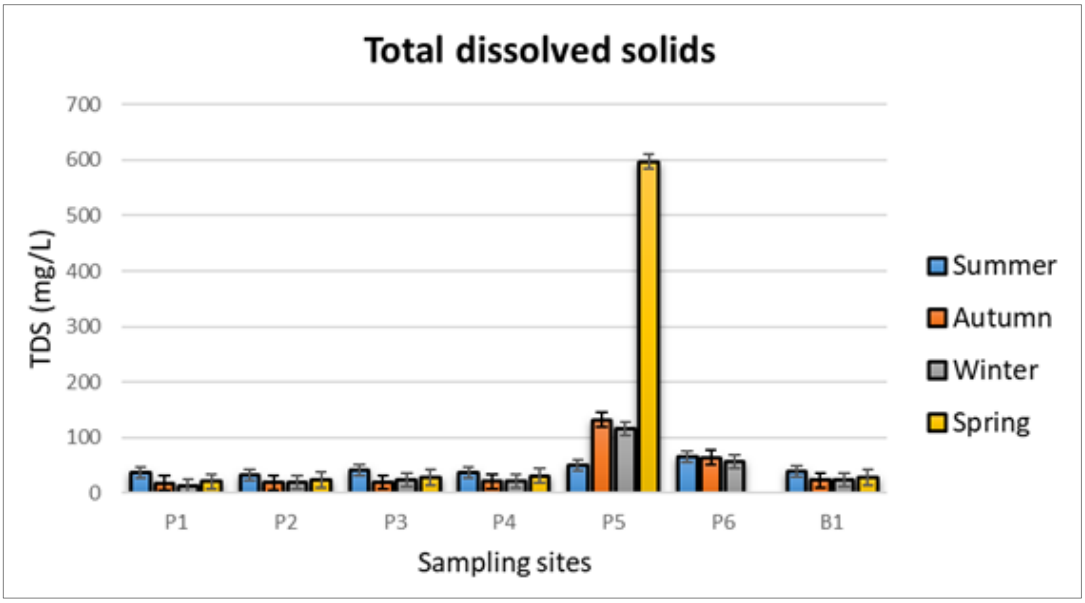

Figure 3.13: Total dissolved solids across sites and sampling periods

3.2.12 Flow velocity

A One Way ANOVA indicated that the flow velocity was significantly different across sites during the course of the study ($F = 2.61$, $p = 0.02$) with the highest velocity (Fig. 3.14) observed at site P3. A post hoc test showed that site P3 was flowing faster than site P1 at ($t_{21} = 2.07$, $p = 0.006$; site P6 at $t_{21} = 2.07$, $p = 0.0006$). A One Way ANOVA indicated no significant variation in terms flow velocity ($F = 1.77$, $p = 0.15$) across periods.

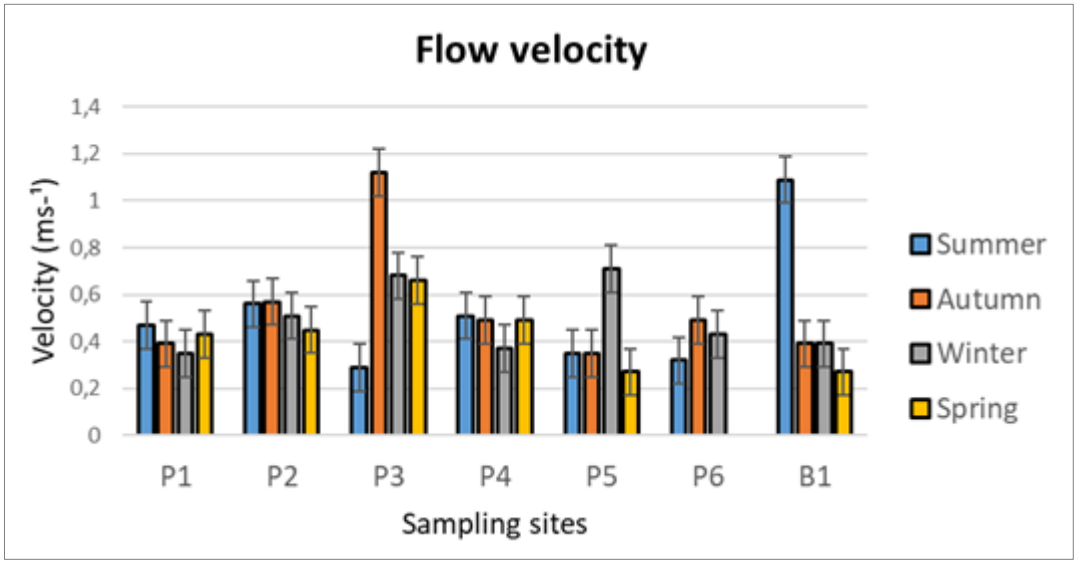

Figure 3.14: Flow velocity across sites and sampling periods

3.2.13 pH

One Way ANOVA indicated that pH changed significantly across sites at (F = 5.63, p = 0.00006) with site P2 and P3 having higher values when compared to downstream sites (Fig. 3.15). A post hoc test indicated that all sites were different when compared to each other. The pH levels varied across periods. A One Way ANOVA indicated significant variations across periods at (F = 2.90, p = 0.03). A post hoc test revealed that there were significant variations when comparing summer and spring at (t_{21} = 2.02, p = 0.04).

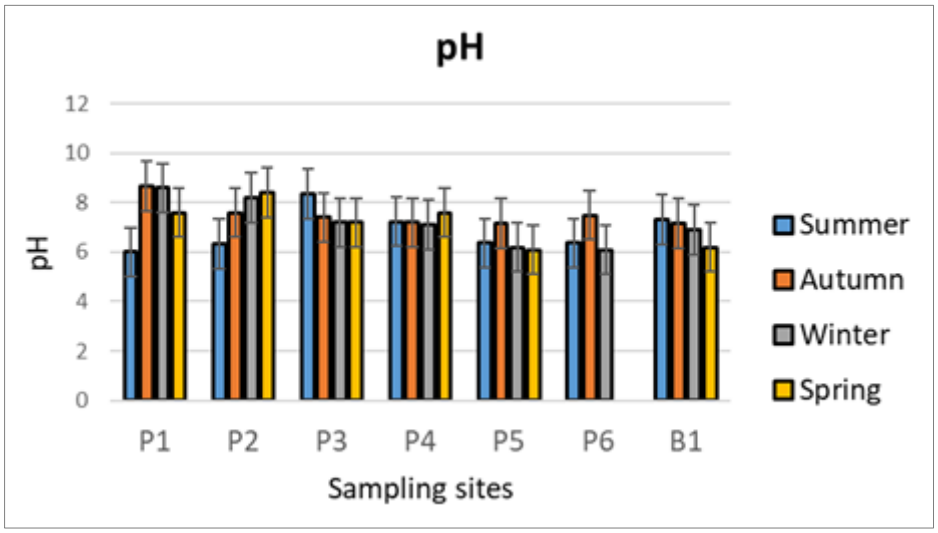

Figure 3.15: pH across sites and sampling periods

3.2.14 Temperature

A One Way ANOVA indicated that there was no significant difference in temperature (Fig. 3.16) across sites at (F = 1.49, p = 0.189). Average water temperatures however varied significantly across periods. A One Way ANOVA indicated significant variations across periods at (F = 34.87, p = 0.01). The highest temperatures were recorded in summer. A post hoc test indicated that summer season varied significantly when compared to other seasons (summer vs autumn; t_{21} = 2.02, p = 0.002: summer vs winter; t_{21} = 2.02, p = 0.003: summer vs spring; t_{21} = 2.02, p = 0.0007).

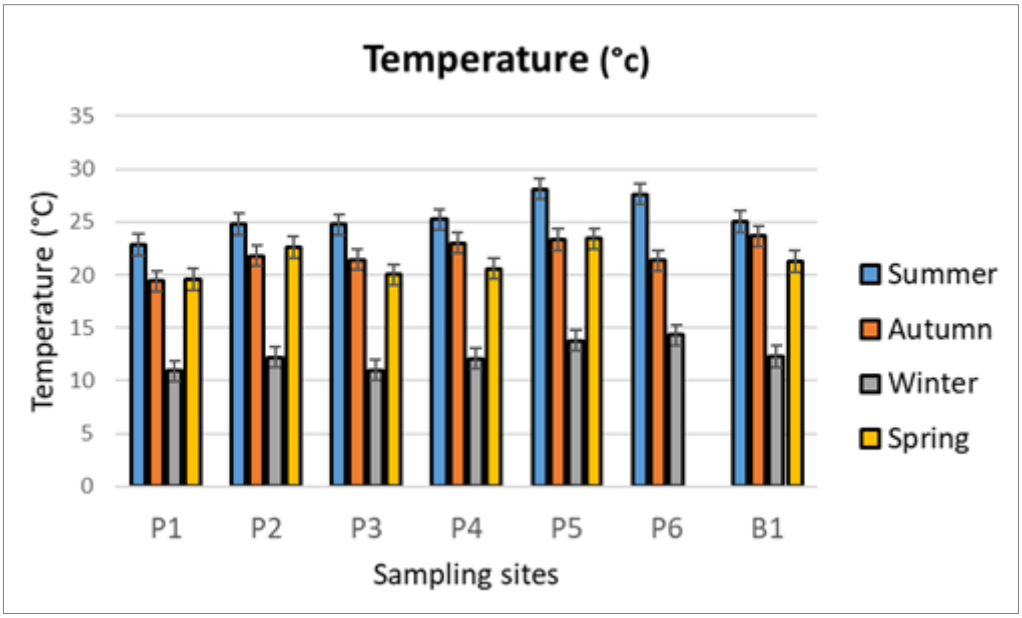

Figure 3.16: Temperature across sites and sampling periods

3.2.15 Water depth

Water depth varied significantly across sites with higher values recorded at sites P3 and P4 (F = 6.57, p = 0.00001) (Fig. 3.17). A post hoc revealed that P3 and P4 varied significantly different when compared to other sites (P2 vs P3; t_{21} = 2.07, p = 0.004: P1 vs P3; t_{21} = 2.07, p = 0.006: P3 vs B1; t_{21} = 2.07, p = 0.005: P5 vs P4; t_{21} = 2.07, p = 0.00001: P4 vs P6; t_{21} = 2.07, p = 0.005 and P4 vs B1; t_{21} = 2.07, p = 0.000005).

A One Way ANOVA indicated that water depth varied significantly across periods with high values recorded during the autumn period. A post hoc test revealed that the autumn had high significance when compared to other periods (autumn vs spring; (t_{21} = 2.02, p = 0.03): autumn vs winter; (t_{21} = 2.02, p = 0.03): autumn vs winter; t_{21} = 2.02, p = 0.03).

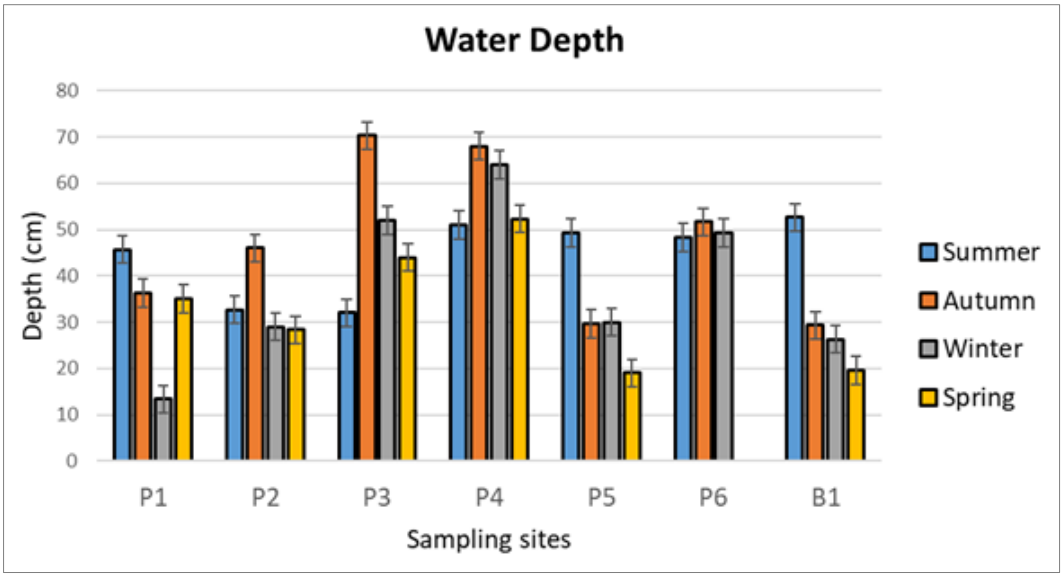

Figure 3.17: Water depth across all sites and sampling periods

3.3 Macroinvertebrates in the Palala River

Very sensitive and moderately tolerant taxa to pollution such as Perlidae, Corduliidae Gomphidae, Hydropsychidae, Oligoneuridae, Polycentropodidae and Teloganodidae were found at upstream and midstream of the Palala River where there are little human activities (Fig. 3.18). Highly tolerant taxa to pollution such as Chironomidae, Hydracarina, and Ceratopogonidae were found (Sites P5 and P6) downstream of the Palala River with high densities of human settlements. The occurrence of the above-mentioned species was represented across all sampling periods (summer, autumn, winter, and spring).

Figure 3.18: A stonefly (A) and a dragonfly (B) found upstream of the study area

The present ecological status at between P1 and P4 of the Palala River falls under class (A) which represents a natural ecological condition. The ecological status at the sites downstream of Lapalala represented by P5 and P6 are seriously modified with classes E and F indicating serious human impact on water quality subsequently impacting on the macroinvertebrates. Furthermore, the river was flowing throughout the year from source to mouth reducing the flow velocity through different sampling periods. The river however declined the flow velocity in October 2022 running very low at site P5 and running completely dry at site P6 (Fig. 3.19).

Figure 3.19: P6 flow during June (A and D) and October (B and C) sampling seasons

3.3.1 Spatial and temporal changes in macroinvertebrate community structure

A total of 4244 individual macroinvertebrates were collected during the past 4 sampling periods i.e., from summer (January 2022) to spring (October 2022) belonging to 59 macroinvertebrates families or taxa (Table D.2, Appendix D). The macroinvertebrates data showed evident patterns in community structure per site. Furthermore, the data showed evident patterns in community structure per sampling period. A One Way ANOVA was employed to indicate significant differences across sites and sampling periods.

3.3.1.1 Taxa richness

One Way ANOVA indicated no significant difference in taxa richness across periods (F = 1.99, p = 0.06) (Fig. 3.20).

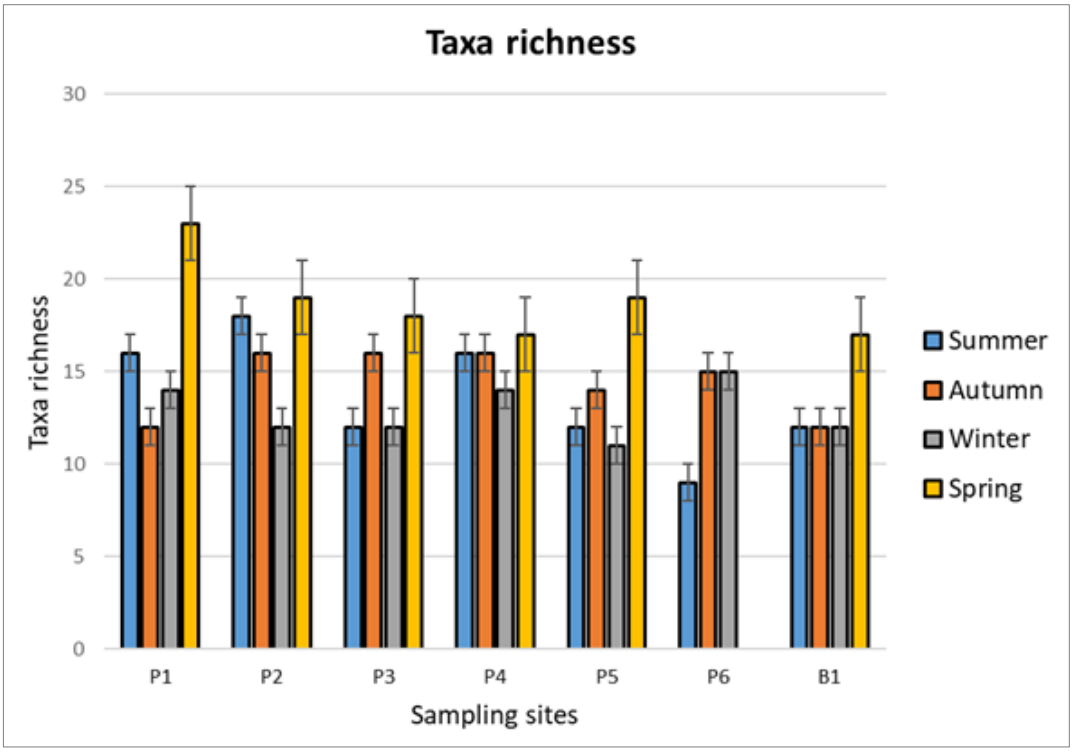

Figure 3.20: Taxa richness across sites and sampling periods

3.3.1.2 Total abundance

A One Way ANOVA revealed that there was no statistical variance in macroinvertebrate abundance across sites (F = 1.34, p = 0.24) (Fig. 3.21). The model however showed a significant variation across sampling periods (F = 7,83, p =0,0001).

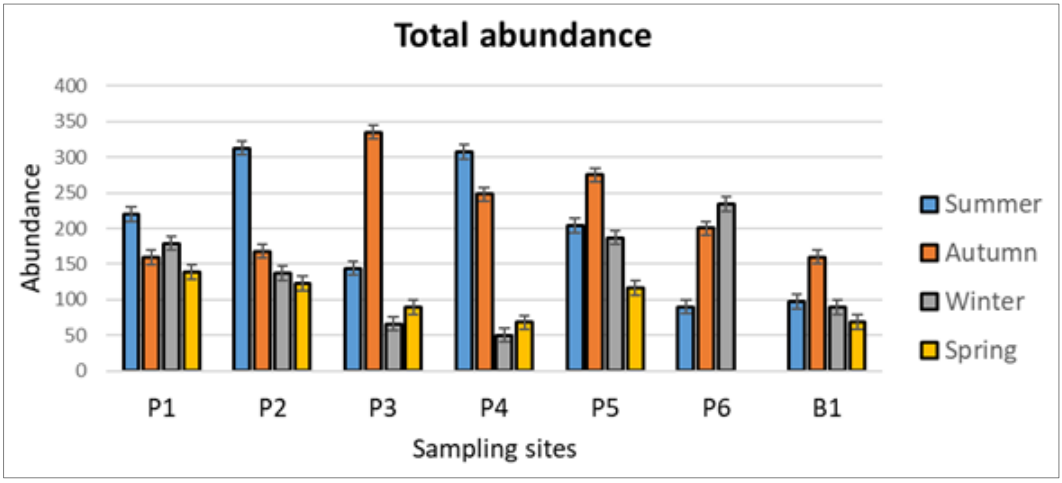

Figure 3.21: Total abundance across sites and sampling periods

3.3.1.3 Taxa diversity

A One Way ANOVA was employed to indicate significant differences in taxa diversity across sites at (F = 6,225877, p = 0,0006) (Fig. 3.22). The post hoc test further

revealed that there was a high level of significance in terms of taxa diversity at (P1 vs P5: t_{21} = 2.07, p = 0.001; P2 vs P6 t_{21} = 2.07, p = 0.0003; P3 vs P5: t_{21} = 2.07, 0.0001, P4 vs P6: t_{21} = 2.07, p = 0.0003, P4 vs P5: t_{21} = 2.07, p = 0002). One Way ANOVA indicated no significant variation in taxa diversity across sampling periods (F = 1.53, p = 0.21).

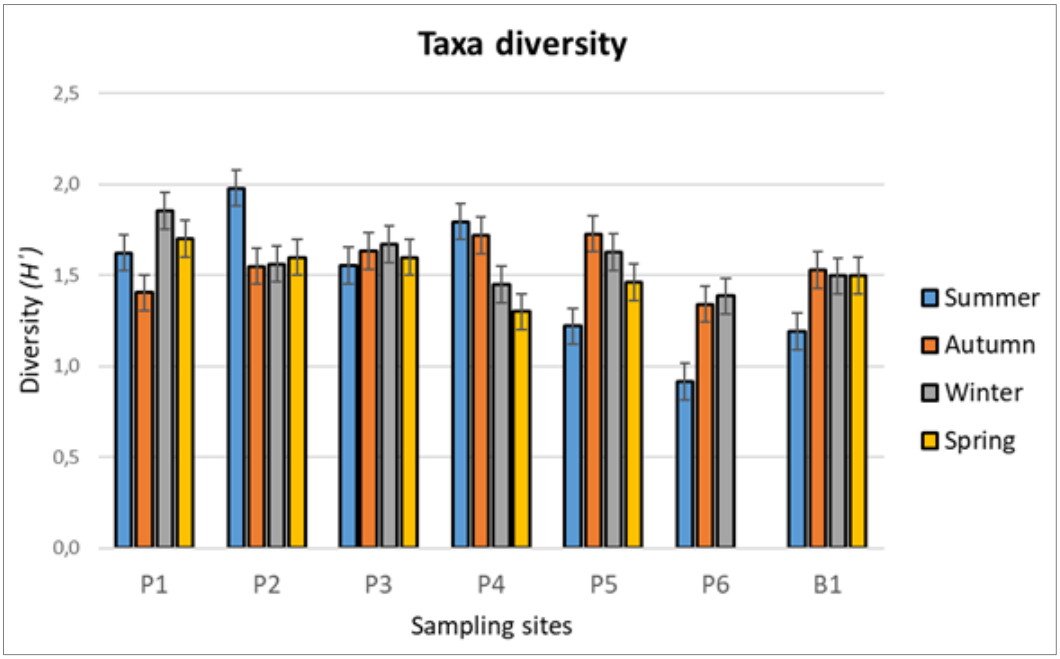

Figure 3.22: Taxa diversity across sites and sampling periods

3.4 Species environment interactions

3.4.1 Factors influencing macroinvertebrates distribution

The relationship test was conducted using the RELATE function within PRIMER V7 to indicate and determine the relationship between the environmental variables and macroinvertebrate communities. The model indicated the important variables that are influential in structuring the communities of the macroinvertebrates across seven sites and four sampling periods (Fig. 3.23A, B). Chloride, total dissolved solids and electrical conductivity were the three environmental variables that were revealed to be significant by a DistLM (distance based linear model) explaining their relationship with macroinvertebrate structure per site and sampling period at (chloride at F = 3.17, p = 0.001; total dissolved solids at (F= 4.17, p = 0.001; electrical conductivity at F = 5.67, p = 0.001). Electrical conductivity had an influence at site P5 and P6 and had major influence during the spring and summer sampling periods. Total dissolved solids had an influence at site P5 and P6 and further influenced macroinvertebrates during winter, summer and autumn sampling periods (Fig. 3.23B). Additionally, relationships were explored for each site and considered statistically significant when $p \leq 0.05$ (indicated in **bold,** Tables 3.2 and 3.3). The equation y = ax+ b was used,

where x = environmental variable, y = water quality index, a = y intercept and b = slope of the regression line.

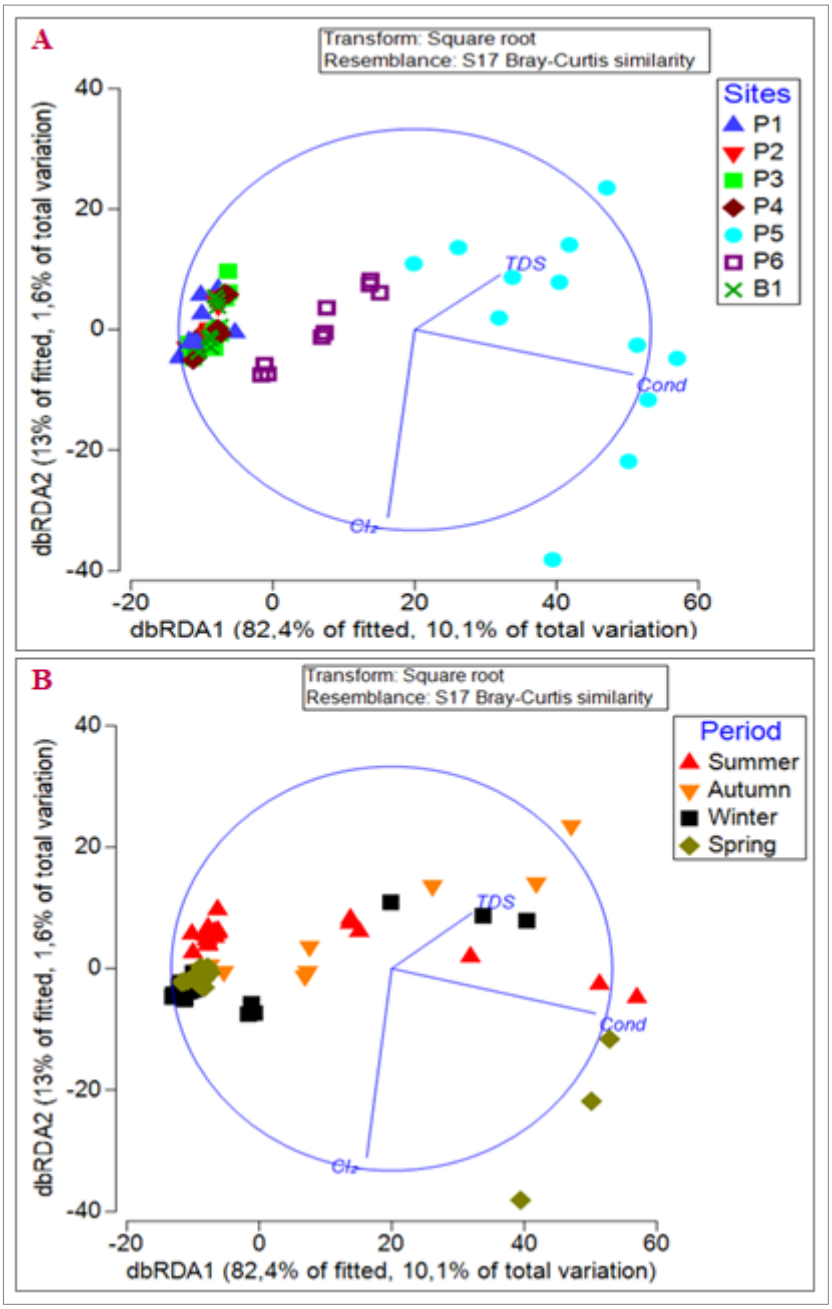

Figure 3.23: dbRDA graphs showing the influential environmental variables in the distribution of macroinvertebrates across sites (A) and sampling periods (B)

Table 3.2: Simple linear regression results showing how the variation in environmental variables affected the water quality scores across sites from January 2022 to October 2022

Sites	Variables	Community indices	R^2	F	*P – value*
P1	Cl_2	**Taxa richness**	**0.79**	**4.54**	**0.02**
		Total abundance	**0.79**	**4.54**	**0.02**
		Diversity (H')	**0.79**	**4.54**	**0.01**
	EC	Taxa richness	0.29	0.50	0.65
		Total abundance	**0.29**	**0.50**	**0.04**
		Diversity (H')	**0.29**	**0.50**	**0.04**
	TDS	Taxa richness	0.48	1.14	0.62
		Total abundance	**0.48**	**1.14**	**0.01**
		Diversity (H')	**0.48**	**1.14**	**0.02**
P2	Cl_2	**Taxa richness**	**0.68**	**2.55**	**0.03**
		Total abundance	**0.68**	**2.55**	**0.02**
		Diversity (H')	**0.68**	**2.55**	**0.02**
	EC	Taxa richness	0.25	0.42	0.47
		Total abundance	**0.25**	**0.42**	**0.01**
		Diversity (H')	0.25	0.42	0.36
	TDS	Taxa richness	0.31	0.54	0.69
		Total abundance	**0.31**	**0.54**	**0.04**
		Diversity (H')	0.31	0.54	0.89
P3	Cl_2	**Taxa richness**	**0.80**	**4.85**	**0.05**
		Total abundance	**0.80**	**4.85**	**0.04**
		Diversity (H')	**0.80**	**4.85**	**0.02**
	EC	Taxa richness	0.42	0.89	0.42
		Total abundance	**0.42**	**0.89**	**0.03**
		Diversity (H')	0.42	0.89	0.56
	TDS	Taxa richness	0.62	2.00	0.71
		Total abundance	**0.62**	**2.00**	**0.03**
		Diversity (H')	0.62	2.00	0.50
P4	Cl_2	Taxa richness	0.25	0.41	0.52
		Total abundance	**0.25**	**0.41**	**0.01**
		Diversity (H')	0.25	0.41	0.70
	EC	Taxa richness	0.54	1.41	0.35
		Total abundance	**0.54**	**1.41**	**0.02**
		Diversity (H')	0.54	1.41	0.10
	TDS	Taxa richness	0.16	0.24	0.44
		Total abundance	**0.16**	**0.24**	**0.01**
		Diversity (H')	0.16	0.24	0.54
P5	Cl_2	Taxa richness	0.36	0.68	0.79
		Total abundance	**0.36**	**0.68**	**0.02**
		Diversity (H')	0.36	0.68	0.55
	EC	Taxa richness	0.35	0.64	0.70
		Total abundance	**0.35**	**0.64**	**0.02**
		Diversity (H')	0.35	0.64	0.55
	TDS	Taxa richness	0.42	0.87	0.88
		Total abundance	**0.42**	**0.87**	**0.03**

		Diversity (H')	0.42	0.87	0.59
P6	Cl_2	Taxa richness	0.57	1.63	0.56
		Total abundance	**0.57**	**1.63**	**0.04**
		Diversity (H')	0.57	1.63	0.86
	EC	Taxa richness	0.32	0.57	0.53
		Total abundance	**0.32**	**0.57**	**0.01**
		Diversity (H')	0.32	0.57	0.82
	TDS	Taxa richness	0.30	0.51	0.56
		Total abundance	**0.30**	**0.51**	**0.03**
		Diversity (H')	0.30	0.51	0.93
B1	Cl_2	Taxa richness	0.27	0.46	0.88
		Total abundance	**0.27**	**0.46**	**0.02**
		Diversity (H')	0.27	0.46	0.99
	EC	Taxa richness	0.29	0.50	0.99
		Total abundance	**0.29**	**0.50**	**0.02**
		Diversity (H')	0.29	0.50	0.78
	TDS	**Taxa richness**	**0.85**	**7.12**	**0.01**
		Total abundance	**0.85**	**7.12**	**0.02**
		Diversity (H')	**0.85**	**7.12**	**0.01**

Table 3.3: Simple linear regression results showing relationships between macroinvertebrate community matrices and influential environmental variables over time

Sampling period	Environmental Variables	Community indices	R^2	F	*P – value*
Summer (January)	Cl-	**Taxa richness**	**0.79**	**4.54**	**0.02**
		Total abundance	**0.79**	**4.54**	**0.02**
		Diversity *(H')*	**0.79**	**0.79**	**0.01**
	EC	Taxa richness	0.29	0.50	0.65
		Total abundance	**0.29**	**0.50**	**0.03**
		Diversity *(H')*	0.29	0.50	0.51
	TDS	Taxa richness	0.48	1.14	0.62
		Total abundance	**0.48**	**1.14**	**0.03**
		Diversity	0.48	1.14	0.42
Autumn (March)	Cl-	Taxa richness	0.68	2.55	0.62
		Total abundance	**0.68**	**2.55**	**0.01**
		Diversity *(H')*	0.68	2.55	0.29
	EC	Taxa richness	0.25	0.42	0.47
		Total abundance	**0.25**	**0.42**	**0.05**
		Diversity *(H')*	0.25	0.42	0.36
	TDS	Taxa richness	0.31	0.54	0.69
		Total abundance	**0.31**	**0.54**	**0.01**
		Diversity *(H')*	0.31	0.54	0.89
Winter (June)	Cl-	**Taxa richness**	**0.80**	**4.85**	**0.05**
		Total abundance	**0.80**	**4.85**	**0.03**
		Diversity *(H')*	**0.80**	**4.85**	**0.02**

	EC	Taxa richness	0.42	0.89	0.42
		Total abundance	**0.42**	**0.89**	**0.01**
		Diversity *(H')*	0.42	0.89	0.56
	TDS	Taxa richness	0.62	2.00	0.71
		Total abundance	**0.62**	**2.00**	**0.05**
		Diversity *(H')*	0.62	2.00	0.50
Spring (October)	Cl-	Taxa richness	0.25	0.41	0.52
		Total abundance	**0.25**	**0.41**	**0.04**
		Diversity *(H')*	0.25	0.41	0.62
	EC	Taxa richness	0.54	1.41	0.35
		Total abundance	**0.54**	**1.41**	**0.02**
		Diversity *(H')*	0.54	1.41	0.10
	TDS	Taxa richness	0.16	0.24	0.44
		Total abundance	**0.16**	**0.24**	**0.01**
		Diversity *(H')*	0.16	0.24	0.54

3.4.2 Distribution of macroinvertebrate based on their level of sensitivity to pollution

Macroinvertebrates have different sensitivity levels based on their level of tolerance to water contamination (Gerber and Gabriel, 2002). Palala 1 (P1) was dominated by intermediate and sensitive taxa across all seasons. Palala 2 (P2) was dominated by intermediate taxa and tolerant taxa across all seasons. Palala 3 (P3), and Palala 4 (P4) were highly dominated by sensitive taxa and intermediate taxa across seasons. Bloklandspruit (B1) was dominated by intermediate taxa through all seasons. However, Palala 5 (P5) and Palala 6 (P6) were highly dominated by tolerant taxa and intermediate taxa through all seasons. Overall, the study indicated that 21% of the macroinvertebrate species collected were sensitive to pollution, 44% fell under the intermediate category and 35% fell under the tolerant category (Fig. 3.24).

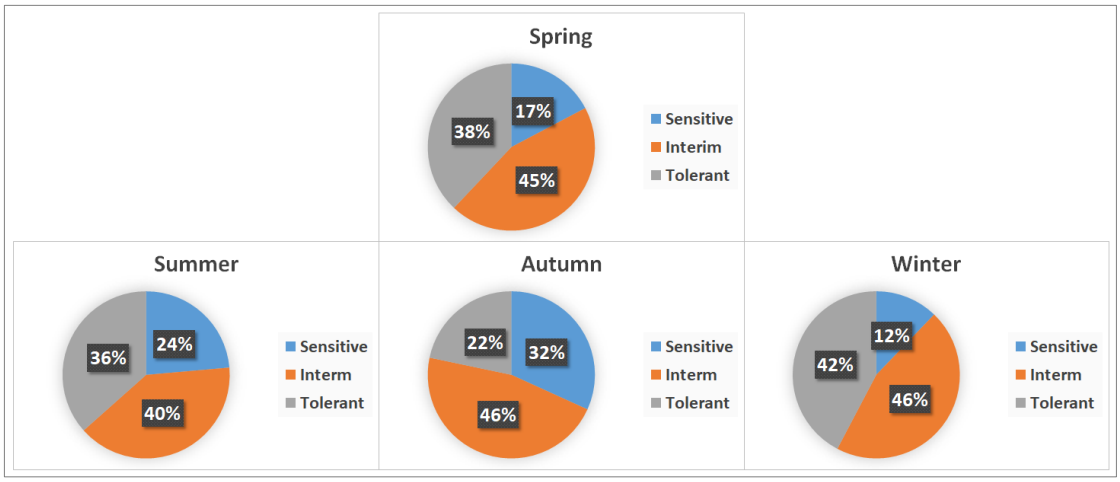

Figure 3.24: The macroinvertebrates sensitivity levels and dominance across sites per season between January and October 2022 in the Palala River

According to the data collected, the following at upstream site before the river enters Lapalala: P1 had 35% sensitive, 54% intermediate and only 11% tolerant taxa, and P2 had 15% sensitive, 54% intermediate and 28% tolerant taxa. Lapalala section: P3 had 46% sensitive, 40% intermediate, 14% tolerant taxa, P4 had 57% sensitive, 28% intermediate and 15% tolerant taxa and B1 (Bloklandspruit) had 16% sensitive, 64% intermediate and 20% tolerant taxa. Downstream sites (Shongoane and Ga Seleka): P5 had 0% sensitive, 25% intermediate and 75% tolerant taxa and P6 had 1% sensitive, 55% intermediate and 44% tolerant taxa (Fig. 3.25).

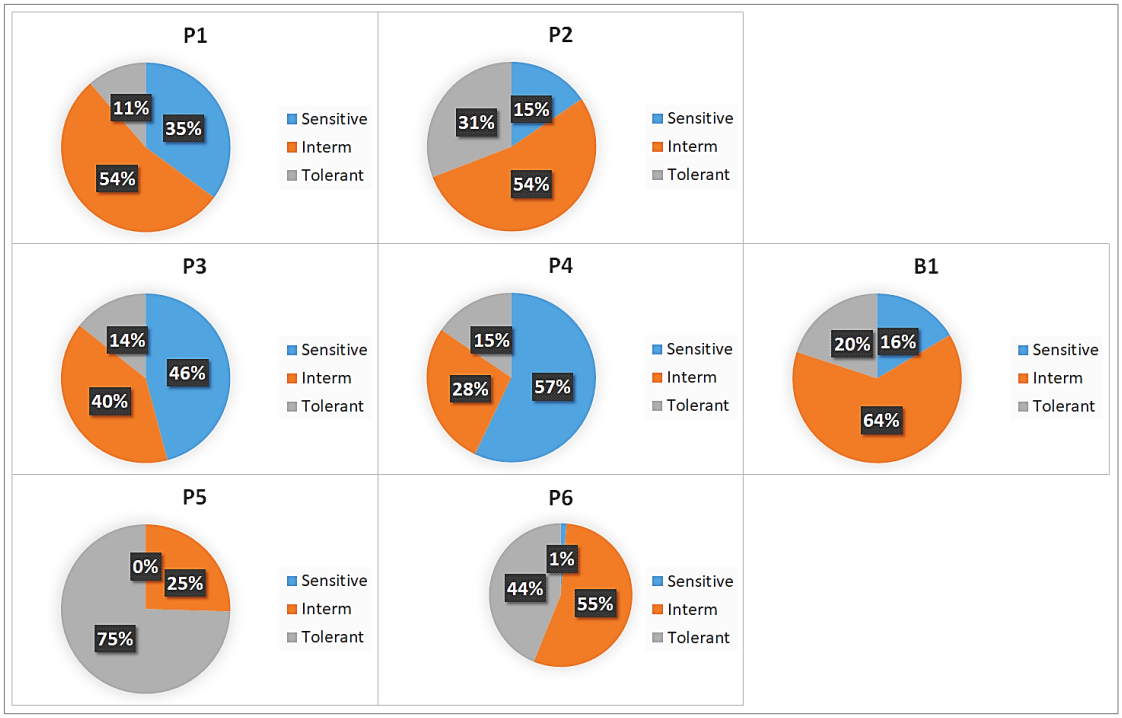

Figure 3.25: The macroinvertebrates sensitivity levels and category dominance across sites, between January and October 2022

3.5 Effects of environmental variables on water quality

3.5.1 General water quality status of the Palala River

Water quality was good in the areas that drain through P1 and P2 upstream and good at P3 and P4 midstream (Lapalala) during this study (Table 3.4). Overall, water quality shifted from naturally clean (upstream and midstream) to generally poor (downstream).

Table 3.4: The ecological status of the Palala River between January 2022 and October 2022 according to classification categories by Dallas (2007)

Site	SASS5 Score	ASPT	IHI Class*	Description
P1	151	7,4	B	Largely natural with few modifications with sensitive taxa present
P2	140	7,1	B	Largely natural with few modifications with sensitive taxa present
P3	161	8,6	B	Largely natural with few modifications with sensitive taxa present
P4	165	7,9	B	Largely natural with few modifications with sensitive taxa present
P5	80	4,5	F	Severely impaired. Only tolerant taxa present.
P6	91	3,8	F	Severely impaired. Only tolerant taxa present.
B1	103	6	B	Largely natural with few modifications with sensitive taxa present

Period	SASS Score	ASPT	IHI Class*	Description
Summer (January)	118	6,9	B	Largely natural with few modifications with sensitive taxa present
Autumn (March)	158	7,2	B	Largely natural with few modifications with sensitive taxa present
Winter (June)	124	6,4	B	Largely natural with few modifications with sensitive taxa present
Spring (October)	109	5,8	D	Considerably impaired. Mostly tolerant taxa present.

*** Class rating: A = Very good; B = Good; C = Moderate; D = Poor; E = Very poor; F = Critically modified**

There were consistent patterns of serious impairment of the ecological integrity at P5 and P6 coupled with the dominance of pollution tolerance taxa throughout all sampling periods. Lower SASS5 scores were noticed as the river drains downstream sites P5 and P6 (Fig. 3.26). Additionally, ASPT scores were noticed downstream as the river drains through rural communities at sites P5 and P6 (Fig. 3.27).

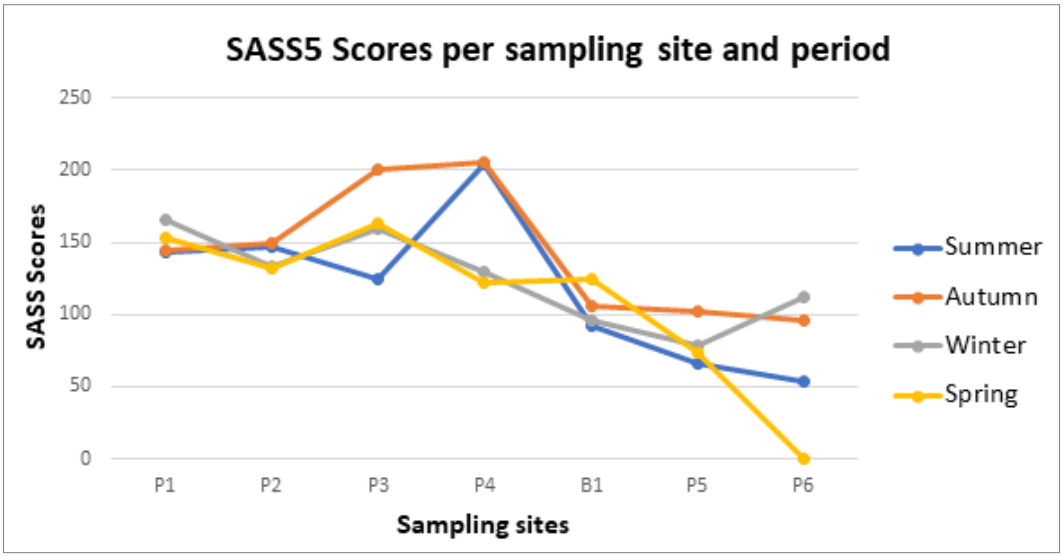

Figure 3.26: SASS5 scores per sampling site and period

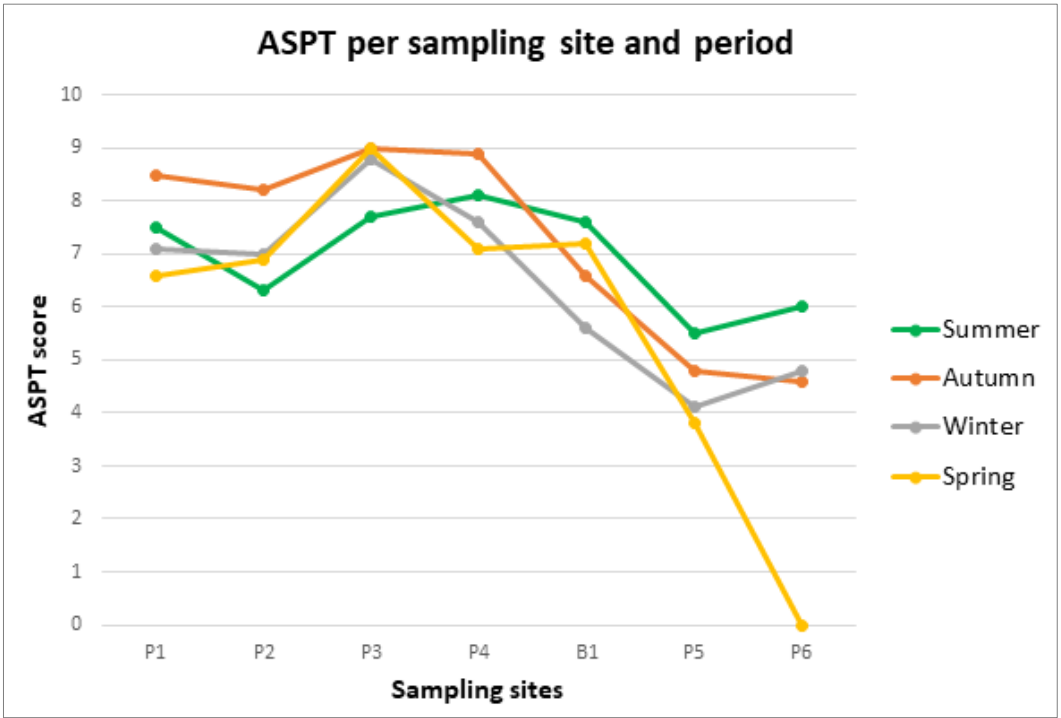

Figure 3.27: scores per sampling site and period

3.5.2 Spatial and temporal changes in water quality

Macroinvertebrates reacted differently to changes in water quality across sites. A One Way ANOVA indicated that macroinvertebrates varied significantly across sites. The scores declined in a downstream direction from source to mouth with lower scores downstream. This proves that water quality declines as the river drains through rural settlements with a

lot of human activities. The SASS5, ASPT and diversity matrices varied significantly (SASS5 F = 10,39265, p = 0.0002; and ASPT F = 16,10917, p = 0.0006).

The SASS5 scores were higher upstream and significantly higher at sites P3 and P4 (Fig. 3.26). The SASS scores decreased significantly downstream as the river drains sections through P5 and P6. A post hoc test revealed that sites P5 and P6 varied significantly when compared to other sites upstream and there was no significant difference when the two sites are compared (SASS: P5 vs P1 at t_{21} = 2,073, p = 0.0001; P5 vs P2 at t_{21} = 2,073, p = 0.0002; P5 vs P3 at t_{21} = 2,073, p = 0.0003; P5 vs P4 at t_{21} = 2,073, p = 0.0004; P5 vs B1 at t_{21} = 2,073, p = 0.002; P6 vs P1 at t_{21} = 2,073, p = 0.0004; P6 vs P2 at t_{21} = 2,073, p = 0.001; P6 vs P3 at t_{21} = 2,073, p = 0.0005, P6 vs P4 at t_{21} = 2,073, p = 0.00006; P6 vs B1 at t_{21} = 2,073, p = 0.003).

The ASPT scores were higher upstream and significantly higher at sites P3 and P4 (Fig. 3.27). The scores decreased significantly downstream as the river drains sections through P5 and P6 supporting the first hypothesis. A post hoc test revealed that sites P5 and P6 varied significantly when compared to other sites upstream and there was no significant difference when the two sites are compared The ASPT score at sites P5 vs P1: t_{21} = 2,073, p = 0.0002; P5 vs P2 at t_{21} = 2,073, p = 0.0001; P5 vs P3 at t_{21} = 2,073, p = 0.004; P5 vs P4 at t_{21} = 2,073, p = 0.0004; P5 vs B1 at t_{21} = 2,073, p = 0.001; P6 vs P1 at t_{21} = 2,073, p = 0.0002; P6 vs P2 at t_{21} = 2,073, p = 0.003; P6 vs P3 at t_{21} = 2,073, p = 0.0007, P6 vs P4 at t_{21} = 2,073, p = 0.00009; P6 vs B1 at t_{21} = 2,073, p = 0.002).

A post hoc test indicated no significant difference between P5 and P6 in SASS scores: t_{21} = 2,073, p = 0,42 and ASPT at t_{21} = 2,073, p = 0,42. Low SASS5 and ASPT scores indicated water quality deterioration at downstream sites supporting the first and third hypothesis.

3.5.3 Environmental variables influencing SASS5 and ASPT scores

Out of the 15 studied environmental variables, only selected ones were significantly associated with the SASS 5 and ASTP water quality matrices during the study. Table 3.5 indicates the significant correlations found between environmental variables and water quality matrices across sampling periods and sites respectively. The equation y = ax+ b was used, where x = environmental variable, y = water quality index, a = y intercept and b = slope of the regression line. Relationships were explored for each site and considered statistically significant when $p \leq 0.05$ (indicated in bold). Cl- = chloride, SASS5 Scores= South African Scoring System Scores, ASPT= Average Scores per Taxon.

Table 3.5: Simple linear regression results showing how the variation in environmental variables affected the water quality scores across sites from January to October 2022

Sites	Variables	Community indices	R^2	F	*P – value*
P5	Cl-	**SASS5**	**0.79**	**4.54**	**0.02**
		ASPT	**0.79**	**4.54**	**0.02**

There was insufficient statistical evidence to suggest that the collected environmental variables were significantly associated with water quality. Only chloride (Cl-) was significantly associated with both SASS5 scores and ASPT at sampling site P5 (Table 3.5).

CHAPTER 4
DISCUSSION OF RESULTS IN RELATION TO LITERATURE

4.1 Introduction

This chapter discusses the results as outlined comprehensively in Chapter 3. The focus is mainly on the factors associated with the variations in environmental variables and macroinvertebrate communities that were assessed during the course of the study. Not all the measured environmental variables changed across sites. Water temperature, dissolved oxygen, ammonia and phosphorus concentrations did not change significantly from site to site, i.e., from the source to the mouth of the Palala River. Furthermore, 9 variables did not change significantly during the four sampling periods. Those variables were: magnesium, sodium, electrical conductivity, ammonia, nitrate, dissolved oxygen, sulphate, total dissolved solids and flow velocity.

The ecological state of the Palala River was clearly revealed by the manner in which the measured environmental variables affected water quality and the macroinvertebrate communities which are found in the river. During this study, water quality was significantly impacted by changes in chloride concentrations. The biological communities of macroinvertebrates were impacted by changes in the concentrations of chlorides (Cl^-), total dissolved solids (TDS), as well as electrical conductivity (EC). The impacts of the above-mentioned nutrients were assessed spatially and temporally to determine their overall ecological importance in the study. The average concentrations indicated increase in a downstream direction.

4.2 Spatial and longitudinal variation of environmental variables

4.2.1 Changes in chemical variables

Only the concentrations of potassium, magnesium, sodium and chloride increased longitudinally in a downstream direction. Such findings partially supported the first hypothesis which stated: "the concentration of total ammonia, dissolved potassium, total phosphorus, nitrates, sulphate, sodium, chloride, total dissolved solids, electrical conductivity and dissolved magnesium will be lower at the sites located at the source and those located within Lapalala, and higher, at sections located downstream of the reserve" (page 12, Subsection 1.11 Research aims, objectives and hypotheses).

The variations in nitrate concentrations showed a different pattern. Nitrates were higher at site P2, downstream of the Lapalala Wilderness Reserve (Lapalala) and inside the reserve, at site B1. The high concentration of nitrates at P2 were attributed to agricultural activities that occurred just above the sampling site, immediately below the source of the river where the first site P1 is located. Although the river continuum concept suggests that human impacts are generally limited upstream at the source of a river, the increase in human populations have put a lot of pressure in the agriculture sector. Large scale food production efforts have utilised water resources from rivers regardless of the location of the water source. Such aggressive agricultural activities have been proven to increase nutrients like nitrogen in nearby rivers (Eger *et al.*, 2023). The use of nitrogen rich fertilisers cause problems in aquatic ecosystems when applied excessively leading to high levels of nitrate concentrations in rivers through leaching and runoff (Wang and Li, 2019).

The second site with high nitrate concentrations was the tributary site B1, the Blok-landspruit River. This site was located approximately 20 m downstream of a Hippo Dam that receives and stores water from anthropogenic activities that occur outside the nature reserve where a local community is situated. Before the Bloklandspruit River enters Lapalala, it cuts through a small isolated village. The villagers use the river for their domestic livelihoods. It was apparent that the activities of the small community could affect the river, especially since the water eventually enters into a man-made dam inside the nature reserve, creating a nutrient pool. A study by Wang *et al.* (2022) suggested that damming of rivers have a potential to change nutrient concentrations and can affect the chemical and biological integrity of surface water leading to significant variations in nutrient concentrations down-stream. Fan *et al.* (2006) has also shown that dams can have dramatic effects on aquatic ecosystems.

The concentrations of all studied chemicals decreased as both the rivers entered Lapalala. The sudden decrease was expected as nature reserves promote and facilitate the conservation of natural environments in their natural state. According to Stolton *et al.* (2015), nature reserves play an important role in restoring ecosystems and sheltering rivers from anthropogenic activities that occur outside them. The importance of Lapalala was clearly visible as the Palala River showed visible signs of being restored as it drained the reserve.

Despite the recovery of the river within the Reserve, it had to exit and cut through rural communities at sites P5 and P6, leading to a dramatic increase in nutrient concentrations. High concentrations of potassium, magnesium, sodium, chloride, electrical

conductivity and total dissolved solids were recorded at downstream sites. The high concentrations were attributed to the prevailing land use practices and multiple activities at the two downstream sites. These sites play an important role in supplying the local rural communities with water for several activities including washing clothes, drinking, bathing and water for livestock. All these activities have been observed occurring onsite during the study. Human settlements and domesticated livestock have been found very influential in increasing chemicals in water resources (Dudgeon *et al.*, 2006).

4.2.1.1 Potassium

In an undisturbed natural river system, potassium concentrations in surface water is typically less than 5 $mg.L^{-1}$ (Skowron *et al.*, 2018). During this study, potassium concentrations were almost double the natural range, especially at site P5 where instream disturbances were constantly prevalent. The washing of clothes and disposal of contaminated water back into the river, severely increased the levels of potassium in this section of the river. Studies who have looked at different sources of potassium in rivers indicated that the levels could increase based on inputs from sources such as sewage, domestic uses and agricultural runoff (Myers and Ludtke, 2017; Rahman and Salbe, 1995; Pillay, 1994; Igbinosa and Okoh, 2009). Although potassium can also be leached from contaminated ground water (Li *et al.*, 2022), there was no evidence to prove that ground water leaching could have been the main cause of elevated potassium concentrations in this study.

4.2.1.2 Magnesium

Magnesium is naturally found in surface river water at approximately 4 $mg.L^{-1}$ and concentrations vary with the type of land use in the catchment areas (Hwang *et al.*, 2012). Concentrations as high as approximately 8 $mg.L^{-1}$ were observed at the site heavily impacted by human interactions (Site P5) during this study. When studying the magnesium and calcium concentrations in the surface water and bottom deposits of Symsarna River and Lake Symsar, Potasznik and Szymczyk (2015) found that magnesium concentrations were somewhat higher in the catchment exposed to increased human activity, than in the catchment occupied mostly by forests. In the current study, natural levels of magnesium were observed upstream and inside Lapalala where limited to no human interactions were prevalent.

4.2.1.3 Chloride

The high chloride concentrations observed at site P5 were also attributed to human interactions, specifically to washing detergents. Chloride is an anion of the chlorine element. Chlorine does not naturally occur, but is found only as chlorides of sodium, potassium, calcium and magnesium which are highly soluble in water (DWAF, 1996). In South Africa, most washing detergents used by low-income households include chlorine-based bleaches that are believed to be powerful stain removers and disinfectants. Most of such detergents are available as sodium chloride derivatives. The use of detergents in in-stream laundry is very common in most rural areas in South Africa, making the findings of the current study not an isolated incident. Data on washing practices in rural areas obtained from a survey undertaken by Lever Bros in KwaZulu-Natal indicated that 16 % of the rural population washed laundry directly at a watercourse (Pillay, 1994). Such activities have detrimental effects on aquatic ecosystems, even though they do not directly affect human livelihoods. The South African Water Quality Guidelines, as laid out by the Department of Water and Sanitation, states that chloride concentrations should exceed 1 200 $mg.L^{-1}$ to be unsafe for human consumption (DWAF, 1996), leaving no room for the determination of acceptable standards for aquatic dwellers of South African rivers.

4.2.1.4 Sodium

The natural sources of sodium are rocks and soils. Rivers contain less than 20 $mgNa.L^{-1}$, while drinking water usually contains more than that (WHO, 2011). In the current study, the high sodium concentrations recorded at site P5 were also due to the observed human activities associated with in-stream laundry and bathing. Fogg *et al.* (1983) suggested that sodium is found in higher concentrations in many washing powders. Moreover, sodium from washing powders is the main contributor to aquatic toxicity than introduced into rivers through acid rain (Warne and Schifko, 1999). The recorded concentrations were above 120 $mgNa.L^{-1}$ in the current study, considerably above natural levels expected in natural rivers. Although such levels may have ecological impacts on aquatic biota, there are no health effects on humans (DWAF, 1996).

4.2.1.5 Sulphate

Typically, the concentration of sulphate in surface water is 5 $mg.L^{-1}$ (DWAF, 1996). In the current study, the highest recorded concentrations were above 80 $mg.L^{-1}$ at site P5 where human interactions were the heaviest. Sulphate is a common constituent of water and

can naturally arise from the dissolution of mineral sulphates in soil and rock, particularly calcium sulphate (gypsum) and other partially soluble sulphate minerals (DWAF, 1996). In addition to the high levels of human induced activities that occurred at P5, high sulphate levels at this site were also attributed to the fact that the site had no canopy cover, resulting in high evaporation rate that in turn affected the overall concentrations of a number of anions and cations. Additionally, water flow was very low with relatively higher water temperatures at this site, contributing greatly on easy evaporation. Although there is no evidence to suggest a positive correlation between low pH and high sulphate concentrations, the acidic conditions observed at site P5 could not be completely eliminated as a possible cause of elevated sulphate concentrations at this site. A study by Guellaf and Kettani (2021) recorded higher concentrations of sulphates in the parts of the Oued Martil River basin that had low flow velocity at high temperatures in North-western Morocco.

4.2.1.6 Phosphorus

Phosphorus concentrations were also highest at the anthropogenically impacted site P5. For decades, studies have shown that excessive delivery of phosphorus in rivers is mainly associated with urbanisation and agriculture (Withers and Jarvie, 2008; Charlton *et al.*, 2018; Timis *et al.*, 2022). Studying phosphorus and its natural occurrence in natural waters is very tricky as there are no known set and specific standards to infer to. Available phosphorus standards calculation methods depend on site conditions such as types of plants present, oxygen availability and potential sources of pollution present. There is very limited phosphorus standards data available in a South African context. Studies in the United Kingdom have indicated that recommended phosphorus standards are changing, and are constantly under study to achieve greater levels of accuracy and reliability (UKTAG, 2014). In the current study, the elevated phosphorus concentrations recorded at site P5 were above 0.4 mg $P.L^{-1}$, a value so small, but yet way above those recorded from all the upstream sites whose values were all below 0.1 mg$P.L^{-1}$. Although phosphorus was higher at P5, there was visible evidence to suggest that eutrophication or any excessive growth of aquatic vegetation was prevalent at the site. Especially since excessive amounts of phosphorus are known to exacerbate plant growth (Timis *et al.*, 2022).

4.2.2 Secondary impacts of elevated chemicals

Conductivity and total dissolved solids (TDS) were higher in the two anthropogenically impacted downstream sites P5 and P6, with the highest values recorded at site P5 (see

Figs. 3.8 and 3.13, respectively). This was due to the elevated concentrations of ionic substances observed at these two sites. High concentrations of magnesium, sulphate and sodium were recorded in both these sites, with the highest values at P5 as mentioned earlier. The combination of ions from the above mentioned chemicals have been proven to increase TDS values (Paul and Sen, 2012). The reduced water flow observed from the two sites was also a contributing factor to high TDS concentrations. When studying the salinity effects of diversions of the Ganges River at Farakka in Bangladesh, Mirza (1998) found that the diversion of water to create easy navigation resulted in an acceleration of sedimentation, reduction in water flow and, therefore, an increase in salinity and TDS in the Gorai and Ganges Rivers. Two years later, an environmental impact assessment study was conducted by Rahman *et al.* (2000) in the same river catchments to determine any existing water quality deteriorations caused by the decreased Ganges outflow. Their study showed that the surface water of the area was sulphate-chloride dominated, which increased salinity, and therefore TDS concentrations.

Studies have proven that there is a relationship between the total dissolved solids and the conductivity of water (Paul and Sen, 2012; Shabalala *et al.*, 2013). By definition, electrical conductivity (EC) is the estimated amount of total dissolved salt/solids (TDS) or the total amount of dissolved ions in the water (Garg *et al.*, 2008) that can allow for the transmission of electrical charges. Since TDS was high downstream of the Palala River in the current study, it is not surprising that the electrical conductivity of the water was also high. Additionally, chemicals such as chlorides, phosphates and nitrates have also been positively associated with increased conductivity of water (Shabalala *et al.*, 2013).

4.2.3 Changes in flow velocity and water depth

Studying flow velocity was important as it helped understand how nutrient concentrations varied from the source to the mouth of the Palala River. Flow velocity was significantly different across sites with highest velocity recorded at sites P3 and B1, inside Lapalala. The habitat gradients, at these sites, was rocky (riffles) and different from the other sampling sites. According to Gerber and Gabriel (2002), rivers usually flowing faster over rocks which leads to turbulent flow. Furthermore, Aliyev (2022) also indicated that flow velocity is usually higher at rocky areas when compared to flat gentle areas of the river. In the current study, flow velocity was mostly affected by an overflow from the Hippo dam that was upstream of sampling site B1 at different sampling times during the study. Yuan *et al.*

(2019) reported that there is generally a significant fluctuation in flow velocity downstream of a dam when precipitation periods change.

Water depth varied significantly across sites with higher values recorded at sites P3 and P4, inside Lapalala. The deep waters were caused by natural erosion as the river meanders through the reserve. Further observations indicated that the river eroded in response to high water flow, eroding the outside of the meander, and depositing on the inside. A similar situation was observed by Ferreira da Silva and Ebrahimi (2017) who suggested that meandering and erosion could significantly affect a channel leading to increase in depth at specifically impacted parts of a river channel.

4.3 Temporal variation of environmental variables

In this study, the highest concentrations of chemicals such as potassium, magnesium, sodium, phosphorus, chloride, sulphate and TDS were recorded in spring. The spring sampling period was characterised by rainfall, which limited the dilution of these chemicals in the water column. Precipitation scarcity resulted in the reduction in water depth and flow during this study. Although water temperature in spring was not as high as it was in summer (Fig. 3.16), the rate of evaporation would have been high enough to cause some level of saturation of chemicals, especially the salts. Summer was the hottest sampling period. However, high rainfall was observed during this period in the study, potentially diluting and washing away most chemicals. High water flow was also observed in summer and autumn due to flooding.

The results of this study were similar to those found by Edokpayi *et al.* (2015) who indicated that nutrient concentrations increased during dry seasons when water levels were low when studying temporal variations in physico-chemical and microbiological characteristics of the Mvudi River in South Africa. Faniran *et al.* (2001) also recorded high levels of nutrients during the spring sampling period during an assessment of the water quality of the Isinuka Springs in the Transkei region of the Eastern Cape. Sulphate and chloride concentrations varied periodically, with higher concentrations recorded during the dry season at sites with reduced flow velocity and runoff levels in a study conducted by Ambani and Annegarn (2015).

The winter sampling period had low chemicals and nutrient concentrations when compared to autumn, summer and spring (Fig. 3.2). This was attributed to the low temperatures that tend to restrict movement of communities to rivers. Activities such as swimming

and bathing were not observed in any part of the river in winter. No visible channel modification was observed during the winter sampling period. It was clear however, that the river is highly useful to the local communities, not only as a water source, but also as a good source of sand. During the spring sampling period, the most downstream site (P6) was heavily affected by sand mining. The channel had no water during this time of the year (see Fig. 3.19), indicating the Palala River is non-perennial and the surrounding communities obtain sand as a usual activity during dry seasons. Sand mining have proven to affect the natural state of rivers, especially when the river is quite close to residential areas (Maeko, 2020). In addition to the effects on the morphological structure of the river, sand mining and changes in the physico-chemical components of a river have noticeable impacts on biological organisms such as macroinvertebrates that occur in the river (Maeko, 2020; Dickens and Graham, 2002; Dallas, 2007).

4.4 Macroinvertebrate community structure and the effects of environmental factors

Macroinvertebrate taxa richness did not change during the study. There was no statistical difference in taxa richness across sites and sampling periods. The diversity of macroinvertebrates remained the same during each sampling period. However, when assessing the longitudinal patterns, it was statistically evident that diversity decreased from source to mouth, with high diversity at the sites located upstream, and the lowest at the downstream site P6. These findings partially supported the second hypothesis which states that "the sites located upstream of Lapalala, and those that are located within the reserve will be characterised by high ASPT, SASS scores, macroinvertebrates abundance, high taxa richness and taxa diversity when compared to those located downstream of the reserve" (page 12, Subsection 1.11 Research aims, objectives and hypotheses).

Certain aspects of the above hypothesis could not be proven statistically true. For example, total macroinvertebrate abundance and taxa richness did not change across sites during the study. Instead, changes in macroinvertebrate abundance were seasonal. Fewer individual macroinvertebrates were collected during the spring sampling period due to the significantly high chemical nutrient concentrations that were observed from the Palala River at that time. A detailed multivariate analysis of the relationship between macroinvertebrates and environmental variables revealed that chloride, TDS and conductivity were the most important environmental variables influencing macroinvertebrates distribution during the course of the study (Fig. 3.23).

4.4.1 Effects of chloride on macroinvertebrates

Chloride levels in rivers vary broadly depending on various factors and sources that may be rising from nature or human induced anthropogenic activities (Corsi *et al.*, 2015). In some areas, the increase of chloride concentrations have been linked to urbanisation and human settlements in general (Conway, 2007). Along the Palala River, the higher than natural chloride concentrations suggested that chloride was derived from both natural and human activities. Visual observations strongly indicated possible influences coming from the way the river was utilised by the surrounding communities. When studying the effects of high chloride concentration on macroinvertebrates, the results from a distance based multivariate analysis showed an existing association. A simple linear regression analysis (Table 3.2) indicated that macroinvertebrate abundance, taxa richness and diversity were higher when chloride concentrations were lower. This was clearly evident at the upstream sites. The results also showed that an increase in chloride concentrations affected macroinvertebrate abundances downstream. The overall impression was that macroinvertebrate responses varied with the variations in chloride concentrations.

Miltner (2021) studied the impacts of chloride and sulphate ions on macroinvertebrate communities in Ohio streams and reported similar findings. In the Ohio streams, some taxa were tolerant to chloride and some were sensitive, which resulted in fluctuations in macroinvertebrate responses as chloride concentrations changed. Studies have shown that some members of the Ephemeroptera taxa, can acclimate to increased salinity by reducing the number of chloride cells on tracheal gills in response to increased salinity (Wichard *et al.*, 1973; Miltner, 2021). It is because of such complex macroinvertebrate behavioural adaptations that researchers warn that in instances where measured chloride concentrations are low, the presence of chloride tolerant taxa may signal a history of exposure (Clements *et al.*, 2016; Miltner, 2021). There is a possibility that chloride concentrations have been a concern in the Palala River for a while. This is because its effects on macroinvertebrates were prevalent at all sampling periods during this study (Table 3.4). Continuous monitoring of the river is important to consider.

4.4.2 Effects of TDS and EC on macroinvertebrates

Although TDS may occur naturally in surface waters, it has the potential to affect colour, alkalinity and conductance of water (Shrestha and Basnet, 2018). TDS contain organic molecules that support concentrations of nutrients and other forms of pollutants (We-

ber-Scannell and Duffy, 2007). Research has shown that TDS concentrations are highly dependent on the type and combination of ions in solution, and that certain combinations of ions increase EC (Timpano *et al.*, 2010; Shrestha and Basnet, 2018). High concentrations of TDS and EC are among the major stressors to aquatic ecosystems (Timpano *et al.*, 2010; Odume *et al.*, 2016). In the current study, the effects of TDS and EC were consistently observed on macroinvertebrate abundance across sites and sampling seasons (Tables 3.2 and 3.3). The multivariate analysis revealed that the most impacted site was P5 (Fig. 3.23A) where the highest concentrations of ionic nutrients were recorded. Macroinvertebrates at site P5 were affected by both EC and TDS on each of the sampling periods during the course of this study (Fig. 3.23B). The site was characterised by high TDS and EC levels.

On average, macroinvertebrate abundance remained above 100 individuals when sampling was done every period at site P5 (Fig. 3.21), proving to be the only site that showed to always have a lot of animals even when conditions were not favourable. A closer look at the types of macroinvertebrates that were abundant at this site showed that 75% were tolerant to pollution (Fig. 3.25). The tolerant taxa included Chironomidae, Hydracarina and Ceratopogonidae. According to literature, these taxa have shown resilience to changes in their habitat, including pollution from different sources (Odume *et al.*, 2016; Olson and Hawkins, 2017; Cormier *et al.*, 2013; Fanton *et al.*, 2023). In the Palala River, the distribution of macroinvertebrates was reflective of changes in water quality during the current study.

4.5 The state of water quality of the Palala River

Chapter 3 of this document showed the results found when the overall river conditions were assessed following the Index of Habitat Integrity (IHI) method. The results revealed that the Palala River was in a natural state, with little modifications occurring upstream, natural conditions occurring within Lapalala, and large modifications occurring downstream where human interactions were prevalent (Table 3.1). The overall water quality of the river was determined by the values of the ASPT and SASS 5 scores as prescribed by Dickens and Graham (2002), where low scores indicate poor quality and high scores indicate natural conditions. The ASPT and SASS5 scores did not change significantly across sampling periods, meaning that the river maintained at a constant state of water quality every time samples were collected during the four sampling periods. However, both the ASPT and SASS5 scores changed significantly across sites. The results suggested that water quality deteriorated at specific sections of the river. Figure 3.27 shows that water quality deteriorated longitudinally, with the most pristine or natural conditions occurring inside Lapalala. The

most affected sites were P5 and P6 where human interactions were highest. Based on visual observations, poor quality of water at site P6 resulted from channel modifications that altered macroinvertebrate communities and, therefore, decreasing the values of the ASPT and SASS5 scores.

A simple linear regression model indicated that the low ASPT and SASS5 scores were associated with the high chloride concentrations observed at site P5 (Table 3.5). These findings supported the parts of hypothesis 3 which states that "the concentration of (...) chloride (...) will have an influence on macroinvertebrate community structure, such that areas with high levels of nutrients will have low ASPT, SASS score (...)." (page 12, Subsection 1.11 Research aims, objectives and hypotheses).

Since the two water quality scores are related to the sensitivity levels of macroinvertebrates, the dominance of pollution tolerant taxa is indicative of the presence of pollutants in a river (Dickens and Graham, 2002). No pollution sensitive taxa were collected at site P5 in the current study (Fig. 3.25). According to the regression results, the collected taxa were tolerant to high chloride concentrations. The most dominant taxa were Culicidae (Mosquito larvae and pupae) from the order Diptera. Studies who have looked at the behaviour of species belonging to the mosquito family have reported that mosquito larvae can survive under high chloride concentrations (Amini *et al.*, 2020; Nikookar *et al.*, 2017). The dominance of tolerant mosquito taxa was observed throughout the sampling period and was positively associated with low ASPT and SASS5 scores, suggesting a deterioration of water quality at the sites with human interactions.

CHAPTER 5
SUMMARY AND RECOMMENDATIONS

5.1 General morphological, chemical and biological characteristics of the Palala River

5.1.1 Morphological characteristics

A naturally undisturbed river system is divided into three different zones namely, headwaters, middle and lower zones (Dallas and Day, 1993). The different zones are characterised by changes in flow velocity, vegetation, stream width, temperature and other important features defining the river as it flows longitudinally (Dallas and Day, 1993). Flow velocity is higher from the source and gradually decreases as the river meanders downstream, slow moving waters towards the mouth. Vegetation cover tends to be dense at the source, with a narrow channel, cold and well oxygenated waters. These are all characteristics laid out by Vannote *et al.* (1980) when explaining the river continuum concept.

The Palala River system was no different from the above-mentioned definition, the morphological makeup of the catchment was as expected, with a few exceptions in areas where human interactions occurred. Although agricultural activities occurred along the first two sampling sites from the source of the river, they posed no visible threats to the physical characteristics of the river. No bank modifications or water abstractions were observed from both sites. The river remained natural as it passed through the Lapalala Wilderness Reserve (Lapalala). No channel modifications were observed within the nature reserve. The presence of the Bloklandspruit tributary did not prove to have any significant influences on the channel structure of the Palala River. The natural state of the Palala River only began to change downstream of Lapalala as the channel widened and communities gained access. The most prevalent changes to the channel resulted from modifications caused by sand mining at the most downstream site. Sand mining appeared to be intense during the dry season (Spring) when water stopped flowing.

5.1.2 Chemical characteristics

During the course of this study, variations in chloride, total ammonia, potassium, sodium, sulphate, nitrate, dissolved magnesium, total dissolved solids (TDS), oxygen absorbed (O_2), electrical conductivity and phosphorus were assessed to determine the chemical

characteristics of the river. There was a longitudinal gradient in the concentration of potassium, magnesium, sodium and chloride, with lower values recorded upstream and higher values recorded downstream. Nitrate concentrations were high upstream due to crop and livestock farming that occurred before the river entered Lapalala. The concentrations of nitrates decreased downstream. Dissolved oxygen, ammonia and the pH of the water did not change across sites. Overall, the Palala River's water chemistry was mostly impacted downstream. The two downstream sites were heavily utilised by the Shongoane, Ga-Monyeki, Thabo Mbeki and Ga-Seleka communities which occurred along the river. Noticeable impacts were observed during the dry season when water levels were lower and temperatures were higher. Most nutrients were washed away during the wet season and the river was not easily accessible to its users. At low flow velocities however, the local communities were observed washing clothes and bathing inside the river channel, increasing the chemical composition of the water.

5.1.3 Biological characteristics

Macroinvertebrates communities were affected by the changes in chemical composition of the river. Macroinvertebrates taxa which are known to be sensitive to pollution, occurred predominately upstream and in the sites that were located inside Lapalala. Low taxa richness, diversity and abundance were associated with high chemical concentrations, specifically downstream where the most impact was observed. The use of macroinvertebrates as indicators of water quality was proven successful during this study as the clear shifts in dominant taxa was observed with the changes in chemical composition. The results indicated that when chemicals increase in the water column, only tolerant macroinvertebrates can survive. The dominance of animals from the order Diptera downstream coupled with the occurrence of stoneflies upstream indicated a clear shift in water quality as the river flows from source to mouth.

5.1.4 The ecological importance of Lapalala on the Palala River

The Lapalala Wilderness Reserve, established in 1981, plays a huge role in protecting any form of land degradation that occurs in the reserve (Walker, 1994). The results of this study indicated that the river was rejuvenated, resembling a headwater zone within Lapalala with clear fast flow waters. Dutta *et al.* (2020) indicated that a river can rejuvenate itself if anthropogenic influences are eliminated. The high nitrate concentrations that were recorded upstream of the reserve decreased as the river drained through the reserve. Proving the self-

rejuvenation power that a river has in the absence of human interactions. Inside Lapalala, the channel was characterised by undisturbed rocks and stones, riffles, gentler slopes and deep pools that provided habitat for hippopotamus and crocodiles. The restricted human activities inside the reserve resulted in high macroinvertebrate richness, diversity and abundance. This elevated the values of the ASPT and SASS5 scores which, in turn is reflective of good water quality. Figures 3.23 and 3.27 clearly show that highest water quality scores were recorded inside the nature reserve. This study provided sufficient evidence to suggest that the placement of the Reserve is of ecological importance to the river as no impacts were visually or statistically observed.

5.2 Conclusion

The main purpose of the study was to determine the ecological status of the Palala River as it flows through various land uses. Necessary data was collected, critically analysed and interpreted. Interesting patterns emerged. The Palala River is, for the most part, fairly natural. A few concerns exist upstream, just below the source of the river and upstream of Lapalala. These concerns were associated to the observed agricultural activities. There was no statistical evidence to suggest that the high nitrate concentrations recorded upstream of the river had any influence on the biological communities of macroinvertebrates in the river at that time. The location of Lapalala provided a rejuvenation period to the river, which unfortunately was lost when the river exited the reserve into human settlements downstream. Changes in water quality were reflected as sensitive macroinvertebrate taxa decreased with an increase in nutrient concentrations. It is, therefore, safe to conclude that human interactions are the driving force behind changes in the morphology, chemical and biological composition of the Palala River.

5.3 Recommendations

Based on the findings of this study, the following recommendations are made:

a) The placement of protected areas should be strategic. A protected area should, among other important factors, be placed in such a way that it can protect or conserve biological diversity that occurs in rivers. A section of a river must be conserved, to ensure that the river has an opportunity for natural rejuvenation. Research has shown that protected areas were previously biased towards terrestrial ecosystems, and suggested that emerging or expanding protected areas should consider rivers and other freshwater ecosystems for conservation purposes (Azevedo-Santos *et al.*, 2019).

b) There must be frequent river health assessments done to monitor and provide accurate water quality results to the communities of Shongoane, Ga-Monyeki, Thabo Mbeki and Ga-Seleka as part of an outreach programs. An environmental education program must be established and involve the communities that depend on the river, train them on how to use citizen science tools to solve their own environmental issues. This will raise awareness and avoid unnecessary waterborne diseases. It will also help the community develop a sense of responsibility and accountability.

c) To be more precise with the identification of sources of pollution into the river, studies that will employ multiple approaches must be conducted. A study by Gininda (2016) used multiple approaches to determine the overall ecological status of the Bloukrans River in Grahamstown and was able to differentiate between pollution derived from domestic, agriculture and sewage sources using stable isotopes. This will help identify the main polluters and help communicate accurate results to decision makers to effect change.

d) More studies focusing on the bacteriology of the Palala River should be conducted. Particularly focusing on the water quality of the sections easily accessible by communities to determine the levels of coliforms and *Escherichia coli (E. coli)*, if any.

CHAPTER 6
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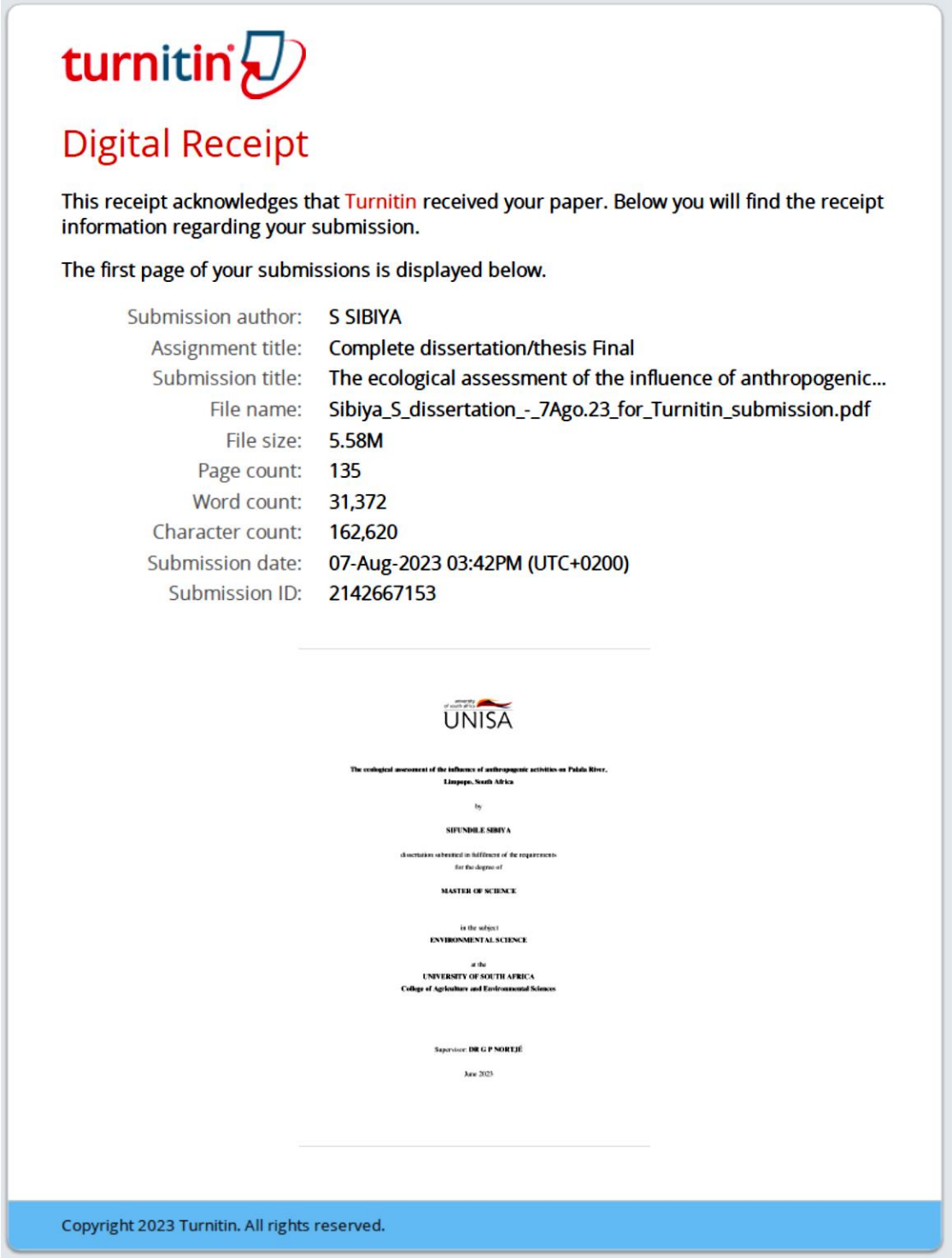
APPENDICES

Appendix A: Turnitin digital receipt



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File size:	5.58M
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UNISA

The ecological assessment of the influence of anthropogenic activities on Palala River, Limpopo, South Africa

by

SIFUNDILE SIBIYA

dissertation submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

in the subject ENVIRONMENTAL SCIENCE

at the UNIVERSITY OF SOUTH AFRICA College of Agriculture and Environmental Sciences

Supervisor: DR G P NORTJÉ

June 2023

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Appendix B: Ethical clearance

UNISA university of south africa

UNISA-CAES ANIMAL RESEARCH ETHICS COMMITTEE

Date: 11/08/2021

NHREC Registration # : N/A
REC Reference # : 2021/CAES_AREC/114
Name : Mr S Sibiya
Student #: 56086172

Dear Mr Sibiya

Decision: Ethics Approval from 06/08/2021 to 31/07/2022

Researcher(s): Mr S Sibiya
sfundosbiyas@gmail.com; 071-062-2365

Supervisor (s): Dr GP Nortjé
nortjgp@unisa.ac.za; 011-471-2286

Working title of research:

The ecological assessment of the influence of anthropogenic activities on Palala River, Limpopo, South Africa

Qualification: MSc Nature Conservation

Thank you for the application for research ethics clearance by the Unisa-CAES Animal Research Ethics Committee for the above mentioned research. Ethics approval is granted for one year.

Ethics clearance is renewable on a yearly basis until completion of the project. **Renewal will be issued upon submission of yearly progress reports.**

Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.

Due date for progress report: 31 July 2022

Please note the following for further action:

1. The committee notes that the researcher will request assistance from Lapalala to ensure the researcher's safety during sampling. The committee requests confirmation that such assistance has been obtained before the researcher may commence with the sampling.

University of South Africa
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PO Box 392 UNISA 0003 South Africa
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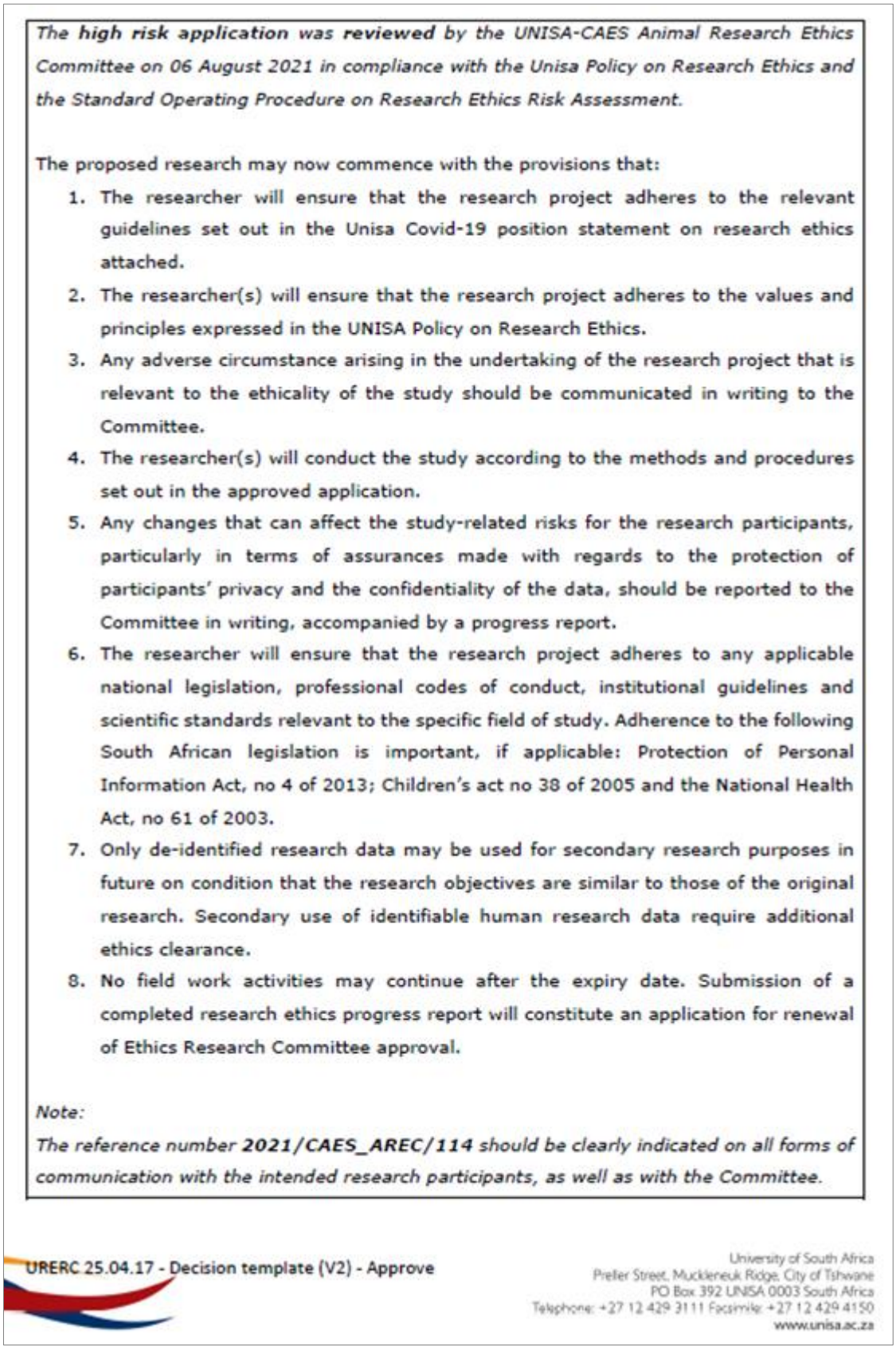
Figure B.1a: Ethical clearance, first page



*The **high risk application** was **reviewed** by the UNISA-CAES Animal Research Ethics Committee on 06 August 2021 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

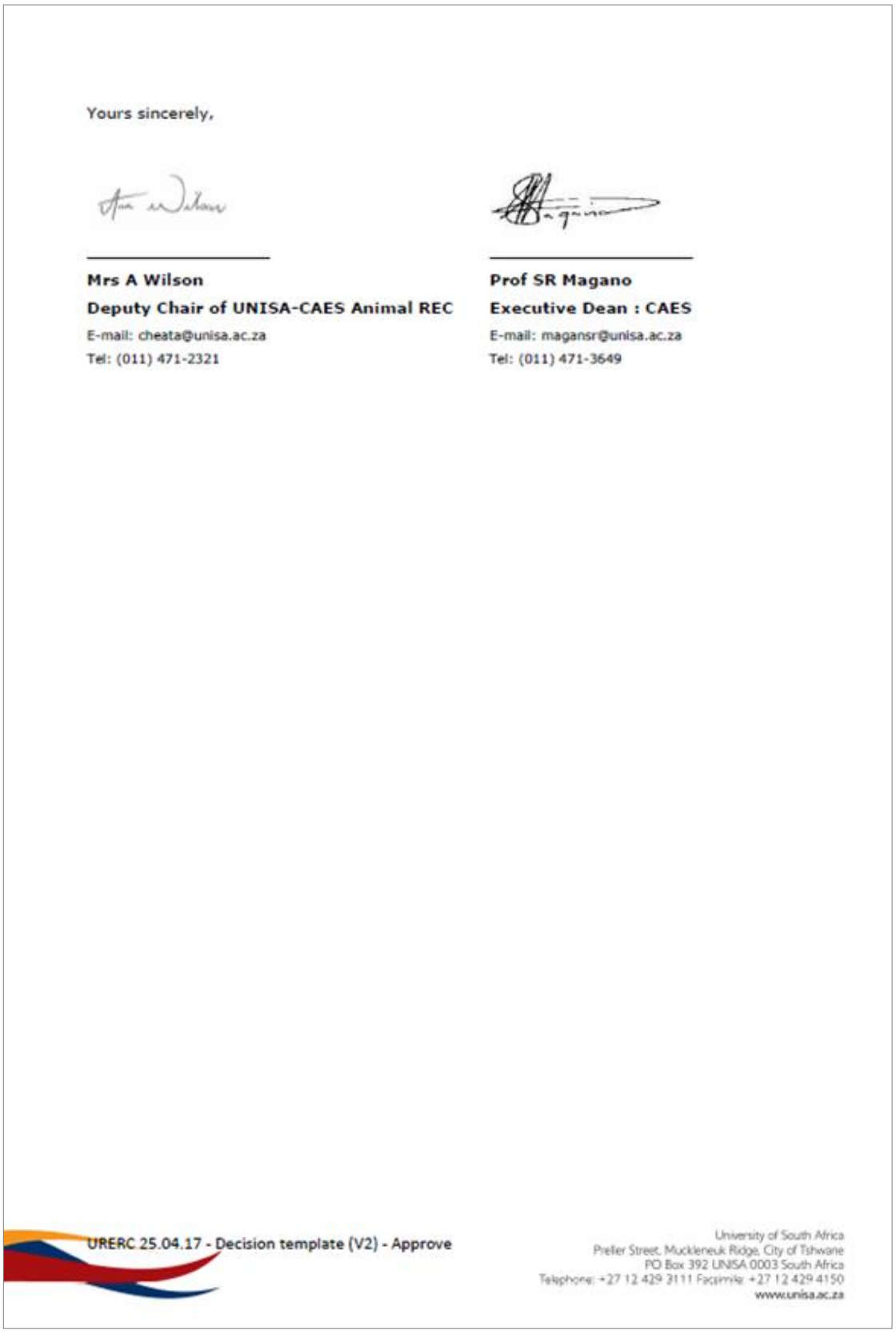
The proposed research may now commence with the provisions that:

1. The researcher will ensure that the research project adheres to the relevant guidelines set out in the Unisa Covid-19 position statement on research ethics attached.
2. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
3. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
4. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
5. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
6. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
7. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
8. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:

*The reference number **2021/CAES_AREC/114** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Figure B.1b: Ethical clearance, second page



Yours sincerely,

Mrs A Wilson
Deputy Chair of UNISA-CAES Animal REC
E-mail: cheata@unisa.ac.za
Tel: (011) 471-2321

Prof SR Magano
Executive Dean : CAES
E-mail: magansr@unisa.ac.za
Tel: (011) 471-3649

URERC 25.04.17 - Decision template (V2) - Approve

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
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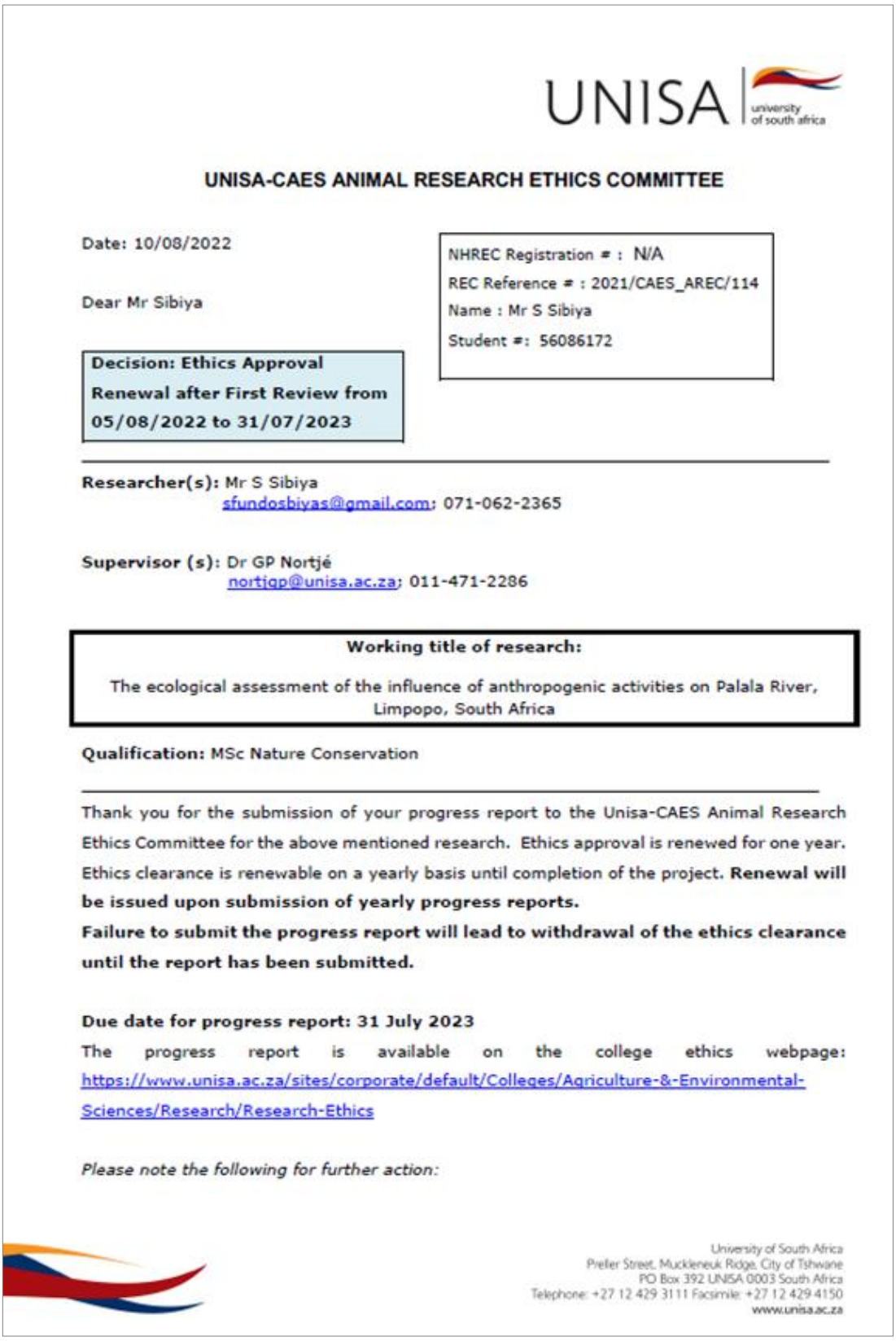
Figure B.1c: Ethical clearance, third page



UNISA university of south africa

UNISA-CAES ANIMAL RESEARCH ETHICS COMMITTEE

Date: 10/08/2022

NHREC Registration # : N/A
REC Reference # : 2021/CAES_AREC/114
Name : Mr S Sibiya
Student #: 56086172

Dear Mr Sibiya

Decision: Ethics Approval
Renewal after First Review from
05/08/2022 to 31/07/2023

Researcher(s): Mr S Sibiya
sfundosbiyas@gmail.com; 071-062-2365

Supervisor (s): Dr GP Nortjé
nortjgp@unisa.ac.za; 011-471-2286

Working title of research:

The ecological assessment of the influence of anthropogenic activities on Palala River, Limpopo, South Africa

Qualification: MSc Nature Conservation

Thank you for the submission of your progress report to the Unisa-CAES Animal Research Ethics Committee for the above mentioned research. Ethics approval is renewed for one year. Ethics clearance is renewable on a yearly basis until completion of the project. **Renewal will be issued upon submission of yearly progress reports.**
Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.

Due date for progress report: 31 July 2023
The progress report is available on the college ethics webpage: https://www.unisa.ac.za/sites/corporate/default/Colleges/Agriculture-&-Environmental-Sciences/Research/Research-Ethics

Please note the following for further action:

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Figure B.2a: Ethical clearance renewal, first page

1. Please note that the risk to the researcher remains high for as long as data collection continues, due to the presence of crocodiles and hippos, as well as other wild animals in the research area.

*The **high risk application** was originally **reviewed** by the UNISA-CAES Animal Research Ethics Committee on 62 August 2021 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

1. The researcher will ensure that the research project adheres to the relevant guidelines set out in the Unisa Covid-19 position statement on research ethics attached.
2. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
3. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
4. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
5. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
6. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
7. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
8. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:

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Figure B.2b: Ethical clearance renewal, second page

*The reference number **2021/CAES_AREC/114** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,

Dr WM Strauss
Chair of UNISA-CAES Animal REC
E-mail: stauwm@unisa.ac.za
Tel: (011) 471-2163

Prof SR Magano
Executive Dean: CAES
E-mail: maganosr@unisa.ac.za
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Figure B.2c: Ethical clearance renewal, third page

Appendix C: Sampling certificates of water analysis from Talbot lab

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[000159/22], [2022/01/19]

Certificate of Analysis

Project details

Customer Details

Customer reference:	MSC PROJECT (UNISA)
Quotation number:	Q2112-086
Company name:	SIFUNDO SIBIYA
Contact address:	01 KARKLOOF ROAD, HOWICK, 3290
Contact person:	SIFUNDO SIBIYA

Sampling Details

Sampled by:	CUSTOMER
Sampled date:	2022/01/06
Additional customer information:	000529/22- SAMPLED BY: SIFUNDO SIBIYA, 000530/22- SAMPLED BY: SIFUNDO SIBIYA, 000531/22- SAMPLED BY: SIFUNDO SIBIYA, 000532/22- SAMPLED BY: SIFUNDO SIBIYA, 000533/22- SAMPLED BY: SIFUNDO SIBIYA, 000534/22- SAMPLED BY: SIFUNDO SIBIYA, 000535/22- SAMPLED BY: SIFUNDO SIBIYA, 000536/22- SAMPLED BY: SIFUNDO SIBIYA, 000537/22- SAMPLED BY: SIFUNDO SIBIYA, 000538/22- SAMPLED BY: SIFUNDO SIBIYA, 000539/22- SAMPLED BY: SIFUNDO SIBIYA, 000540/22- SAMPLED BY: SIFUNDO SIBIYA, 000541/22- SAMPLED BY: SIFUNDO SIBIYA, 000542/22- SAMPLED BY: SIFUNDO SIBIYA, 000543/22- SAMPLED BY: SIFUNDO SIBIYA, 000544/22- SAMPLED BY: SIFUNDO SIBIYA, 000545/22- SAMPLED BY: SIFUNDO SIBIYA, 000546/22- SAMPLED BY: SIFUNDO SIBIYA, 000547/22- SAMPLED BY: SIFUNDO SIBIYA, 000548/22- SAMPLED BY: SIFUNDO SIBIYA, 000549/22- SAMPLED BY: SIFUNDO SIBIYA

Sample Details

Sample type(s):	SURFACE WATER SAMPLES
Date received:	2022/01/07
Delivered by:	CUSTOMER
Temperature at sample receipt (°C):	12.8

Report Details

Testing commenced:	2022/01/07
Testing completed:	2022/01/19
Report date:	2022/01/19
Our reference:	000159/22

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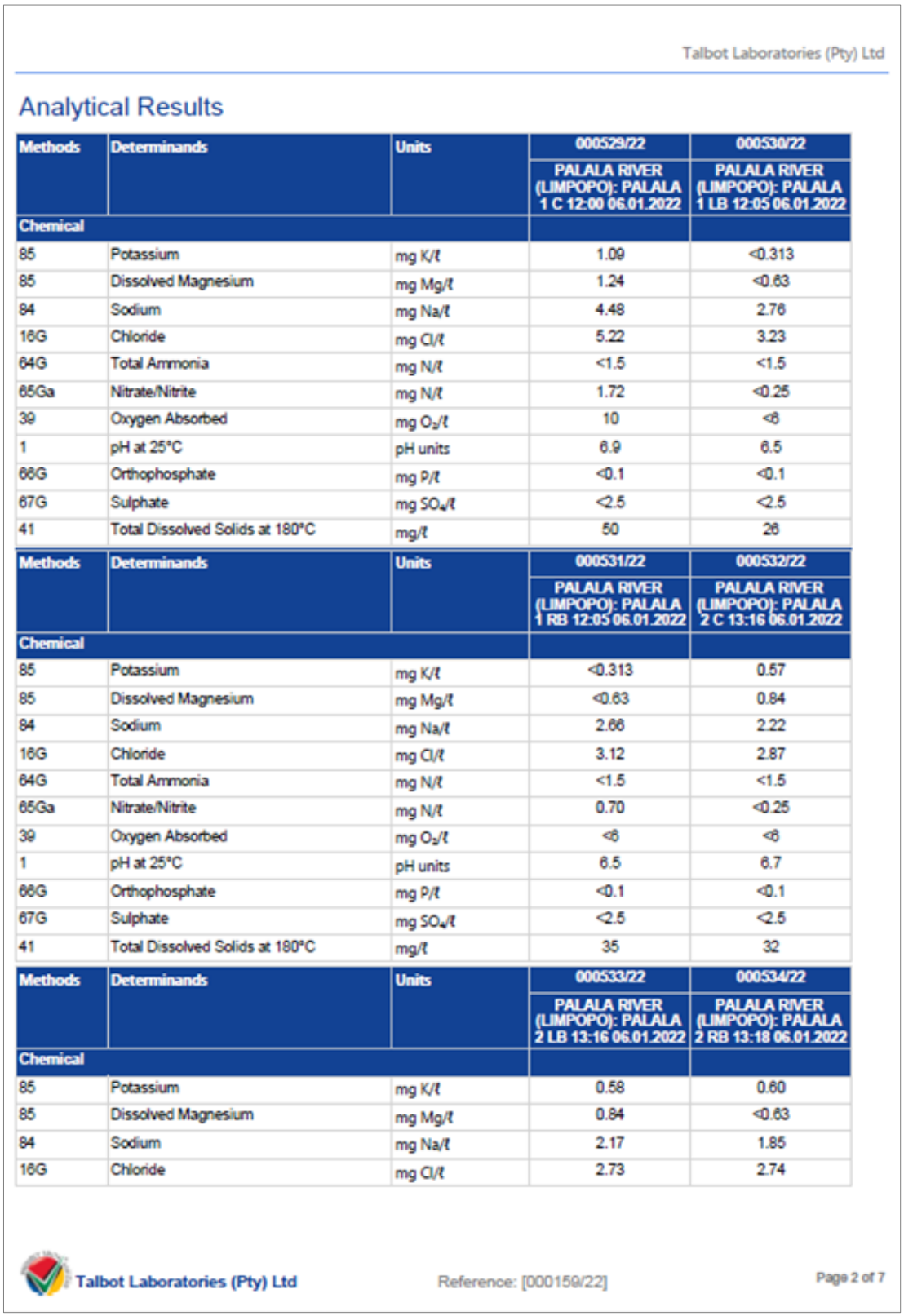
Figure C.1a: First sampling period certificate of water analysis from Talbot lab, first page



Talbot Laboratories (Pty) Ltd

Analytical Results

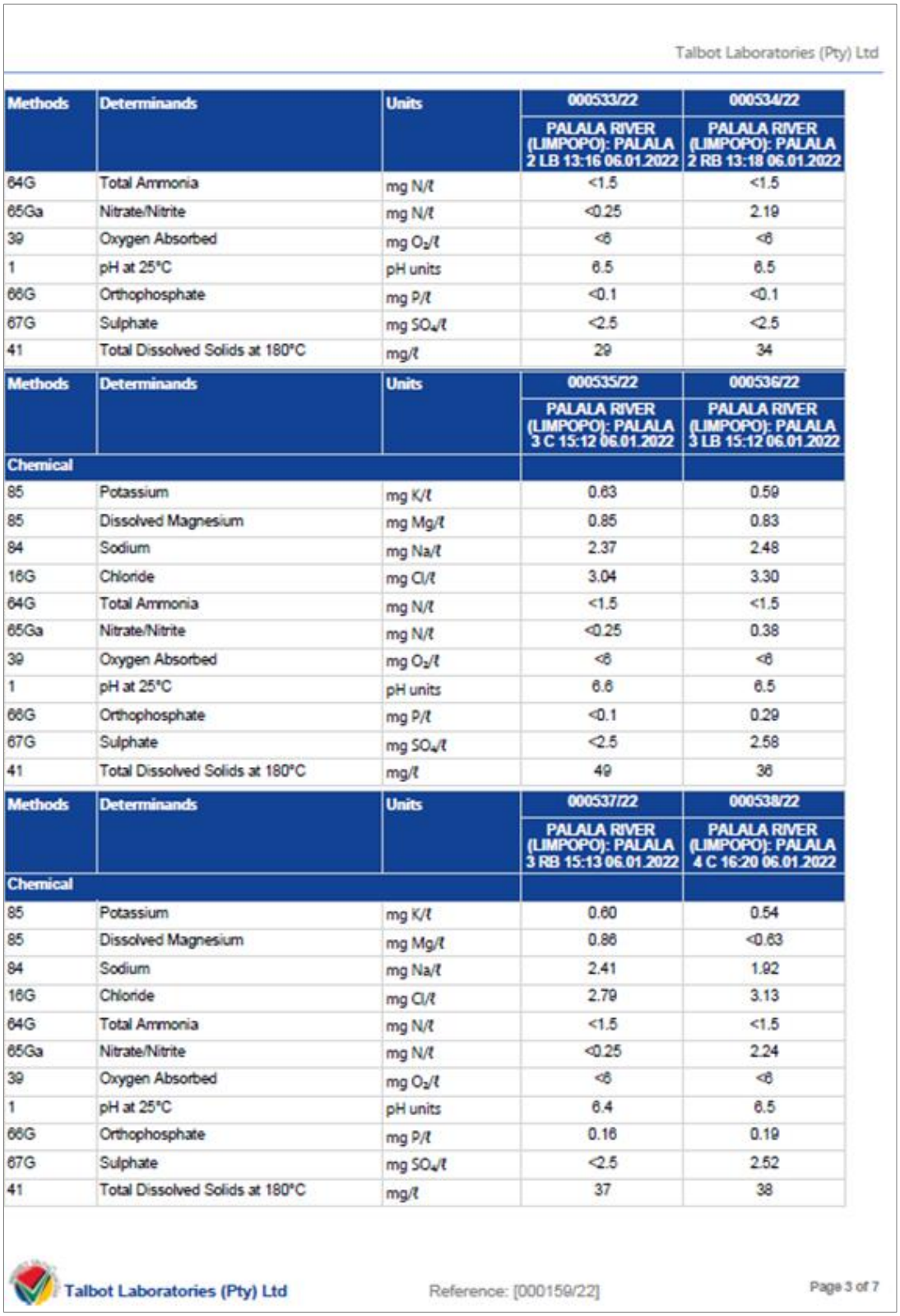
Methods	Determinands	Units	000529/22 PALALA RIVER (LIMPOPO): PALALA 1 C 12:00 06.01.2022	000530/22 PALALA RIVER (LIMPOPO): PALALA 1 LB 12:05 06.01.2022
Chemical				
85	Potassium	mg K/ℓ	1.09	<0.313
85	Dissolved Magnesium	mg Mg/ℓ	1.24	<0.63
84	Sodium	mg Na/ℓ	4.48	2.76
16G	Chloride	mg Cl/ℓ	5.22	3.23
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	1.72	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	10	<6
1	pH at 25°C	pH units	6.9	6.5
66G	Orthophosphate	mg P/ℓ	<0.1	<0.1
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	50	26

Methods	Determinands	Units	000531/22 PALALA RIVER (LIMPOPO): PALALA 1 RB 12:05 06.01.2022	000532/22 PALALA RIVER (LIMPOPO): PALALA 2 C 13:16 06.01.2022
Chemical				
85	Potassium	mg K/ℓ	<0.313	0.57
85	Dissolved Magnesium	mg Mg/ℓ	<0.63	0.84
84	Sodium	mg Na/ℓ	2.66	2.22
16G	Chloride	mg Cl/ℓ	3.12	2.87
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	0.70	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
1	pH at 25°C	pH units	6.5	6.7
66G	Orthophosphate	mg P/ℓ	<0.1	<0.1
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	35	32

Methods	Determinands	Units	000533/22 PALALA RIVER (LIMPOPO): PALALA 2 LB 13:16 06.01.2022	000534/22 PALALA RIVER (LIMPOPO): PALALA 2 RB 13:18 06.01.2022
Chemical				
85	Potassium	mg K/ℓ	0.58	0.60
85	Dissolved Magnesium	mg Mg/ℓ	0.84	<0.63
84	Sodium	mg Na/ℓ	2.17	1.85
16G	Chloride	mg Cl/ℓ	2.73	2.74

Talbot Laboratories (Pty) Ltd Reference: [000159/22] Page 2 of 7

Figure C.1b: First sampling period certificate of water analysis from Talbot lab, second page



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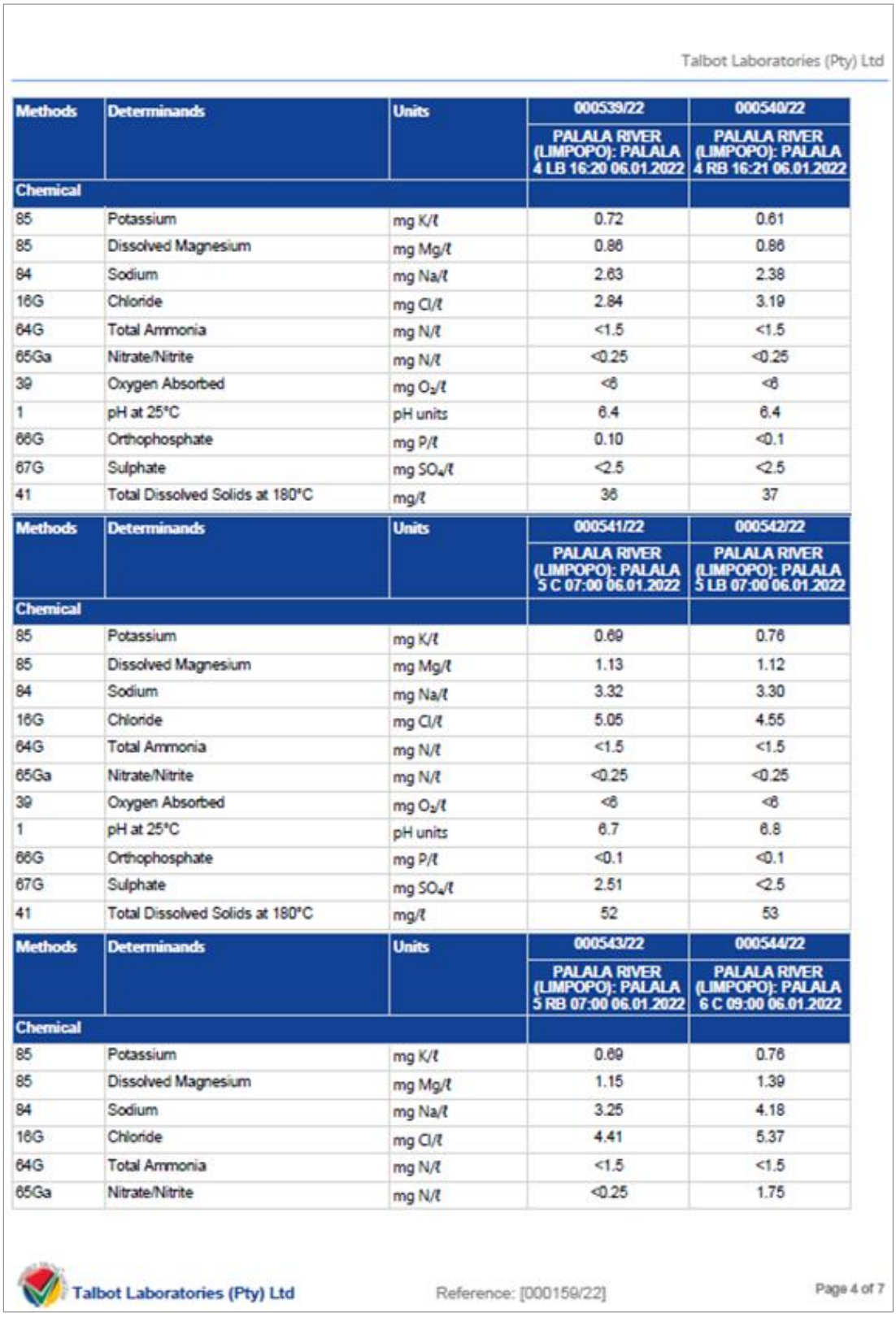
Methods	Determinands	Units	000533/22	000534/22
			PALALA RIVER (LIMPOPO): PALALA 2 LB 13:16 06.01.2022	PALALA RIVER (LIMPOPO): PALALA 2 RB 13:18 06.01.2022
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	2.19
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
1	pH at 25°C	pH units	6.5	6.5
66G	Orthophosphate	mg P/ℓ	<0.1	<0.1
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	29	34

Methods	Determinands	Units	000535/22	000536/22
			PALALA RIVER (LIMPOPO): PALALA 3 C 15:12 06.01.2022	PALALA RIVER (LIMPOPO): PALALA 3 LB 15:12 06.01.2022
Chemical				
85	Potassium	mg K/ℓ	0.63	0.59
85	Dissolved Magnesium	mg Mg/ℓ	0.85	0.83
84	Sodium	mg Na/ℓ	2.37	2.48
16G	Chloride	mg Cl/ℓ	3.04	3.30
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	0.38
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
1	pH at 25°C	pH units	6.6	6.5
66G	Orthophosphate	mg P/ℓ	<0.1	0.29
67G	Sulphate	mg SO_4/ℓ	<2.5	2.58
41	Total Dissolved Solids at 180°C	mg/ℓ	49	36

Methods	Determinands	Units	000537/22	000538/22
			PALALA RIVER (LIMPOPO): PALALA 3 RB 15:13 06.01.2022	PALALA RIVER (LIMPOPO): PALALA 4 C 16:20 06.01.2022
Chemical				
85	Potassium	mg K/ℓ	0.60	0.54
85	Dissolved Magnesium	mg Mg/ℓ	0.86	<0.63
84	Sodium	mg Na/ℓ	2.41	1.92
16G	Chloride	mg Cl/ℓ	2.79	3.13
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	2.24
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
1	pH at 25°C	pH units	6.4	6.5
66G	Orthophosphate	mg P/ℓ	0.16	0.19
67G	Sulphate	mg SO_4/ℓ	<2.5	2.52
41	Total Dissolved Solids at 180°C	mg/ℓ	37	38

Talbot Laboratories (Pty) Ltd Reference: [000159/22] Page 3 of 7

Figure C.1c: First sampling period certificate of water analysis from Talbot lab, third page



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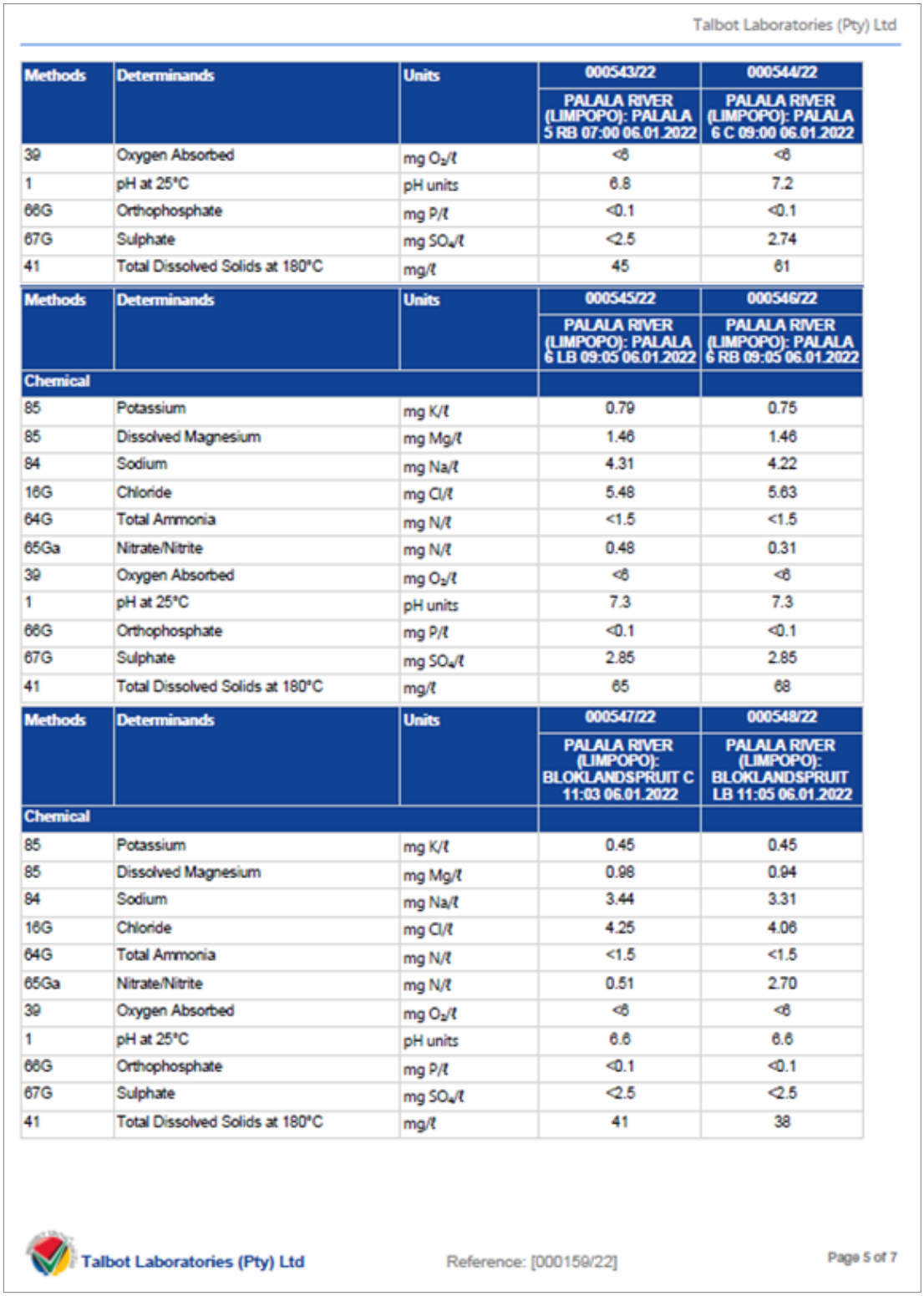
Methods	Determinands	Units	000539/22	000540/22
			PALALA RIVER (LIMPOPO): PALALA 4 LB 16:20 06.01.2022	PALALA RIVER (LIMPOPO): PALALA 4 RB 16:21 06.01.2022
Chemical				
85	Potassium	mg K/ℓ	0.72	0.61
85	Dissolved Magnesium	mg Mg/ℓ	0.86	0.86
84	Sodium	mg Na/ℓ	2.63	2.38
16G	Chloride	mg Cl/ℓ	2.84	3.19
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
1	pH at 25°C	pH units	6.4	6.4
66G	Orthophosphate	mg P/ℓ	0.10	<0.1
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	36	37

Methods	Determinands	Units	000541/22	000542/22
			PALALA RIVER (LIMPOPO): PALALA 5 C 07:00 06.01.2022	PALALA RIVER (LIMPOPO): PALALA 5 LB 07:00 06.01.2022
Chemical				
85	Potassium	mg K/ℓ	0.69	0.76
85	Dissolved Magnesium	mg Mg/ℓ	1.13	1.12
84	Sodium	mg Na/ℓ	3.32	3.30
16G	Chloride	mg Cl/ℓ	5.05	4.55
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
1	pH at 25°C	pH units	6.7	6.8
66G	Orthophosphate	mg P/ℓ	<0.1	<0.1
67G	Sulphate	mg SO_4/ℓ	2.51	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	52	53

Methods	Determinands	Units	000543/22	000544/22
			PALALA RIVER (LIMPOPO): PALALA 5 RB 07:00 06.01.2022	PALALA RIVER (LIMPOPO): PALALA 6 C 09:00 06.01.2022
Chemical				
85	Potassium	mg K/ℓ	0.69	0.76
85	Dissolved Magnesium	mg Mg/ℓ	1.15	1.39
84	Sodium	mg Na/ℓ	3.25	4.18
16G	Chloride	mg Cl/ℓ	4.41	5.37
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	1.75

Talbot Laboratories (Pty) Ltd Reference: [000159/22] Page 4 of 7

Figure C.1d: First sampling period certificate of water analysis from Talbot lab, fourth page



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Methods	Determinands	Units	000543/22	000544/22
			PALALA RIVER (LIMPOPO): PALALA 5 RB 07:00 06.01.2022	PALALA RIVER (LIMPOPO): PALALA 6 C 09:00 06.01.2022
39	Oxygen Absorbed	mg O_2/l	<6	<6
1	pH at 25°C	pH units	6.8	7.2
66G	Orthophosphate	mg P/l	<0.1	<0.1
67G	Sulphate	mg SO_4/l	<2.5	2.74
41	Total Dissolved Solids at 180°C	mg/l	45	61

Methods	Determinands	Units	000545/22	000546/22
			PALALA RIVER (LIMPOPO): PALALA 6 LB 09:05 06.01.2022	PALALA RIVER (LIMPOPO): PALALA 6 RB 09:05 06.01.2022
Chemical				
85	Potassium	mg K/l	0.79	0.75
85	Dissolved Magnesium	mg Mg/l	1.46	1.46
84	Sodium	mg Na/l	4.31	4.22
16G	Chloride	mg Cl/l	5.48	5.63
64G	Total Ammonia	mg N/l	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/l	0.48	0.31
39	Oxygen Absorbed	mg O_2/l	<6	<6
1	pH at 25°C	pH units	7.3	7.3
66G	Orthophosphate	mg P/l	<0.1	<0.1
67G	Sulphate	mg SO_4/l	2.85	2.85
41	Total Dissolved Solids at 180°C	mg/l	65	68

Methods	Determinands	Units	000547/22	000548/22
			PALALA RIVER (LIMPOPO): BLOKLANDSPRUIT C 11:03 06.01.2022	PALALA RIVER (LIMPOPO): BLOKLANDSPRUIT LB 11:05 06.01.2022
Chemical				
85	Potassium	mg K/l	0.45	0.45
85	Dissolved Magnesium	mg Mg/l	0.98	0.94
84	Sodium	mg Na/l	3.44	3.31
16G	Chloride	mg Cl/l	4.25	4.06
64G	Total Ammonia	mg N/l	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/l	0.51	2.70
39	Oxygen Absorbed	mg O_2/l	<6	<6
1	pH at 25°C	pH units	6.6	6.6
66G	Orthophosphate	mg P/l	<0.1	<0.1
67G	Sulphate	mg SO_4/l	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/l	41	38

Talbot Laboratories (Pty) Ltd Reference: [000159/22] Page 5 of 7

Figure C.1e: First sampling period certificate of water analysis from Talbot lab, fifth page

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Methods	Determinands	Units	000549/22
			PALALA RIVER (LIMPOPO): BLOKLANDSPRUIT RB 11:07 06.01.2022
Chemical			
85	Potassium	mg K/ℓ	0.48
85	Dissolved Magnesium	mg Mg/ℓ	0.96
84	Sodium	mg Na/ℓ	3.53
16G	Chloride	mg Cl/ℓ	4.23
64G	Total Ammonia	mg N/ℓ	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6
1	pH at 25°C	pH units	6.5
66G	Orthophosphate	mg P/ℓ	<0.1
67G	Sulphate	mg SO_4/ℓ	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	36

Refer to the "Notes" section at the end of this report for further explanations.

Specific Observations

None

Talbot Laboratories (Pty) Ltd Reference: [000159/22] Page 6 of 7

Figure C.1f: First sampling period certificate of water analysis from Talbot lab, sixth page

TALBOT
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[002820/22], [2022/04/21

Certificate of Analysis

Project details

Customer Details

Customer reference:	MSC PROJECT (UNISA)
Company name:	SIFUNDO SIBIYA
Contact address:	01 KARKLOOF ROAD, HOWICK, 3290
Contact person:	SIFUNDO SIBIYA

Sampling Details

Sampled by:	CUSTOMER
Sampled date:	2022/03/31
Additional customer information:	009074/22- SAMPLED BY: SIFUNDO SIBIYA, 009075/22- SAMPLED BY: SIFUNDO SIBIYA, 009076/22- SAMPLED BY: SIFUNDO SIBIYA, 009077/22- SAMPLED BY: SIFUNDO SIBIYA, 009078/22- SAMPLED BY: SIFUNDO SIBIYA, 009079/22- SAMPLED BY: SIFUNDO SIBIYA, 009080/22- SAMPLED BY: SIFUNDO SIBIYA, 009081/22- SAMPLED BY: SIFUNDO SIBIYA, 009082/22- SAMPLED BY: SIFUNDO SIBIYA, 009083/22- SAMPLED BY: SIFUNDO SIBIYA, 009084/22- SAMPLED BY: SIFUNDO SIBIYA, 009085/22- SAMPLED BY: SIFUNDO SIBIYA, 009086/22- SAMPLED BY: SIFUNDO SIBIYA, 009087/22- SAMPLED BY: SIFUNDO SIBIYA, 009088/22- SAMPLED BY: SIFUNDO SIBIYA, 009089/22- SAMPLED BY: SIFUNDO SIBIYA, 009090/22- SAMPLED BY: SIFUNDO SIBIYA, 009091/22- SAMPLED BY: SIFUNDO SIBIYA, 009092/22- SAMPLED BY: SIFUNDO SIBIYA, 009093/22- SAMPLED BY: SIFUNDO SIBIYA

Sample Details

Sample type(s):	SURFACE WATER SAMPLES
Date received:	2022/04/01
Delivered by:	CUSTOMER
Temperature at sample receipt (°C):	18.3

Report Details

Testing commenced:	2022/04/01
Testing completed:	2022/04/21
Report date:	2022/04/21
Our reference:	002820/22

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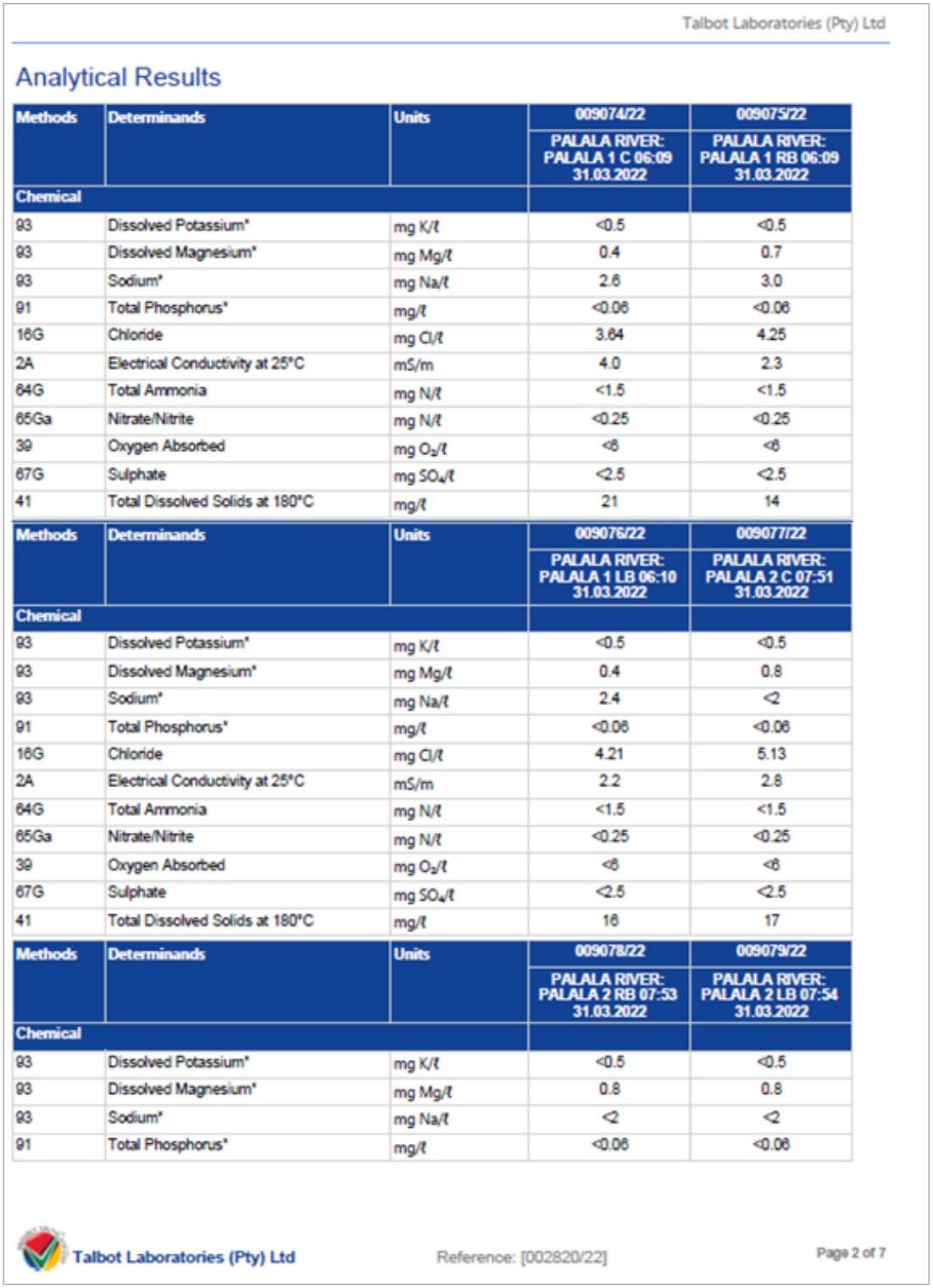
Figure C.2a: Second sampling period certificate of water analysis from Talbot lab, first page



Talbot Laboratories (Pty) Ltd

Analytical Results

Methods	Determinands	Units	009074/22 PALALA RIVER: PALALA 1 C 06:09 31.03.2022	009075/22 PALALA RIVER: PALALA 1 RB 06:09 31.03.2022
Chemical				
93	Dissolved Potassium*	mg K/ℓ	<0.5	<0.5
93	Dissolved Magnesium*	mg Mg/ℓ	0.4	0.7
93	Sodium*	mg Na/ℓ	2.6	3.0
91	Total Phosphorus*	mg/ℓ	<0.06	<0.06
16G	Chloride	mg Cl/ℓ	3.64	4.25
2A	Electrical Conductivity at 25°C	mS/m	4.0	2.3
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	21	14

Methods	Determinands	Units	009076/22 PALALA RIVER: PALALA 1 LB 06:10 31.03.2022	009077/22 PALALA RIVER: PALALA 2 C 07:51 31.03.2022
Chemical				
93	Dissolved Potassium*	mg K/ℓ	<0.5	<0.5
93	Dissolved Magnesium*	mg Mg/ℓ	0.4	0.8
93	Sodium*	mg Na/ℓ	2.4	<2
91	Total Phosphorus*	mg/ℓ	<0.06	<0.06
16G	Chloride	mg Cl/ℓ	4.21	5.13
2A	Electrical Conductivity at 25°C	mS/m	2.2	2.8
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	16	17

Methods	Determinands	Units	009078/22 PALALA RIVER: PALALA 2 RB 07:53 31.03.2022	009079/22 PALALA RIVER: PALALA 2 LB 07:54 31.03.2022
Chemical				
93	Dissolved Potassium*	mg K/ℓ	<0.5	<0.5
93	Dissolved Magnesium*	mg Mg/ℓ	0.8	0.8
93	Sodium*	mg Na/ℓ	<2	<2
91	Total Phosphorus*	mg/ℓ	<0.06	<0.06

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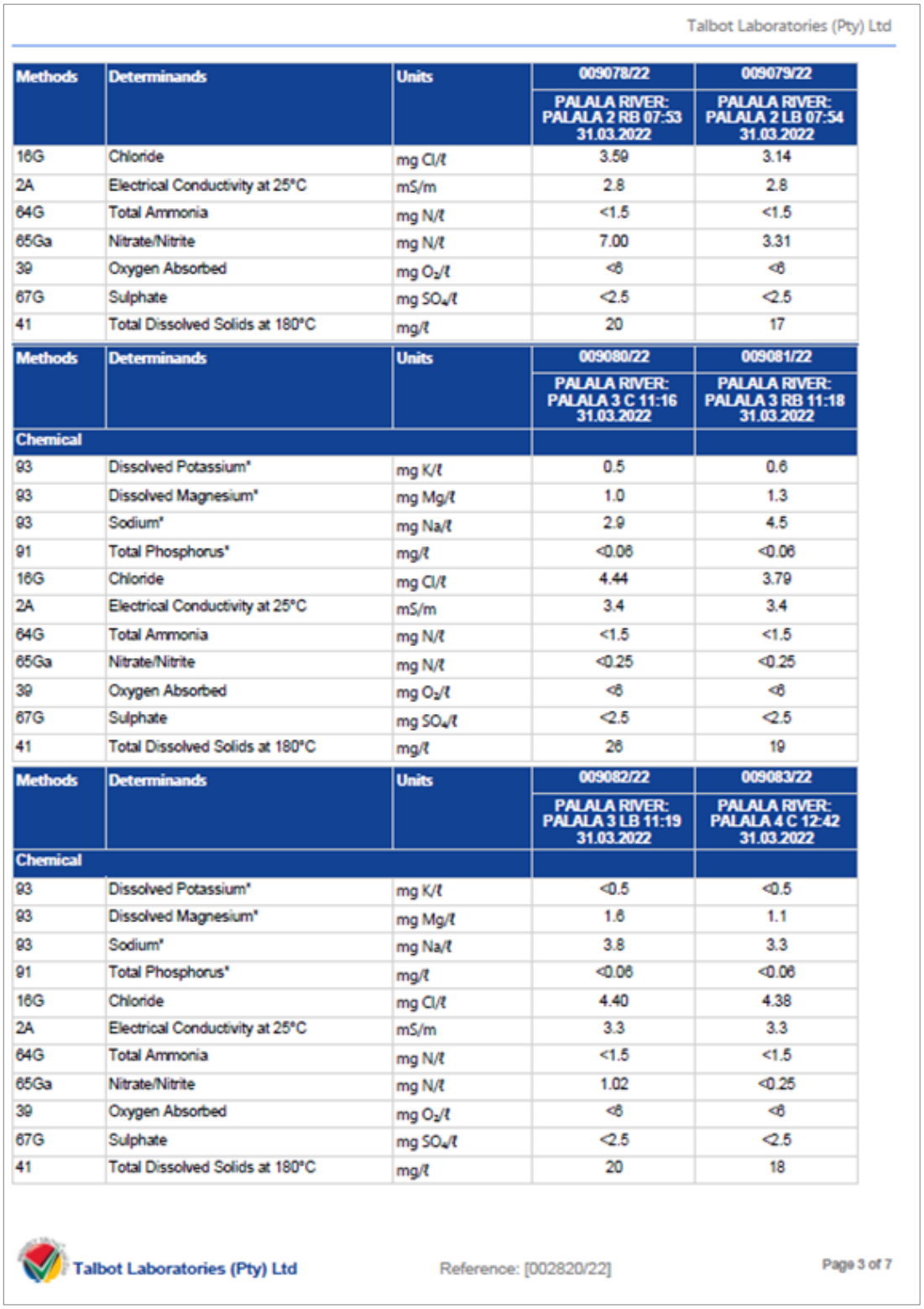
Figure C.2b: Second sampling period certificate of water analysis from Talbot lab, second page



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Methods	Determinands	Units	009078/22	009079/22
			PALALA RIVER: PALALA 2 RB 07:53 31.03.2022	PALALA RIVER: PALALA 2 LB 07:54 31.03.2022
16G	Chloride	mg Cl/ℓ	3.59	3.14
2A	Electrical Conductivity at 25°C	mS/m	2.8	2.8
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	7.00	3.31
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	20	17

Methods	Determinands	Units	009080/22	009081/22
			PALALA RIVER: PALALA 3 C 11:16 31.03.2022	PALALA RIVER: PALALA 3 RB 11:18 31.03.2022
Chemical				
93	Dissolved Potassium*	mg K/ℓ	0.5	0.6
93	Dissolved Magnesium*	mg Mg/ℓ	1.0	1.3
93	Sodium*	mg Na/ℓ	2.9	4.5
91	Total Phosphorus*	mg/ℓ	<0.06	<0.06
16G	Chloride	mg Cl/ℓ	4.44	3.79
2A	Electrical Conductivity at 25°C	mS/m	3.4	3.4
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	26	19

Methods	Determinands	Units	009082/22	009083/22
			PALALA RIVER: PALALA 3 LB 11:19 31.03.2022	PALALA RIVER: PALALA 4 C 12:42 31.03.2022
Chemical				
93	Dissolved Potassium*	mg K/ℓ	<0.5	<0.5
93	Dissolved Magnesium*	mg Mg/ℓ	1.6	1.1
93	Sodium*	mg Na/ℓ	3.8	3.3
91	Total Phosphorus*	mg/ℓ	<0.06	<0.06
16G	Chloride	mg Cl/ℓ	4.40	4.38
2A	Electrical Conductivity at 25°C	mS/m	3.3	3.3
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	1.02	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	20	18

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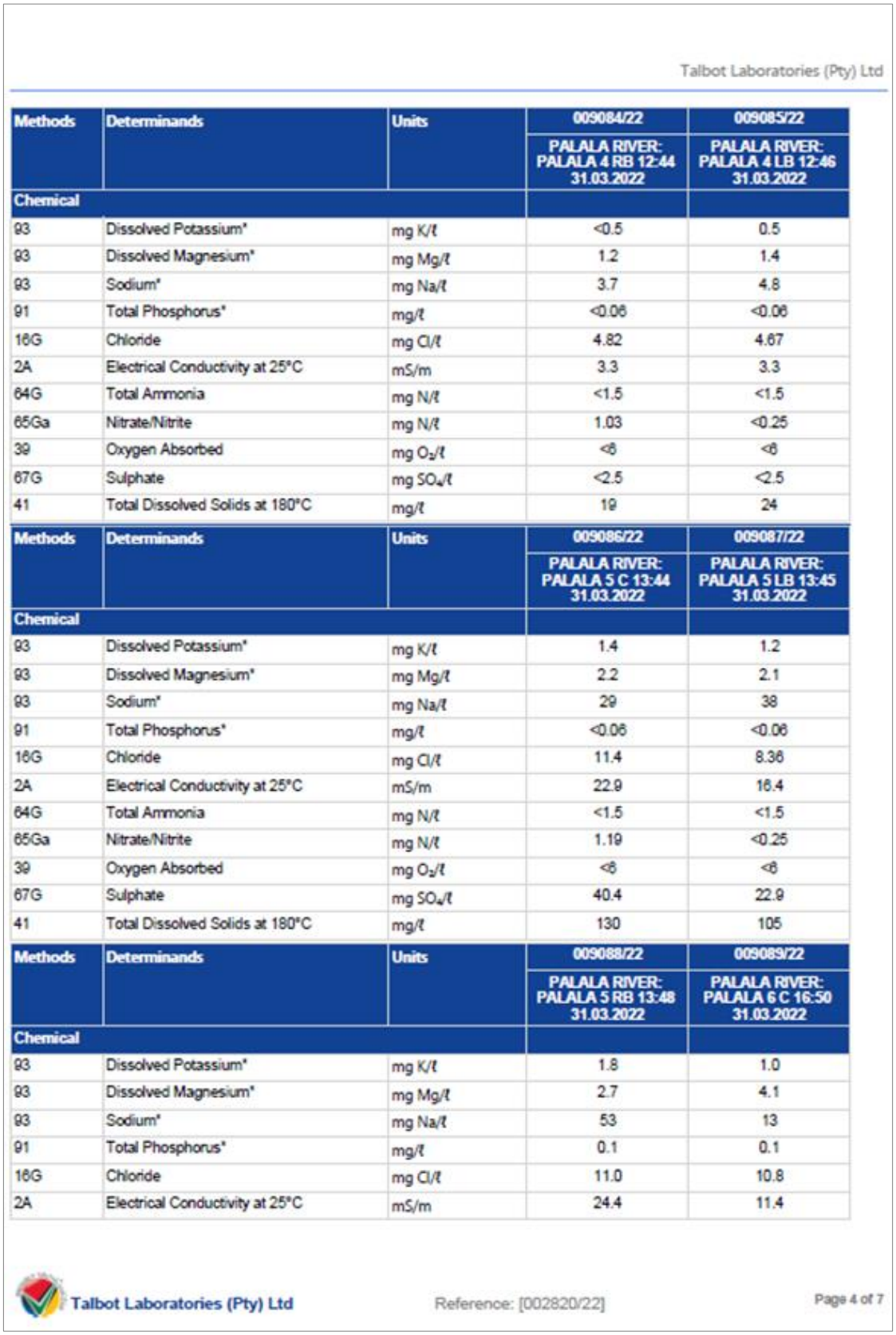
Figure C.2c: Second sampling period certificate of water analysis from Talbot lab, third page



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Methods	Determinands	Units	009084/22	009085/22
			PALALA RIVER: PALALA 4 RB 12:44 31.03.2022	PALALA RIVER: PALALA 4 LB 12:46 31.03.2022
Chemical				
93	Dissolved Potassium*	mg K/ℓ	<0.5	0.5
93	Dissolved Magnesium*	mg Mg/ℓ	1.2	1.4
93	Sodium*	mg Na/ℓ	3.7	4.8
91	Total Phosphorus*	mg/ℓ	<0.06	<0.06
16G	Chloride	mg Cl/ℓ	4.82	4.67
2A	Electrical Conductivity at 25°C	mS/m	3.3	3.3
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	1.03	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	19	24

Methods	Determinands	Units	009086/22	009087/22
			PALALA RIVER: PALALA 5 C 13:44 31.03.2022	PALALA RIVER: PALALA 5 LB 13:45 31.03.2022
Chemical				
93	Dissolved Potassium*	mg K/ℓ	1.4	1.2
93	Dissolved Magnesium*	mg Mg/ℓ	2.2	2.1
93	Sodium*	mg Na/ℓ	29	38
91	Total Phosphorus*	mg/ℓ	<0.06	<0.06
16G	Chloride	mg Cl/ℓ	11.4	8.36
2A	Electrical Conductivity at 25°C	mS/m	22.9	16.4
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	1.19	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	40.4	22.9
41	Total Dissolved Solids at 180°C	mg/ℓ	130	105

Methods	Determinands	Units	009088/22	009089/22
			PALALA RIVER: PALALA 5 RB 13:48 31.03.2022	PALALA RIVER: PALALA 6 C 16:50 31.03.2022
Chemical				
93	Dissolved Potassium*	mg K/ℓ	1.8	1.0
93	Dissolved Magnesium*	mg Mg/ℓ	2.7	4.1
93	Sodium*	mg Na/ℓ	53	13
91	Total Phosphorus*	mg/ℓ	0.1	0.1
16G	Chloride	mg Cl/ℓ	11.0	10.8
2A	Electrical Conductivity at 25°C	mS/m	24.4	11.4

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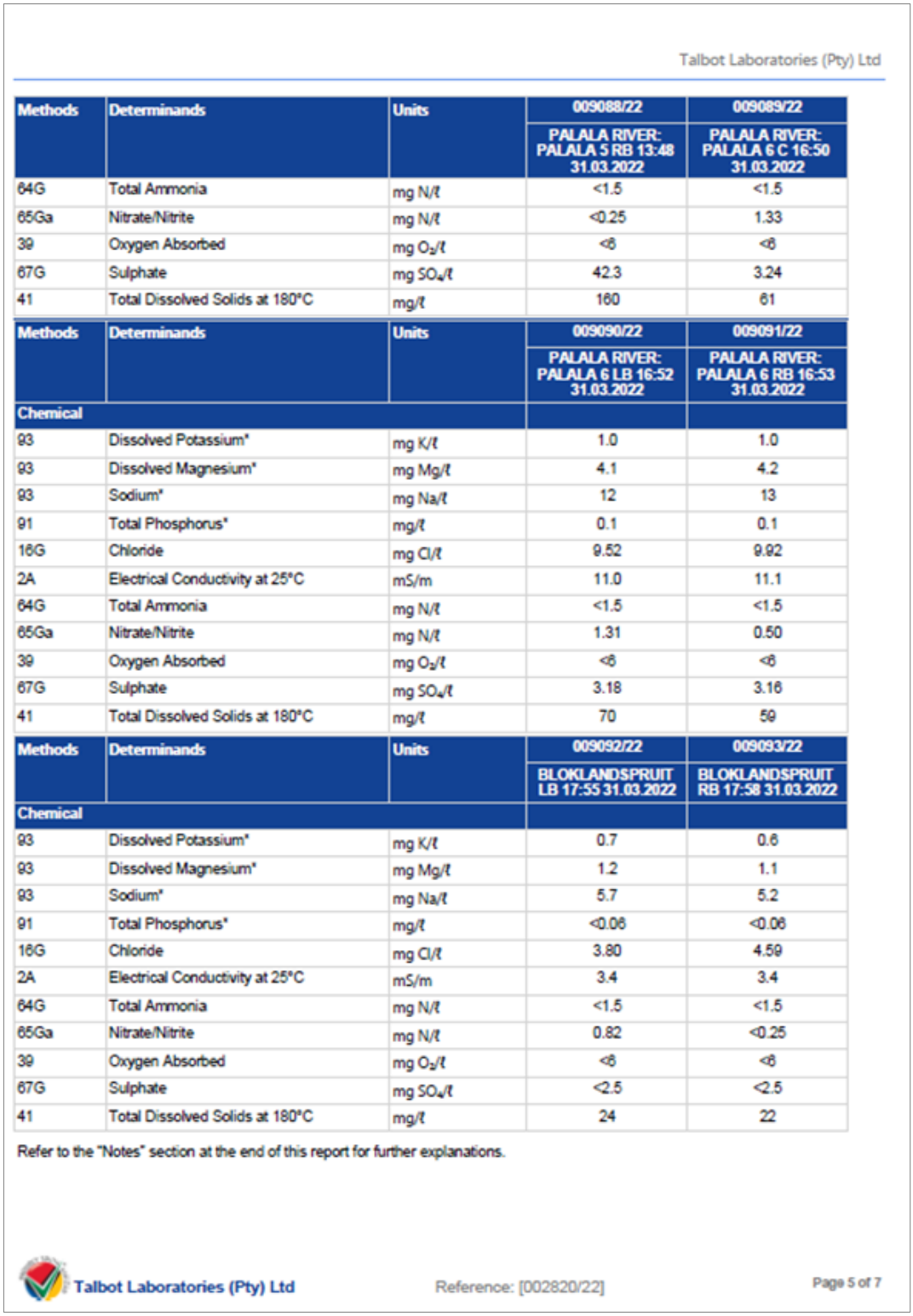
Figure C.2d: Second sampling period certificate of water analysis from Talbot lab, fourth page



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Methods	Determinands	Units	009088/22 PALALA RIVER: PALALA 5 RB 13:48 31.03.2022	009089/22 PALALA RIVER: PALALA 6 C 16:50 31.03.2022
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	1.33
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	42.3	3.24
41	Total Dissolved Solids at 180°C	mg/ℓ	160	61

Methods	Determinands	Units	009090/22 PALALA RIVER: PALALA 6 LB 16:52 31.03.2022	009091/22 PALALA RIVER: PALALA 6 RB 16:53 31.03.2022
Chemical				
93	Dissolved Potassium*	mg K/ℓ	1.0	1.0
93	Dissolved Magnesium*	mg Mg/ℓ	4.1	4.2
93	Sodium*	mg Na/ℓ	12	13
91	Total Phosphorus*	mg/ℓ	0.1	0.1
16G	Chloride	mg Cl/ℓ	9.52	9.92
2A	Electrical Conductivity at 25°C	mS/m	11.0	11.1
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	1.31	0.50
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	3.18	3.16
41	Total Dissolved Solids at 180°C	mg/ℓ	70	59

Methods	Determinands	Units	009092/22 BLOKLANDSPRUIT LB 17:55 31.03.2022	009093/22 BLOKLANDSPRUIT RB 17:58 31.03.2022
Chemical				
93	Dissolved Potassium*	mg K/ℓ	0.7	0.6
93	Dissolved Magnesium*	mg Mg/ℓ	1.2	1.1
93	Sodium*	mg Na/ℓ	5.7	5.2
91	Total Phosphorus*	mg/ℓ	<0.06	<0.06
16G	Chloride	mg Cl/ℓ	3.80	4.59
2A	Electrical Conductivity at 25°C	mS/m	3.4	3.4
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	0.82	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	24	22

Refer to the "Notes" section at the end of this report for further explanations.

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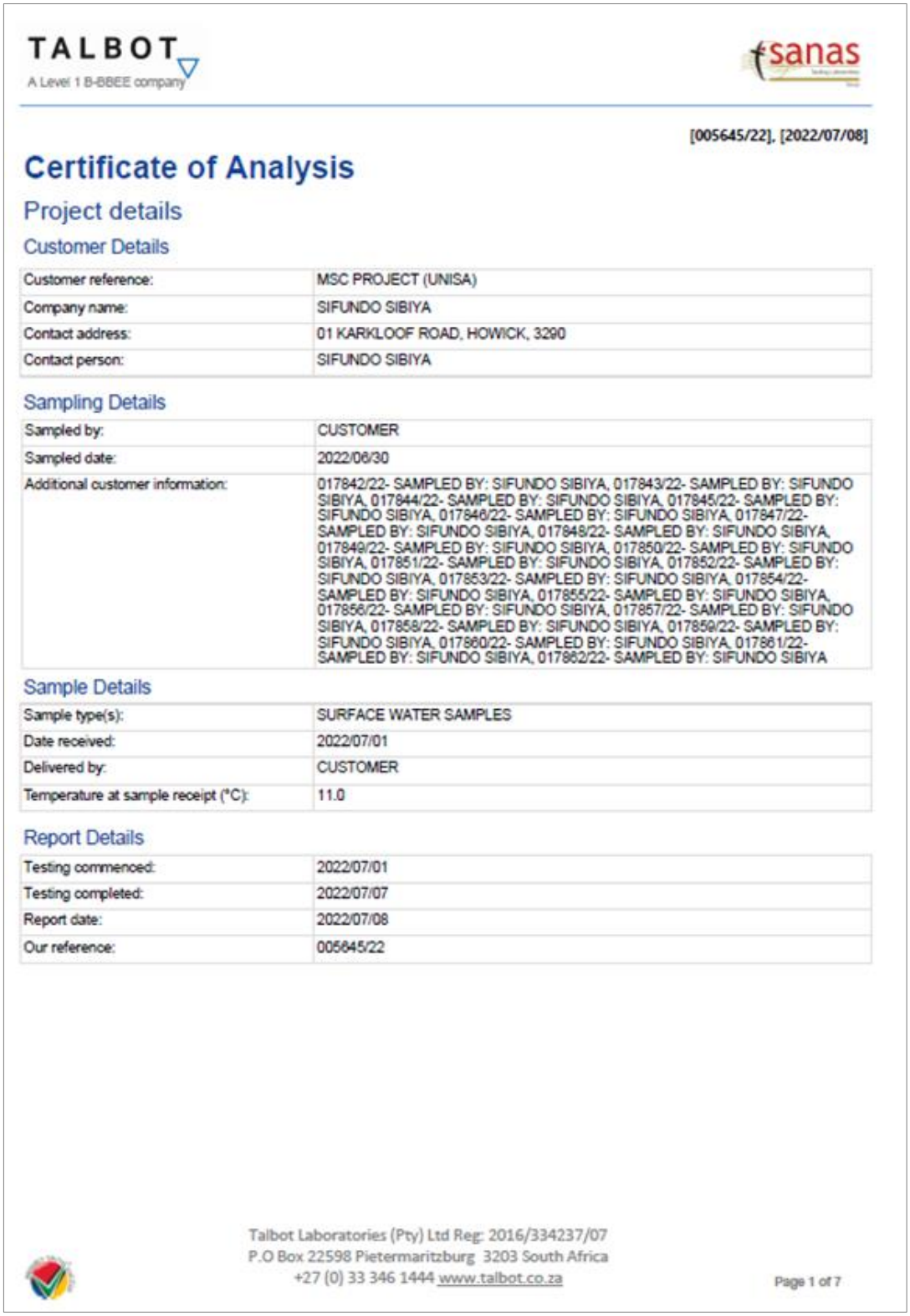
Figure C.2e: Second sampling period certificate of water analysis from Talbot lab, fifth page



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[005645/22], [2022/07/08]

Certificate of Analysis

Project details

Customer Details

Customer reference:	MSC PROJECT (UNISA)
Company name:	SIFUNDO SIBIYA
Contact address:	01 KARKLOOF ROAD, HOWICK, 3290
Contact person:	SIFUNDO SIBIYA

Sampling Details

Sampled by:	CUSTOMER
Sampled date:	2022/06/30
Additional customer information:	017842/22- SAMPLED BY: SIFUNDO SIBIYA, 017843/22- SAMPLED BY: SIFUNDO SIBIYA, 017844/22- SAMPLED BY: SIFUNDO SIBIYA, 017845/22- SAMPLED BY: SIFUNDO SIBIYA, 017846/22- SAMPLED BY: SIFUNDO SIBIYA, 017847/22- SAMPLED BY: SIFUNDO SIBIYA, 017848/22- SAMPLED BY: SIFUNDO SIBIYA, 017849/22- SAMPLED BY: SIFUNDO SIBIYA, 017850/22- SAMPLED BY: SIFUNDO SIBIYA, 017851/22- SAMPLED BY: SIFUNDO SIBIYA, 017852/22- SAMPLED BY: SIFUNDO SIBIYA, 017853/22- SAMPLED BY: SIFUNDO SIBIYA, 017854/22- SAMPLED BY: SIFUNDO SIBIYA, 017855/22- SAMPLED BY: SIFUNDO SIBIYA, 017856/22- SAMPLED BY: SIFUNDO SIBIYA, 017857/22- SAMPLED BY: SIFUNDO SIBIYA, 017858/22- SAMPLED BY: SIFUNDO SIBIYA, 017859/22- SAMPLED BY: SIFUNDO SIBIYA, 017860/22- SAMPLED BY: SIFUNDO SIBIYA, 017861/22- SAMPLED BY: SIFUNDO SIBIYA, 017862/22- SAMPLED BY: SIFUNDO SIBIYA

Sample Details

Sample type(s):	SURFACE WATER SAMPLES
Date received:	2022/07/01
Delivered by:	CUSTOMER
Temperature at sample receipt (°C):	11.0

Report Details

Testing commenced:	2022/07/01
Testing completed:	2022/07/07
Report date:	2022/07/08
Our reference:	005645/22

Talbot Laboratories (Pty) Ltd Reg: 2016/334237/07
P.O Box 22598 Pietermaritzburg 3203 South Africa
+27 (0) 33 346 1444 www.talbot.co.za

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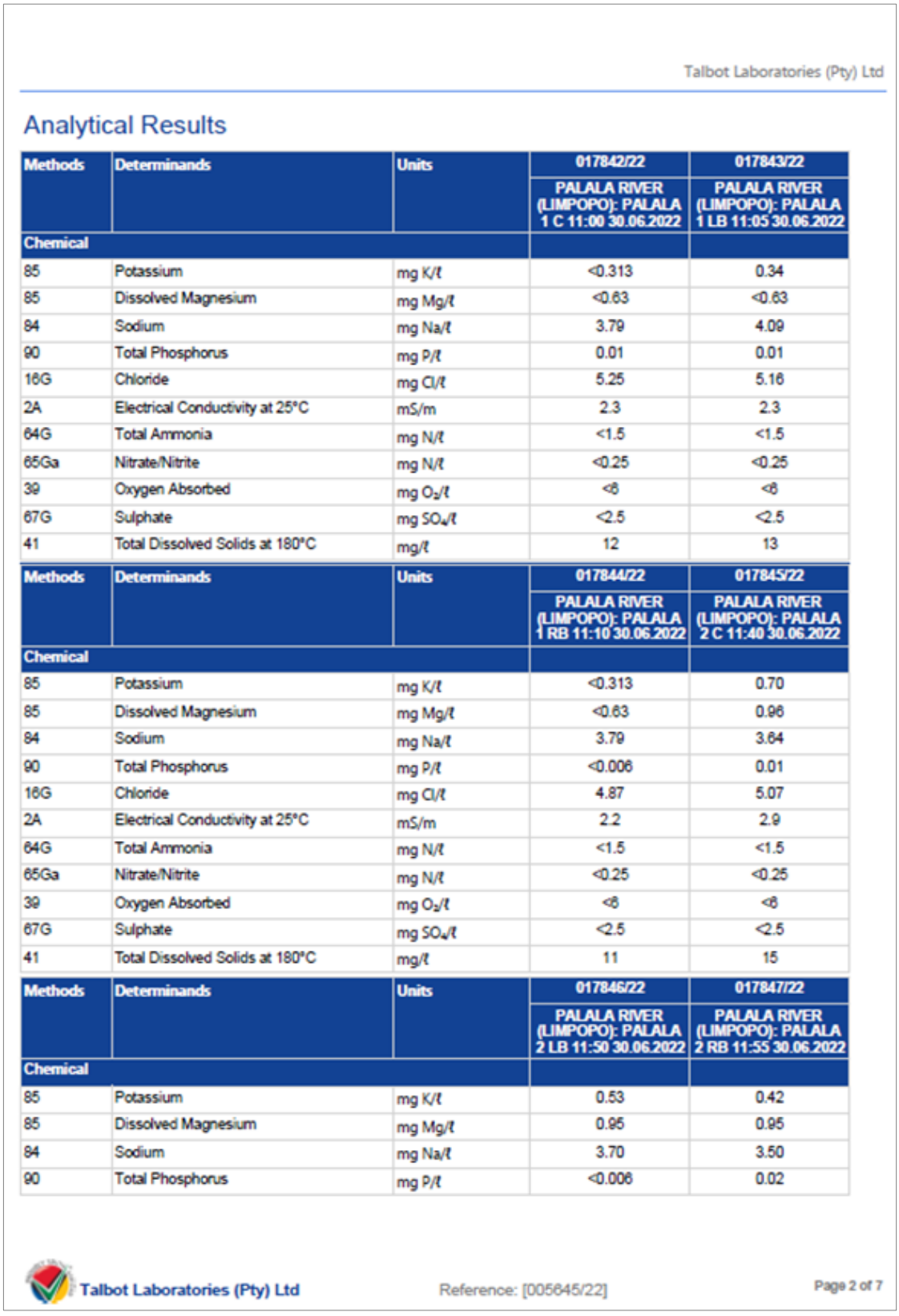
Figure C.3a: Third sampling period certificate of water analysis from Talbot lab, first page



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Analytical Results

Methods	Determinands	Units	017842/22	017843/22
			PALALA RIVER (LIMPOPO): PALALA 1 C 11:00 30.06.2022	PALALA RIVER (LIMPOPO): PALALA 1 LB 11:05 30.06.2022
Chemical				
85	Potassium	mg K/ℓ	<0.313	0.34
85	Dissolved Magnesium	mg Mg/ℓ	<0.63	<0.63
84	Sodium	mg Na/ℓ	3.79	4.09
90	Total Phosphorus	mg P/ℓ	0.01	0.01
16G	Chloride	mg Cl/ℓ	5.25	5.16
2A	Electrical Conductivity at 25°C	mS/m	2.3	2.3
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	12	13

Methods	Determinands	Units	017844/22	017845/22
			PALALA RIVER (LIMPOPO): PALALA 1 RB 11:10 30.06.2022	PALALA RIVER (LIMPOPO): PALALA 2 C 11:40 30.06.2022
Chemical				
85	Potassium	mg K/ℓ	<0.313	0.70
85	Dissolved Magnesium	mg Mg/ℓ	<0.63	0.96
84	Sodium	mg Na/ℓ	3.79	3.64
90	Total Phosphorus	mg P/ℓ	<0.006	0.01
16G	Chloride	mg Cl/ℓ	4.87	5.07
2A	Electrical Conductivity at 25°C	mS/m	2.2	2.9
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	11	15

Methods	Determinands	Units	017846/22	017847/22
			PALALA RIVER (LIMPOPO): PALALA 2 LB 11:50 30.06.2022	PALALA RIVER (LIMPOPO): PALALA 2 RB 11:55 30.06.2022
Chemical				
85	Potassium	mg K/ℓ	0.53	0.42
85	Dissolved Magnesium	mg Mg/ℓ	0.95	0.95
84	Sodium	mg Na/ℓ	3.70	3.50
90	Total Phosphorus	mg P/ℓ	<0.006	0.02

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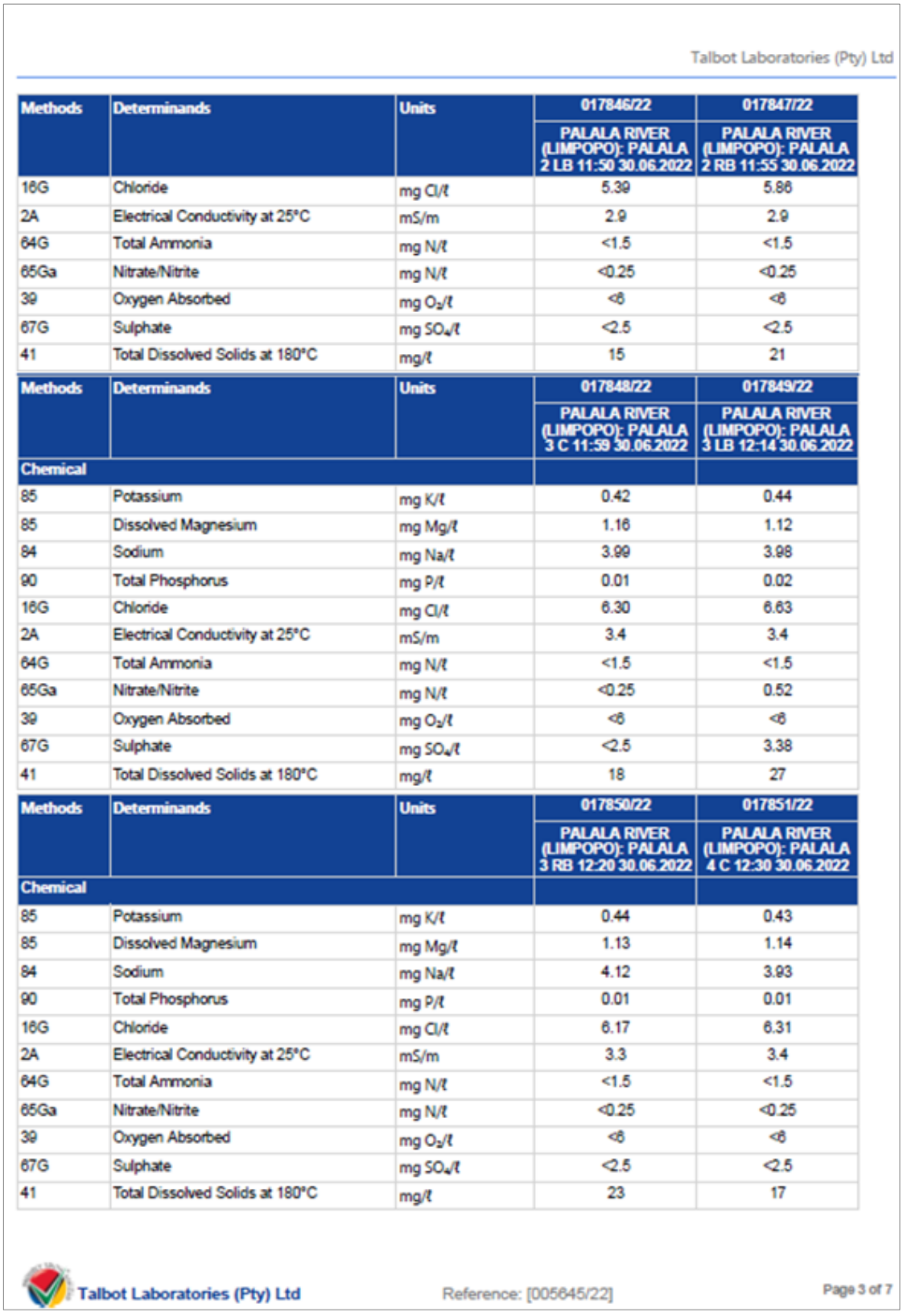
Figure C.3b: Third sampling period certificate of water analysis from Talbot lab, second page



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Methods	Determinands	Units	017846/22	017847/22
			PALALA RIVER (LIMPOPO): PALALA 2 LB 11:50 30.06.2022	PALALA RIVER (LIMPOPO): PALALA 2 RB 11:55 30.06.2022
16G	Chloride	mg Cl/ℓ	5.39	5.86
2A	Electrical Conductivity at 25°C	mS/m	2.9	2.9
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	15	21

Methods	Determinands	Units	017848/22	017849/22
			PALALA RIVER (LIMPOPO): PALALA 3 C 11:59 30.06.2022	PALALA RIVER (LIMPOPO): PALALA 3 LB 12:14 30.06.2022
Chemical				
85	Potassium	mg K/ℓ	0.42	0.44
85	Dissolved Magnesium	mg Mg/ℓ	1.16	1.12
84	Sodium	mg Na/ℓ	3.99	3.98
90	Total Phosphorus	mg P/ℓ	0.01	0.02
16G	Chloride	mg Cl/ℓ	6.30	6.63
2A	Electrical Conductivity at 25°C	mS/m	3.4	3.4
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	0.52
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	3.38
41	Total Dissolved Solids at 180°C	mg/ℓ	18	27

Methods	Determinands	Units	017850/22	017851/22
			PALALA RIVER (LIMPOPO): PALALA 3 RB 12:20 30.06.2022	PALALA RIVER (LIMPOPO): PALALA 4 C 12:30 30.06.2022
Chemical				
85	Potassium	mg K/ℓ	0.44	0.43
85	Dissolved Magnesium	mg Mg/ℓ	1.13	1.14
84	Sodium	mg Na/ℓ	4.12	3.93
90	Total Phosphorus	mg P/ℓ	0.01	0.01
16G	Chloride	mg Cl/ℓ	6.17	6.31
2A	Electrical Conductivity at 25°C	mS/m	3.3	3.4
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	23	17

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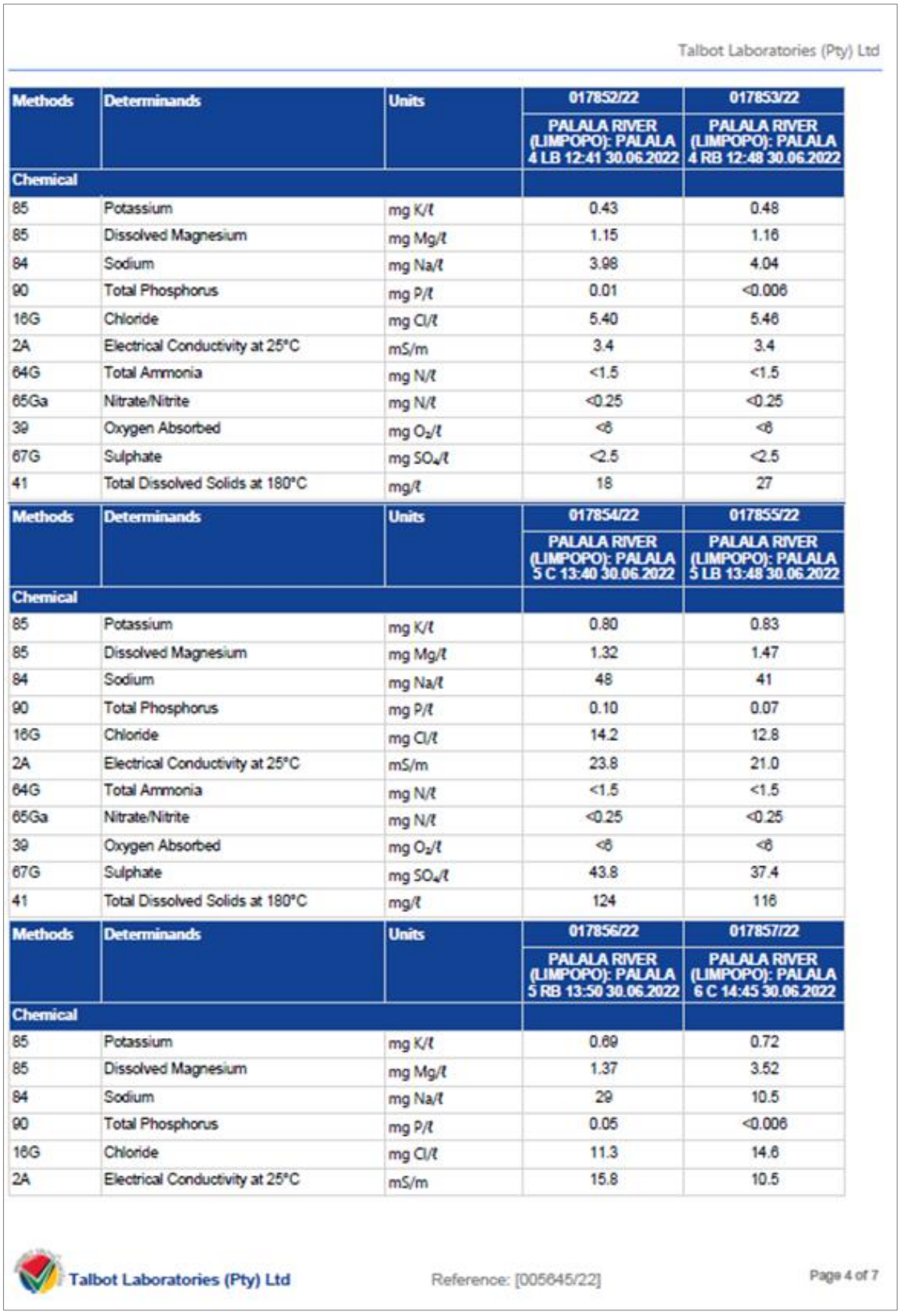
Figure C.3c: Third sampling period certificate of water analysis from Talbot lab, third page



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Methods	Determinands	Units	017852/22	017853/22
			PALALA RIVER (LIMPOPO): PALALA 4 LB 12:41 30.06.2022	PALALA RIVER (LIMPOPO): PALALA 4 RB 12:48 30.06.2022
Chemical				
85	Potassium	mg K/ℓ	0.43	0.48
85	Dissolved Magnesium	mg Mg/ℓ	1.15	1.16
84	Sodium	mg Na/ℓ	3.98	4.04
90	Total Phosphorus	mg P/ℓ	0.01	<0.006
16G	Chloride	mg Cl/ℓ	5.40	5.46
2A	Electrical Conductivity at 25°C	mS/m	3.4	3.4
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	18	27

Methods	Determinands	Units	017854/22	017855/22
			PALALA RIVER (LIMPOPO): PALALA 5 C 13:40 30.06.2022	PALALA RIVER (LIMPOPO): PALALA 5 LB 13:48 30.06.2022
Chemical				
85	Potassium	mg K/ℓ	0.80	0.83
85	Dissolved Magnesium	mg Mg/ℓ	1.32	1.47
84	Sodium	mg Na/ℓ	48	41
90	Total Phosphorus	mg P/ℓ	0.10	0.07
16G	Chloride	mg Cl/ℓ	14.2	12.8
2A	Electrical Conductivity at 25°C	mS/m	23.8	21.0
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	43.8	37.4
41	Total Dissolved Solids at 180°C	mg/ℓ	124	116

Methods	Determinands	Units	017856/22	017857/22
			PALALA RIVER (LIMPOPO): PALALA 5 RB 13:50 30.06.2022	PALALA RIVER (LIMPOPO): PALALA 6 C 14:45 30.06.2022
Chemical				
85	Potassium	mg K/ℓ	0.69	0.72
85	Dissolved Magnesium	mg Mg/ℓ	1.37	3.52
84	Sodium	mg Na/ℓ	29	10.5
90	Total Phosphorus	mg P/ℓ	0.05	<0.006
16G	Chloride	mg Cl/ℓ	11.3	14.6
2A	Electrical Conductivity at 25°C	mS/m	15.8	10.5

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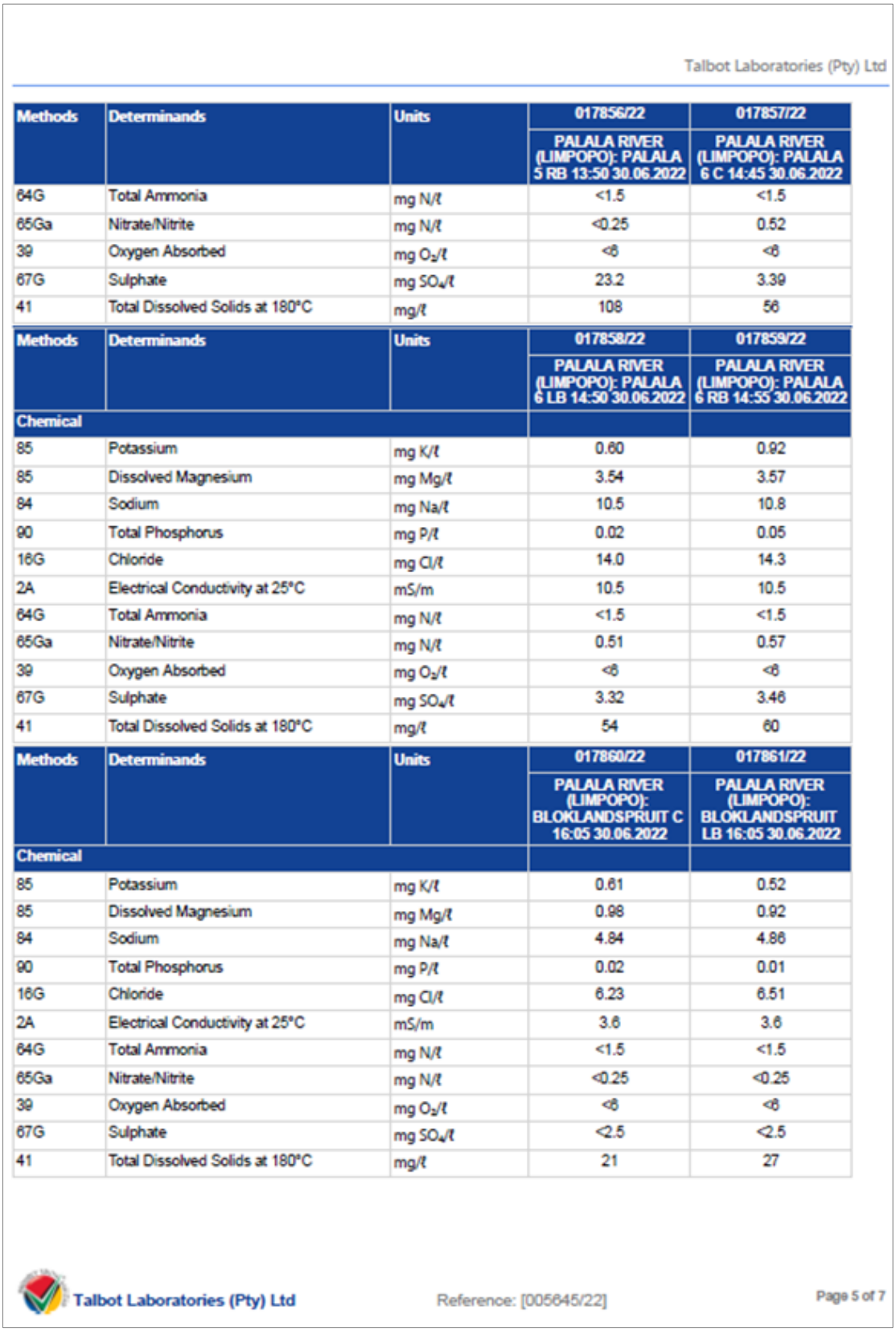
Figure C.3d: Third sampling period certificate of water analysis from Talbot lab, fourth page



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Methods	Determinands	Units	017856/22	017857/22
			PALALA RIVER (LIMPOPO): PALALA 5 RB 13:50 30.06.2022	PALALA RIVER (LIMPOPO): PALALA 6 C 14:45 30.06.2022
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	0.52
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	23.2	3.39
41	Total Dissolved Solids at 180°C	mg/ℓ	108	56

Methods	Determinands	Units	017858/22	017859/22
			PALALA RIVER (LIMPOPO): PALALA 6 LB 14:50 30.06.2022	PALALA RIVER (LIMPOPO): PALALA 6 RB 14:55 30.06.2022
Chemical				
85	Potassium	mg K/ℓ	0.60	0.92
85	Dissolved Magnesium	mg Mg/ℓ	3.54	3.57
84	Sodium	mg Na/ℓ	10.5	10.8
90	Total Phosphorus	mg P/ℓ	0.02	0.05
16G	Chloride	mg Cl/ℓ	14.0	14.3
2A	Electrical Conductivity at 25°C	mS/m	10.5	10.5
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	0.51	0.57
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	3.32	3.46
41	Total Dissolved Solids at 180°C	mg/ℓ	54	60

Methods	Determinands	Units	017860/22	017861/22
			PALALA RIVER (LIMPOPO): BLOKLANDSPRUIT C 16:05 30.06.2022	PALALA RIVER (LIMPOPO): BLOKLANDSPRUIT LB 16:05 30.06.2022
Chemical				
85	Potassium	mg K/ℓ	0.61	0.52
85	Dissolved Magnesium	mg Mg/ℓ	0.98	0.92
84	Sodium	mg Na/ℓ	4.84	4.86
90	Total Phosphorus	mg P/ℓ	0.02	0.01
16G	Chloride	mg Cl/ℓ	6.23	6.51
2A	Electrical Conductivity at 25°C	mS/m	3.6	3.6
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	21	27

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Figure C.3e: Third sampling period certificate of water analysis from Talbot lab, fifth page

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Methods	Determinands	Units	017862/22
			PALALA RIVER (LIMPOPO): BLOKLAND$PRUIT RB 16:20 30.06.2022
Chemical			
85	Potassium	mg K/ℓ	0.53
85	Dissolved Magnesium	mg Mg/ℓ	0.93
84	Sodium	mg Na/ℓ	4.72
90	Total Phosphorus	mg P/ℓ	0.01
16G	Chloride	mg Cl/ℓ	6.56
2A	Electrical Conductivity at 25°C	mS/m	3.6
64G	Total Ammonia	mg N/ℓ	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6
67G	Sulphate	mg SO_4/ℓ	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	25

Refer to the "Notes" section at the end of this report for further explanations.

Specific Observations

None

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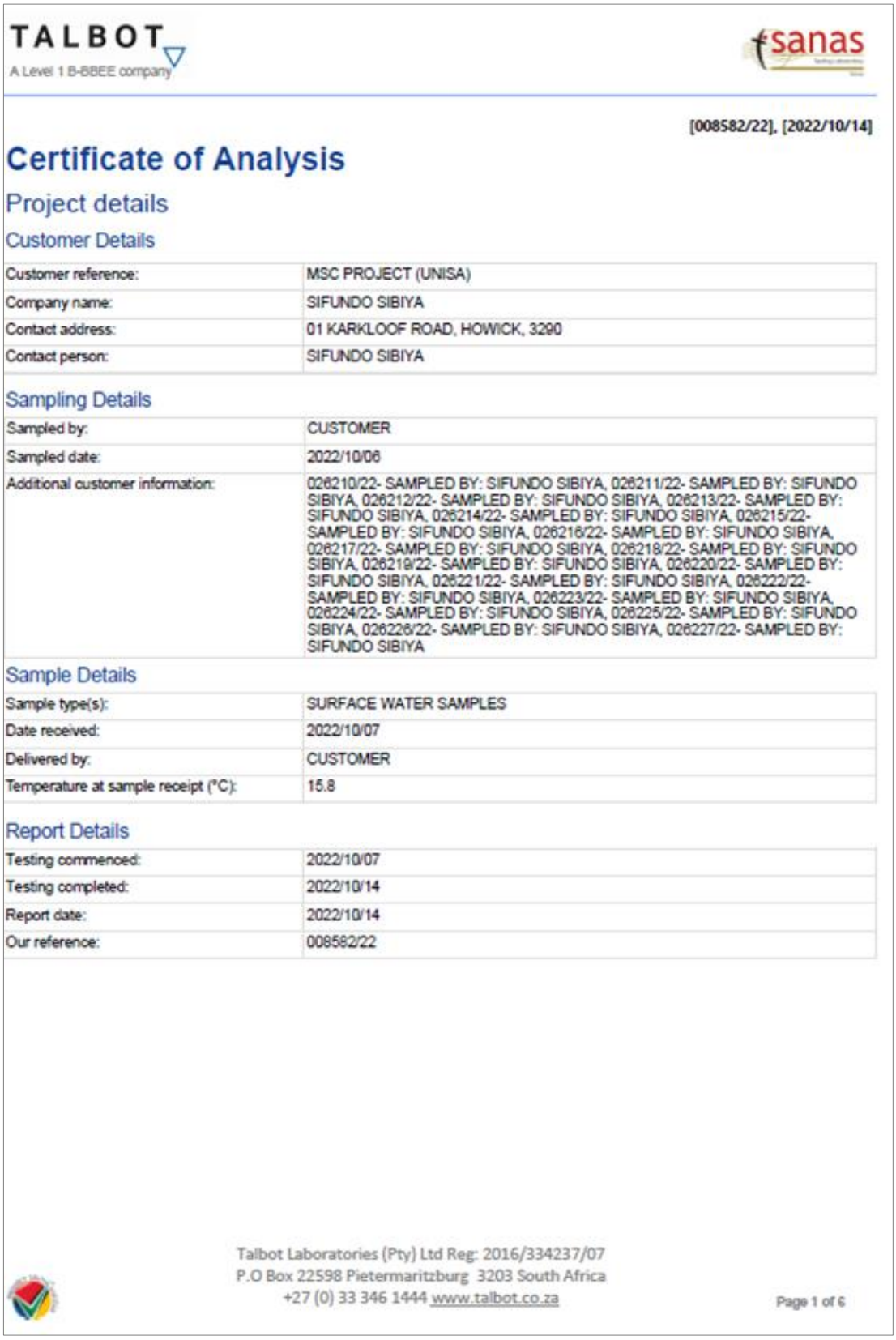
Figure C.3d: Third sampling period certificate of water analysis from Talbot lab, sixth page



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[008582/22], [2022/10/14]

Certificate of Analysis

Project details

Customer Details

Customer reference:	MSC PROJECT (UNISA)
Company name:	SIFUNDO SIBIYA
Contact address:	01 KARKLOOF ROAD, HOWICK, 3290
Contact person:	SIFUNDO SIBIYA

Sampling Details

Sampled by:	CUSTOMER
Sampled date:	2022/10/06
Additional customer information:	026210/22- SAMPLED BY: SIFUNDO SIBIYA, 026211/22- SAMPLED BY: SIFUNDO SIBIYA, 026212/22- SAMPLED BY: SIFUNDO SIBIYA, 026213/22- SAMPLED BY: SIFUNDO SIBIYA, 026214/22- SAMPLED BY: SIFUNDO SIBIYA, 026215/22- SAMPLED BY: SIFUNDO SIBIYA, 026216/22- SAMPLED BY: SIFUNDO SIBIYA, 026217/22- SAMPLED BY: SIFUNDO SIBIYA, 026218/22- SAMPLED BY: SIFUNDO SIBIYA, 026219/22- SAMPLED BY: SIFUNDO SIBIYA, 026220/22- SAMPLED BY: SIFUNDO SIBIYA, 026221/22- SAMPLED BY: SIFUNDO SIBIYA, 026222/22- SAMPLED BY: SIFUNDO SIBIYA, 026223/22- SAMPLED BY: SIFUNDO SIBIYA, 026224/22- SAMPLED BY: SIFUNDO SIBIYA, 026225/22- SAMPLED BY: SIFUNDO SIBIYA, 026226/22- SAMPLED BY: SIFUNDO SIBIYA, 026227/22- SAMPLED BY: SIFUNDO SIBIYA

Sample Details

Sample type(s):	SURFACE WATER SAMPLES
Date received:	2022/10/07
Delivered by:	CUSTOMER
Temperature at sample receipt (°C):	15.8

Report Details

Testing commenced:	2022/10/07
Testing completed:	2022/10/14
Report date:	2022/10/14
Our reference:	008582/22

Talbot Laboratories (Pty) Ltd Reg: 2016/334237/07
P.O Box 22598 Pietermaritzburg 3203 South Africa
+27 (0) 33 346 1444 www.talbot.co.za

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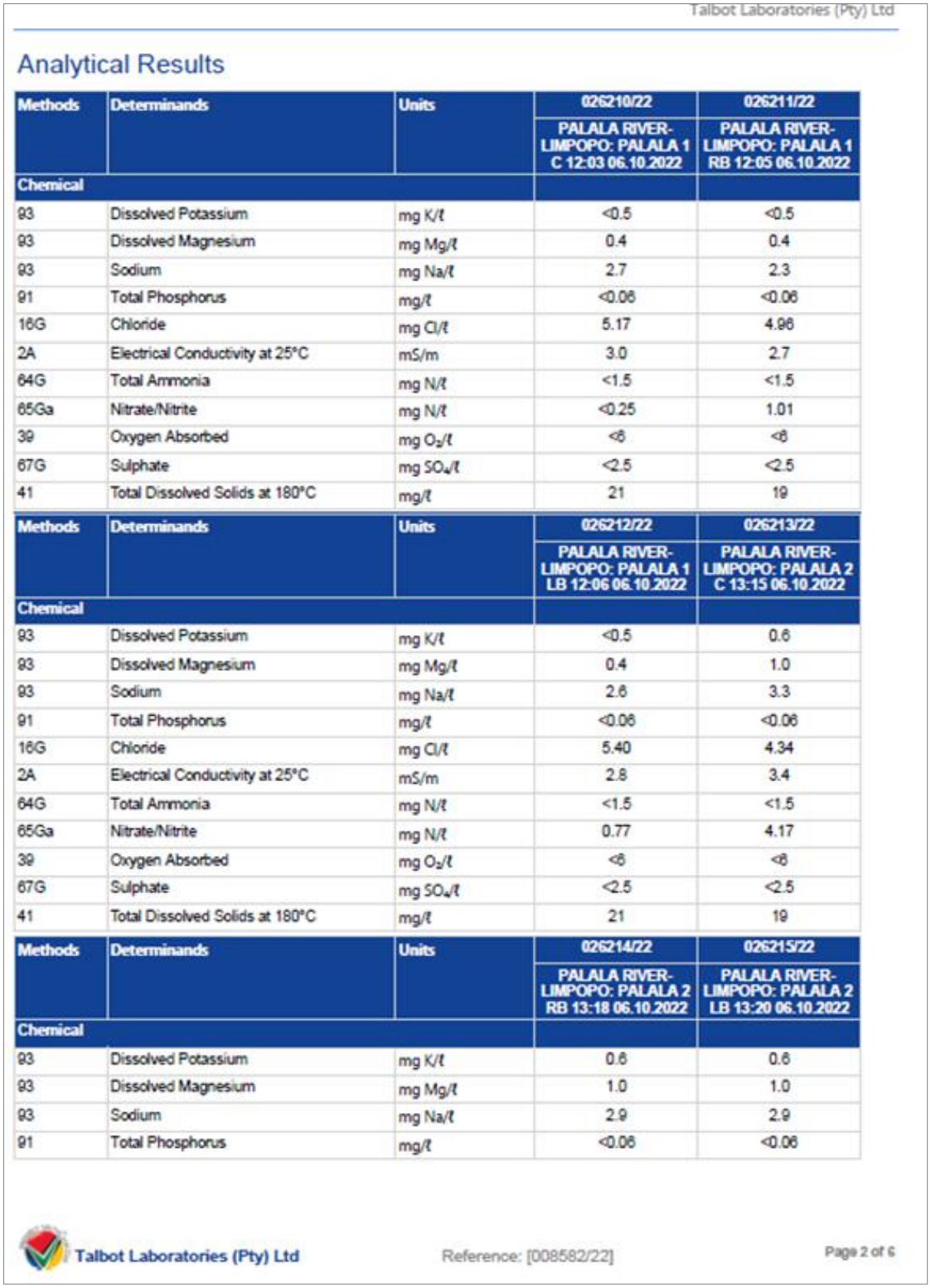
Figure C.4a: Fourth sampling period certificate of water analysis from Talbot lab, first page



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Analytical Results

Methods	Determinands	Units	026210/22	026211/22
			PALALA RIVER-LIMPOPO: PALALA 1 C 12:03 06.10.2022	PALALA RIVER-LIMPOPO: PALALA 1 RB 12:05 06.10.2022
Chemical				
93	Dissolved Potassium	mg K/ℓ	<0.5	<0.5
93	Dissolved Magnesium	mg Mg/ℓ	0.4	0.4
93	Sodium	mg Na/ℓ	2.7	2.3
91	Total Phosphorus	mg/ℓ	<0.06	<0.06
16G	Chloride	mg Cl/ℓ	5.17	4.96
2A	Electrical Conductivity at 25°C	mS/m	3.0	2.7
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	1.01
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	21	19

Methods	Determinands	Units	026212/22	026213/22
			PALALA RIVER-LIMPOPO: PALALA 1 LB 12:06 06.10.2022	PALALA RIVER-LIMPOPO: PALALA 2 C 13:15 06.10.2022
Chemical				
93	Dissolved Potassium	mg K/ℓ	<0.5	0.6
93	Dissolved Magnesium	mg Mg/ℓ	0.4	1.0
93	Sodium	mg Na/ℓ	2.6	3.3
91	Total Phosphorus	mg/ℓ	<0.06	<0.06
16G	Chloride	mg Cl/ℓ	5.40	4.34
2A	Electrical Conductivity at 25°C	mS/m	2.8	3.4
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	0.77	4.17
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	21	19

Methods	Determinands	Units	026214/22	026215/22
			PALALA RIVER-LIMPOPO: PALALA 2 RB 13:18 06.10.2022	PALALA RIVER-LIMPOPO: PALALA 2 LB 13:20 06.10.2022
Chemical				
93	Dissolved Potassium	mg K/ℓ	0.6	0.6
93	Dissolved Magnesium	mg Mg/ℓ	1.0	1.0
93	Sodium	mg Na/ℓ	2.9	2.9
91	Total Phosphorus	mg/ℓ	<0.06	<0.06

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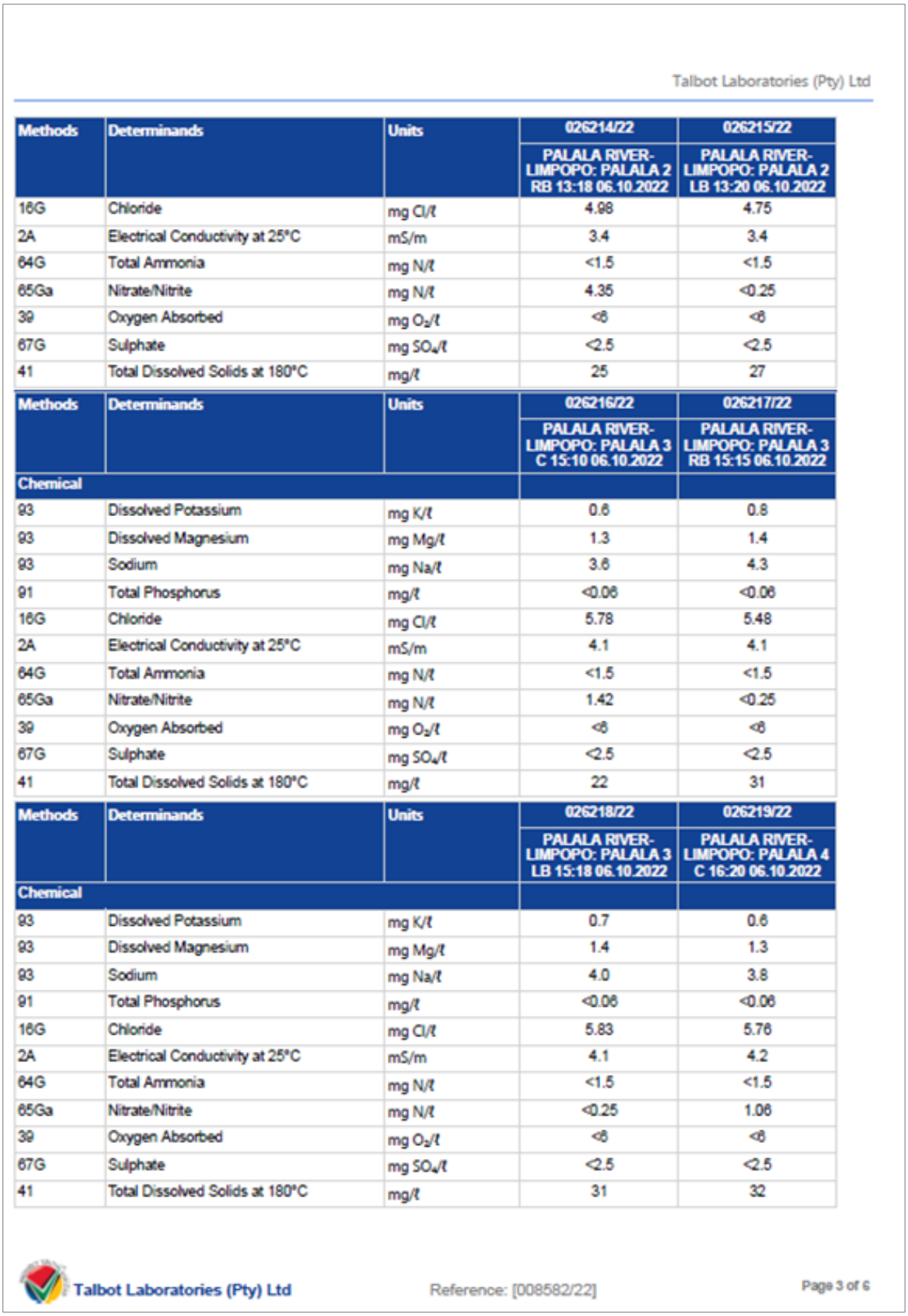
Figure C.4b: Fourth sampling period certificate of water analysis from Talbot lab, second page



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Methods	Determinands	Units	026214/22	026215/22
			PALALA RIVER-LIMPOPO: PALALA 2 RB 13:18 06.10.2022	PALALA RIVER-LIMPOPO: PALALA 2 LB 13:20 06.10.2022
16G	Chloride	mg Cl/ℓ	4.98	4.75
2A	Electrical Conductivity at 25°C	mS/m	3.4	3.4
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	4.35	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	25	27

Methods	Determinands	Units	026216/22	026217/22
			PALALA RIVER-LIMPOPO: PALALA 3 C 15:10 06.10.2022	PALALA RIVER-LIMPOPO: PALALA 3 RB 15:15 06.10.2022
Chemical				
93	Dissolved Potassium	mg K/ℓ	0.6	0.8
93	Dissolved Magnesium	mg Mg/ℓ	1.3	1.4
93	Sodium	mg Na/ℓ	3.6	4.3
91	Total Phosphorus	mg/ℓ	<0.06	<0.06
16G	Chloride	mg Cl/ℓ	5.78	5.48
2A	Electrical Conductivity at 25°C	mS/m	4.1	4.1
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	1.42	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	22	31

Methods	Determinands	Units	026218/22	026219/22
			PALALA RIVER-LIMPOPO: PALALA 3 LB 15:18 06.10.2022	PALALA RIVER-LIMPOPO: PALALA 4 C 16:20 06.10.2022
Chemical				
93	Dissolved Potassium	mg K/ℓ	0.7	0.6
93	Dissolved Magnesium	mg Mg/ℓ	1.4	1.3
93	Sodium	mg Na/ℓ	4.0	3.8
91	Total Phosphorus	mg/ℓ	<0.06	<0.06
16G	Chloride	mg Cl/ℓ	5.83	5.76
2A	Electrical Conductivity at 25°C	mS/m	4.1	4.2
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	1.06
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	31	32

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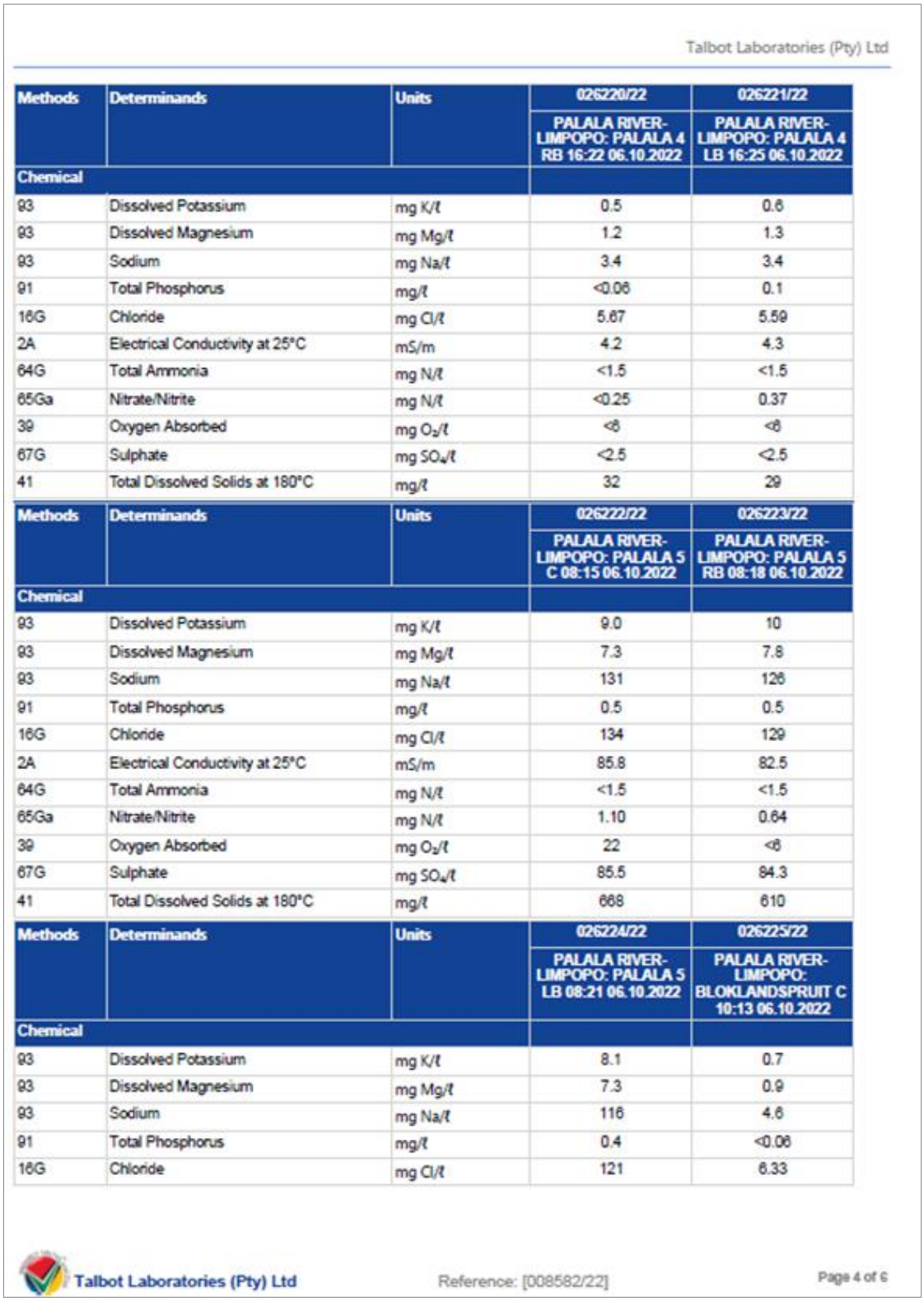
Figure C.4c: Fourth sampling period certificate of water analysis from Talbot lab, third page



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Methods	Determinands	Units	026220/22	026221/22
			PALALA RIVER-LIMPOPO: PALALA 4 RB 16:22 06.10.2022	PALALA RIVER-LIMPOPO: PALALA 4 LB 16:25 06.10.2022
Chemical				
93	Dissolved Potassium	mg K/ℓ	0.5	0.6
93	Dissolved Magnesium	mg Mg/ℓ	1.2	1.3
93	Sodium	mg Na/ℓ	3.4	3.4
91	Total Phosphorus	mg/ℓ	<0.06	0.1
16G	Chloride	mg Cl/ℓ	5.67	5.59
2A	Electrical Conductivity at 25°C	mS/m	4.2	4.3
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	0.37
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	32	29

Methods	Determinands	Units	026222/22	026223/22
			PALALA RIVER-LIMPOPO: PALALA 5 C 08:15 06.10.2022	PALALA RIVER-LIMPOPO: PALALA 5 RB 08:18 06.10.2022
Chemical				
93	Dissolved Potassium	mg K/ℓ	9.0	10
93	Dissolved Magnesium	mg Mg/ℓ	7.3	7.8
93	Sodium	mg Na/ℓ	131	126
91	Total Phosphorus	mg/ℓ	0.5	0.5
16G	Chloride	mg Cl/ℓ	134	129
2A	Electrical Conductivity at 25°C	mS/m	85.8	82.5
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	1.10	0.64
39	Oxygen Absorbed	mg O_2/ℓ	22	<6
67G	Sulphate	mg SO_4/ℓ	85.5	84.3
41	Total Dissolved Solids at 180°C	mg/ℓ	668	610

Methods	Determinands	Units	026224/22	026225/22
			PALALA RIVER-LIMPOPO: PALALA 5 LB 08:21 06.10.2022	PALALA RIVER-LIMPOPO: BLOKLANDSPRUIT C 10:13 06.10.2022
Chemical				
93	Dissolved Potassium	mg K/ℓ	8.1	0.7
93	Dissolved Magnesium	mg Mg/ℓ	7.3	0.9
93	Sodium	mg Na/ℓ	116	4.6
91	Total Phosphorus	mg/ℓ	0.4	<0.06
16G	Chloride	mg Cl/ℓ	121	6.33

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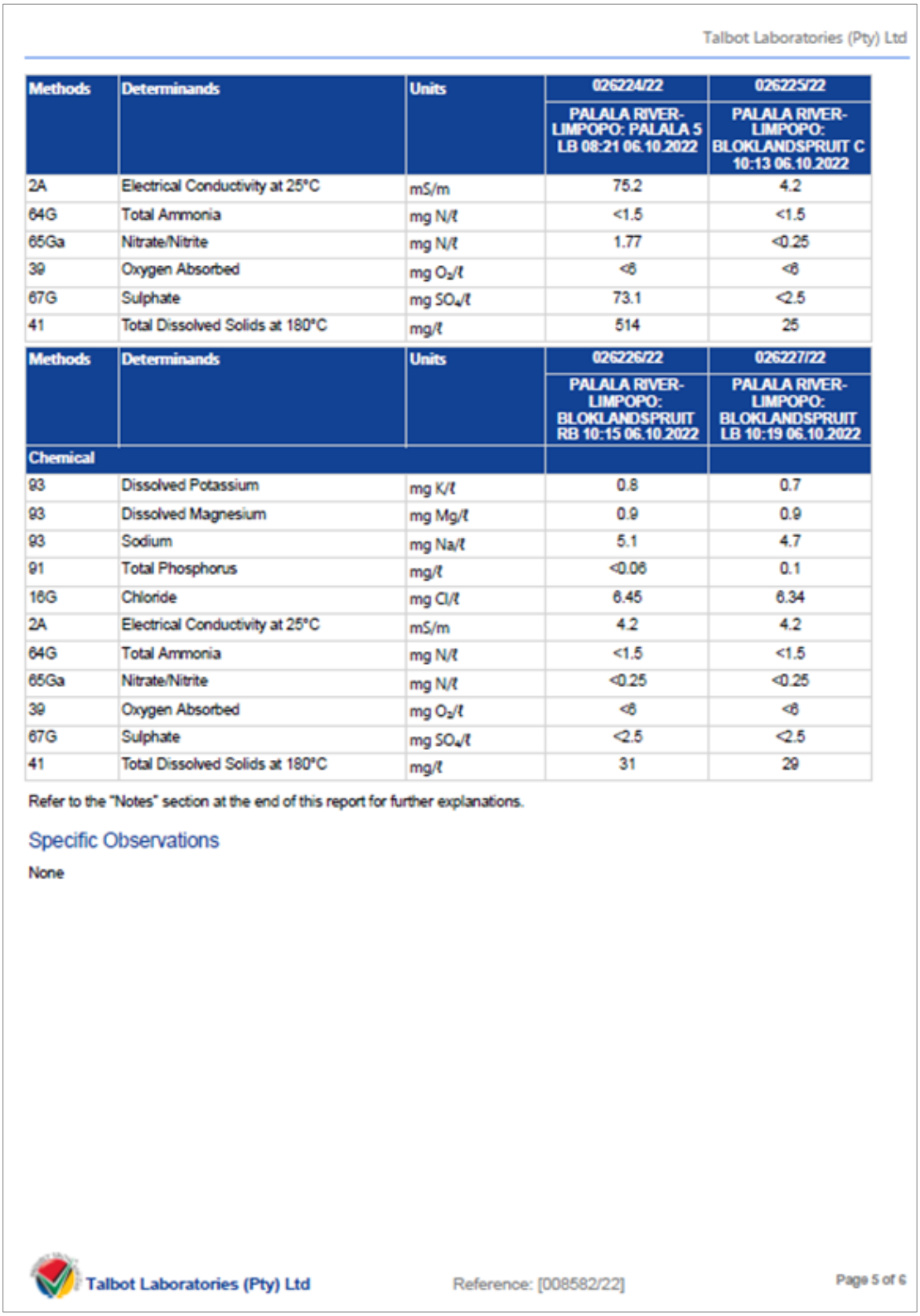
Figure C.4d: Fourth sampling period certificate of water analysis from Talbot lab, fourth page



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Methods	Determinands	Units	026224/22	026225/22
			PALALA RIVER-LIMPOPO: PALALA 5 LB 08:21 06.10.2022	PALALA RIVER-LIMPOPO: BLOKLANDSPRUIT C 10:13 06.10.2022
2A	Electrical Conductivity at 25°C	mS/m	75.2	4.2
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	1.77	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	73.1	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	514	25

Methods	Determinands	Units	026226/22	026227/22
			PALALA RIVER-LIMPOPO: BLOKLANDSPRUIT RB 10:15 06.10.2022	PALALA RIVER-LIMPOPO: BLOKLANDSPRUIT LB 10:19 06.10.2022
Chemical				
93	Dissolved Potassium	mg K/ℓ	0.8	0.7
93	Dissolved Magnesium	mg Mg/ℓ	0.9	0.9
93	Sodium	mg Na/ℓ	5.1	4.7
91	Total Phosphorus	mg/ℓ	<0.06	0.1
16G	Chloride	mg Cl/ℓ	6.45	6.34
2A	Electrical Conductivity at 25°C	mS/m	4.2	4.2
64G	Total Ammonia	mg N/ℓ	<1.5	<1.5
65Ga	Nitrate/Nitrite	mg N/ℓ	<0.25	<0.25
39	Oxygen Absorbed	mg O_2/ℓ	<6	<6
67G	Sulphate	mg SO_4/ℓ	<2.5	<2.5
41	Total Dissolved Solids at 180°C	mg/ℓ	31	29

Refer to the "Notes" section at the end of this report for further explanations.

Specific Observations

None

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Figure C.4e: Fourth sampling period certificate of water analysis from Talbot lab, fifth page

Appendix D: Mean and standard deviation of all environmental parameters and total macroinvertebrates collected across sites and seasons

Table D.1: Mean and standard deviation of all environmental parameters measured across sites, between January and June 2022 in the Palala River

	Sites						
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**B1**
Environmental Parameters	upstream (Source)	upstream (Melkriever)	Lapalala Wilderness Reserve (DL)	Lapalala Wilderness Reserve (Mudumela)	Communities (Shongoane)	Communities (Ga-Seleka)	Lapalala Wilderness Reserve (Tributary)
	January (Summer)						
Potassium K (mg K/L)	0.6±0.4	0.6	0.5±0.1	0.6±0.1	0.7	0.8±0.1	0.5
Dissolved **Magnesium Mg** (Mg/L)	0.8±0.4	0.8±0.1	0.8	0.8±0.1	1.1	1.4	1.0
Sodium Na (mg Na/L)	3.3±1.0	2.1±0.2	2.4±0.1	2.3±0.4	3.3	4.2±0.1	3.4±0.1
Phosphorus P (mg/L)	0.1	0.1	2.1±1.8	1.1±1.8	0.1	0.1	0.1
Chloride Cl_2 (mg Cl/L)	3.9±1.2	2.8±0.1	3.0±0.3	3.1±0.2	4.7±0.3	5.5±0.1	4.2±0.1
Electrical conductivity (mS/m)	2.8±1.0	2.8	3.4±0.1	3.3	21.2±4.3	11.2±0.2	3.4±0.1
Ammonia NH_3 (mg N/L)	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Nitrate $N0_3$ (mg N/L)	0.9±0.8	0.9±1.1	0.3±0.1	0.9±1.1	0.8±0.9	0.8±0.8	1.2±1.3
Oxygen absorbed (mg O_2/L)	7.3±2.3	6.0	6.0	6.0	6.0	6.0	6.0
Sulphate SO_4 (mg SO_4/L)	2.5	2.5	2.5June	2.5	2.5	2.8±0.1	2.5
Total dissolved solids TDS	37.0±0.2	31.7±2.5	40.7±0.2	37.0±1.0	50.0±4.4	64.7±3.5	38.3±2.5

Flow **velocity** ($m.s^{-1}$)	0.5±0.1	0.6±0.2	0.3±0.1	0.5±0.1	0.4±0.1	0.3±0.1	1.1±0.1
pH	6.4±0.4	7.6±0.3	8.6±0.3	6.5±0.4	6.7±0.2	7.5±0.3	7.5±0.2
Temperature (°C)	22.7±0.4	23.0±0.9	24.5±0.5	24.5±0.3	25.7±0.2	25.3±0.2	26.5±0.2
Depth (cm)	45.7±0.2	32.7±0.2	32.0±4.0	51.0±0.2	49.3±0.2	48.3±2.5	52.7±3.8
	March (Autumn)						
Potassium K (mg K/L)	0.5	0.5	0.5±0.1	0.5±0.1	1.5±0.3	1.0	0.6±0.1
Dissolved **Magnesium Mg** (Mg/L)	0.5±0.2	0.8	1.3±0.3	1.2±0.2	2.3±0.3	4.1±0.1	1.2±0.1
Sodium Na (mg Na/L)	2.7±0.3	2.0	3.7±0.8	3.9±0.8	40.0±0.2	12.7±0.6	5.5±0.3
Phosphorus P (mg/L)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Chloride Cl_2 (mg Cl/L)	4.0±0.3	4.0±1.0	4.2±0.4	4.6±0.2	10.3±1.7	10.1±0.7	4.0±0.5
Electrical conductivity (mS/m)	2.8±1.0	2.8	3.4±0.1	3.3	21.2±4.3	11.2±0.2	3.3±0.2
Ammonia NH_3 (mg N/L)	1.5	1.5	1.5	1.5	1.5	1.5	1.4±0.1
Nitrate $N0_3$ (mg N/L)	0.3	3.5±3.4	0.5±0.4	0.5±0.5	0.6±0.5	1.0±0.5	0.5±0.3
Oxygen absorbed (mg O_2/L)	6.0	6.0	6.0	6.0	6.0	6.0	5.7±0.6
Sulphate SO_4 (mg SO_4/L)	2.5	2.5	2.5	2.5	35.1±0.2	3.2	2.7±0.3
Total dissolved solids TDS	17.0±3.6	18.0±1.7	19.0±1.0	20.3±3.2	131.7±0.2	63.3±0.2	22.3±1.5
Flow **velocity** ($m.s^{-1}$)	0.4±0.2	0.6±0.1	1.1±0.5	0.5±0.1	0.4±0.1	0.5±0.1	0.4±0.2
pH	8.2±0.2	7.6±0.4	7.4±0.2	7.2±0.1	6.5±0.3	7.3±0.3	7.0±0.3
Temperature (°C)	19.2±0.1	22.8±0.7	21.4±0.5	23.0±1.0	23.3±1.5	21.3±1.5	23.0±1.0

Depth (cm)	36.3±2.1	46.0±0.2	70.3±0.6	68.0±3.0	29.7±0.2	51.7±3.1	29.3±1.5
	June (Winter)						
Potassium K (mg K/L)	0.3	0.6±0.1	0.4	0.4	0.8±0.1	0.7±0.2	0.6
Dissolved **Magnesium Mg** (Mg/L)	0.6	1.0	1.1	1.2	1.4±0.1	3.5	0.9
Sodium Na (mg Na/L)	3.9±0.2	3.6±0.1	4.0±0.1	4.0	39.3±2.5	10.6±0.2	4.8±0.1
Phosphorus P (mg/L)	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Chloride Cl_2 (mg Cl/L)	5.1±0.2	5.4±0.4	6.4±0.2	5.7±0.5	12.8±1.5	14.3±0.3	6.4±0.2
Electrical conductivity (mS/m)	2.3±0.1	2.9	3.4±0.1	3.4	20.2±4.1	10.5	3.6
Ammonia NH_3 (mg N/L)	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Nitrate $N0_3$ (mg N/L)	0.3	0.3	0.3±0.2	0.3	0.3	0.5	0.3
Oxygen absorbed (mg O_2/L)	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Sulphate SO_4 (mg SO_4/L)	2.5	2.5	2.8±0.5	2.5	34.5±2.5	3.4±0.1	2.5
Total dissolved solids TDS	12.0±1.0	19.0±3.5	22.7±4.5	20.7±0.5	116.0±2.5	56.7±3.1	24.3±3.1
Flow **velocity** (m.s-¹)	0.4±0.1	0.5±0.3	0.7±0.1	0.4±0.2	0.7±0.5	0.4±0.2	0.4±0.2
pH	8.2±0.2	8.2±0.2	7.3±0.1	7.2±0.1	6.3±0.2	6.5±0.4	6.8±0.2
Temperature (°C)	11.0±0.2	12.5±0.4	11±0.2	12.1±0.1	14.0±0.2	14.3±0.2	12.1±0.2
Depth (cm)	13.3±1.2	29.3±1.5	52.0±1.0	64.0±2.0	30.0±2.5	49.3±0.6	26.3±3.8
	October (Spring)						
Potassium K (mg K/L)	0.5±0.0	0.6±0.0	0.7±0.1	0.6±0.1	9.0±0.9	0.0±0.0	0.7±0.1

Dissolved **Magnesium Mg** (Mg/L)	0.4±0.0	1.0±0.0	1.4±0.1	1.3±0.1	7.5±0.3	0.0±0.0	0.9±0.0
Sodium Na (mg Na/L)	2.5±0.2	3.0±0.2	4.0±0.4	3.5±0.2	124.3±7.6	0.0±0.0	4.8±0.3
Phosphorus P (mg/L)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.5±0.1	0.0±0.0	0.1±0.0
Chloride Cl_2 (mg Cl/L)	5.2±0.2	4.7±0.3	5.7±0.2	5.7±0.1	128.0±6.6	0.0±0.0	6.4±0.1
Electrical conductivity (mS/m)	2.8±0.2	3.4±0.0	4.1±0.0	4.2±0.1	81.2±5.4	0.0±0.0	4.2±0.0
Ammonia NH_3 (mg N/L)	1.5±0.0	1.5±0.0	1.5±0.0	1.5±0.0	1.5±0.0	0.0±0.0	1.5±0.0
Nitrate $N0_3$ (mg N/L)	0.7±0.4	2.9±2.3	0.6±0.7	0.6±0.4	1.2±0.6	0.0±0.0	0.3±0.0
Oxygen absorbed (mg O_2/L)	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	0.0±0.0	6.0±0.0
Sulphate SO_4 (mg SO_4/L)	2.5±0.0	2.5±0.0	2.5±0.0	2.5±0.0	2.5±0.0	0.0±0.0	2.5±0.0
Total dissolved solids TDS	20.3±1.2	23.7±4.2	28.0±5.2	31.0±1.7	597.3±77.8	0.0±0.0	28.3±3.1
Flow **velocity** ($m.s^{-1}$)	0.4±0.0	0.5±0.1	0.7±0.2	0.5±0.1	0.3±0.1	0.0±0.0	0.3±0.1
pH	7.6±0.4	8.4±0.5	7.2±0.2	7.6±0.5	6.1±0.2	0.0±0.0	6.2±0.1
Temperature (°C)	19.7±1.2	22.7±1.2	20.0±1.0	20.7±2.3	23.4±0.4	0.0±0.0	21.3±1.2
Depth (cm)	35.0±2.6	28.3±1.5	44.0±3.6	52.3±2.5	19.0±1.0	0.0±0.0	19.7±0.6

Table D.2: The macroinvertebrates collected across sites per season, between January and October 2022 in the Palala River

Families	(Summer) January							(Autumn) March							(Winter) June							(Spring) October						
	P1	P2	P3	P4	P5	P6	B1	P1	P2	P3	P4	P5	P6	B1	P1	P2	P3	P4	P5	P6	B1	P1	P2	P3	P4	P5	P6	B1
Aeshnidae	11	7	4	7	-	5	2	8	-	3	9	-	8	30	14	8	4	-	-	5	-	9	-	7	-	-	-	-
Ancylidae	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-
Athericidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Baetidae 2 > sp	72	-	40	40	-	-	9	59	32	38	39	-	-	22	24	23	10	18	-	-	26	2-	27	17	18	-	-	15
Baetidae 2 sp	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Baetidae 1 sp	-	18	-	-	21	18	-	-	-	-	-	4	8	-	-	-	-	-	-	-	-	-	-	-	-	23	-	-
Barbaroch-thonidae	-	11	-	-	-	-	-	-	-	46	60	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbaroch-thonidae	2	18	-	-	14	-	-	-	-	4	8	-	-	-	-	-	-	-	19	15	-	-	-	-	-	-	-	-
Caenidae	-	16	-	-	-	-	-	-	7	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ceratopo-gonidae	-	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	63	-	-	-	-	-	-	-	-
Chironomidae	7	-	-	-	20	6	-	8	3	3	-	40	11	5	4	6	-	1	21	17	4	4	-	-	2	12	-	5
Coanagrionidae	-	46	-	-	-	-	-	-	-	-	-	-	4	2	-	-	-	-	-	21	4	-	-	-	-	-	-	-
Chlorocyphidae	-	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
Corbiculidae	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Corixidae	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
Corduliidae	28	19	19	-	-	-	-	6	13	9	8	6	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Corydalidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	3	-	-	-	-	-	-	-	-	-	-
Culicidae	-	15	19	-	28	-	-	-	-	-	-	65	2	-	-	-	-	-	19	6	-	-	-	-	-	23	-	-
Dixidae	-	-	6	-	-	-	-	6	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	11	-	-	-	-
Dytiscidae	-	-	-	13	-	-	-	-	-	-	-	-	-	-	-	9	-	-	-	-	-	-	9	-	-	-	-	-
Elmidae	9	4	4	-	-	21	-	-	-	15	-	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ecnomidae	-	-	-	11	-	-	6	-	-	-	6	-	-	21	25	19	-	-	-	-	-	-	17	-	-	-	-	-

Gerridae	-	-	-	11	-	-	-	-	-	-	5	-	-	-	-	2	-	7	5	-	-	-	-	-	1-	5	-	-
Gomphidae	-	-	-	24	22	5	-	3	6	14	4	43	3	4	29	23	2	2	21	32	7	25	32	9	9	5	-	7
Heptageniidae	-	-	-	11	-	-	-	-	-	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gyrinidae	-	17	15	-	8	-	3	-	-	-	-	-	-	-	5	-	-	-	3	-	-	-	-	-	-	8	-	-
Helodidae	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	3	-	-	-
Hydracarina (Water mites)	-	-	-	-	-	-	-	-	-	-	-	7	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrophylidae	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydropsychidae 2 sp	-	45	-	-	-	-	21	8	16	6	-	-	-	-	-	-	6	-	-	-	7	-	-	7	12	-	-	-
Hydropsychidae	-	-	-	-	4	-	-	-	-	-	-	17	-	-	-	-	-	-	38	3	-	-	-	-	-	-	-	7
Hydroptilidae	-	8	-	-	7	10	8	-	7	-	-	-	-	-	-	-	-	-	-	-	2	7	-	-	-	-	-	7
Lepidos-tomatidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-
Leptoceridae	5	30	-	6	3	16	-	4	15	-	-	9	-	24	11	4	-	-	-	-	-	-	-	-	-	-	-	-
Lestidae	19	-	-	-	-	-	12	-	12	-	13	-	-	16	7	15	10	3	15	7	-	13	1-	1-	-	-	-	-
Leptoceridae	-	-	12	-	-	7	-	-	-	8	-	-	45	-	-	-	-	-	-	-	-	18	-	-	-	-	-	-
Libellulidae (Darters)	-	-	6	-	-	-	-	-	3	-	-	-	-	-	3	2	3	2	-	9	2	-	-	7	1	-	-	4
Polycen-tropodidae	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Naucoridae	-	-	-	9	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
Notonectidae	-	-	-	-	-	-	-	2	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Notonemouridae	-	-	-	-	-	-	-	-	28	44	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oligochaeta	-	-	-	-	6	-	-	-	-	-	-	9	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oligoneuridae	-	-	-	93	-	-	-	-	-	-	46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Perlidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	5	-	-	-	-
Philopotamidae	15	15	-	-	-	-	12	-	6	22	11	-	-	13	11	-	-	-	-	-	-	8	-	-	-	-	-	-
Platycnemidae	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Pisuliidae	13	3	-	11	-	-	-	24	13	7	7	16	39	11	18	-	8	2	10	39	11	11	-	14	-	1-	-	7
Pleidae	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polycentro	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Potamonautidae	-	-	-	-	5	-	-	-	2	-	2	5	1	3	11	-	1	3	-	-	4	-	-	-	2	-	-	2
Psychomyiidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-
Simulidae	3	27	-	27	66	7	12	-	-	-	-	23	-	-	-	4	4	3	29	6	12	-	6	2	3	23	-	6
Sphaeriidae	-	-	-	-	-	-	-	-	-	-	-	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Synlestidae	10	-	-	-	-	-	-	-	-	-	-	12	-	-	-	-	-	3	-	-	7	-	-	-	6	-	-	9
Tabanidae	-	-	-	12	-	-	-	-	-	3	-	-	-	9	-	-	-	-	7	7	-	-	-	-	-	-	-	-
Teloganodidae	-	-	-	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichorythidae	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turbelarria	-	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Veliidae	3	-	2	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
TOTAL ABUNDANCE	**220**	**314**	**144**	**307**	**204**	**95**	**97**	**159**	**175**	**224**	**248**	**281**	**200**	**160**	**174**	**117**	**66**	**53**	**187**	**234**	**89**	**95**	**91**	**79**	**56**	**106**	**0**	**69**

Sites: P1 upstream (Source); P2 Upstream (Melkriever); P3 Reserve (Doreenlegte); P4 Reserve (Mudumela); P5 Communities (Shongoane); P6 Communities (Ga-Seleka); B1 Reserve (Tributary)