An investigation of water quality regulation by the Karsriviervlei, Bredasdorp

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DECLARATION

I declare that AN INVESTIGATION OF WATER QUALITY REGULATION BY THE KARSRIVIERVLEI, BREDASDORP is my own work, that has not been submitted before for any degree or examination in any other university, and that all sources I have used or quoted have been indicated and acknowledged by complete references.

Signed: ___________

Magen Munnik

Date: 14 August 2017

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ABSTRACT

Studies have shown that ecosystem services that are provided by wetlands are beneficial to the improvement of water quality regulation. Some of these ecosystem services may include sequestration of sediment, toxicants and nutrients by wetlands, which contributes to the quality of water in rivers downstream and thereby, the health and well-being of humanity and the environment. However, studies have also shown that there has been insufficient research done on how natural wetlands regulate water quality. Therefore, this study investigated the regulation of water quality by a wetland located in an agricultural setting in the Western Cape. This type of research was essential to South Africa as the country is experiencing a great loss and degradation of wetlands, even though national policies and legislation are geared towards their protection and rehabilitation. The study was aimed at evaluating the assumption that wetlands improve the quality of water in river systems, using the Karsriviervlei as a case study and by invoking two objectives. The first objective was to investigate the spatial and temporal variation in selected water quality variables upstream, through the wetland and downstream. The second objective was to investigate the hydrogeomorphic characteristics and processes of the Karsriviervlei that determined the effectiveness of wetlands, in regulating water quality. Furthermore, the study also consisted of two methods that provided an understanding of how natural wetlands regulate water quality. These methods were namely the WET-EcoServices Assessment as well as fieldwork & laboratory analysis. The WET-EcoServices method is a rapid assessment tool that was based on document analysis and observation. However, the fieldwork & laboratory analysis were based on observation as well as the collection of water samples - that were tested in laboratories - and field measurements using specific equipment. The results showed that the Karsriviervlei is influencing and to some extent improving the quality of water, through fluctuations and decreases in certain physico-chemical parameters. An essential decrease in specific parameters from the river inlet to the outlet downstream included nitrate, nitrite, turbidity and conductivity; which decreased from 1.25mg/l to 0.4mg/l, 9.5mg/l to 6.5mg/l, 34.45NTU to 14.95NTU and 4439µs/cm to 2794.5µs/cm respectively. Additional findings included the presence of the pathogen indicator – *Escherichia coli* - and the possibility of the wetland indicating a 'Severely Impacted Ecological Category'. The category is based on the values of nutrients as compared to previous research done on water quality boundary values (delimiting of Ecological Categories). Even though this was anticipated because modification to the Karsriviervlei is evident (i.e. implementation of agricultural drainage system), more research is required.

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GLOSSARY OF TERMS

Absorption	: refers to the taking up of one substance into the body of another
Adsorption	**:** is the physical adherence to the surface of a molecule or particle.
Agricultural drainage system	**:** refers to a system by which water is drained on or in the soil
Ammonification	: also known as nitrogen mineralization, is the transformation of organically bound nitrogen to ammonium-nitrogen
Constructed / Artificial wetlands	**:** are man-made wastewater treatment systems consisting of shallow ponds or channels which have been planted with aquatic plants, and which rely upon natural microbial, biological, physical and chemical processes to treat wastewater.
Berm	**:** refers to mound or bank of earth used as a barrier against flooding of land
Ecosystem services	**:** refers to the direct / indirect benefits people obtain from ecosystems
Evaporation	**:** refers to the physical process of molecular transfer by which a liquid is changed into a gas
Evapotranspiration	**:** can be defined as The loss of moisture from the terrain by direct evaporation plus transpiration from vegetation
Degradation	**:** refers to the general and progressive lessening of stream or channel profiles, or earth's surface, due to long-term periods of water induced erosion and/or scour
Denitrification	**:** refers to the process whereby nitrogen is lost by the transformation of nitrate to nitrous oxide and molecular nitrogen by bacteria under an anaerobic environment
Diffuse flow	**:** refers to flow that is spread as sheet-flow on the ground surface or as seepage below the ground surface
Dissolution	**:** refers to the process of dissolving
Fixation (Nitrogen Cycle)	: is the process by which nitrogen gas (N_2) in the Earth's atmosphere converts to ammonia (NH_3).
Hydrogeomorphic	: is the combination of hydrology and geomorphology
Hydrogeomorphic unit	**:** refers to the noticeable physiographic wetland-unit based on geomorphic setting, water source and water flow patterns
Hydrology	**:** is the study of the properties, distribution, and circulation of water on the earth
Macrophyte	**:** is a plant that grows in or near water which can either be classified as emergent, submerged or floating

Nitrification	**:** is the biological oxidation of ammonia or ammonium to nitrite. This is followed by the oxidation of nitrite to nitrate
Photosynthesis	: is the process by which plants generate food molecules from carbon dioxide and water by trapping solar energy from the sun
Rehabilitation	is the process of assisting a wetland in its recovery from degradation or preserving a wetland that is in the process of degradation in order to improve the wetland's capacity to provide services to society
Sedimentation	**:** refers to the settlement or deposition of sediments
Volatilization	: is the process whereby organic nitrogen (i.e. urea or animal fertilizer) converts to ammonia gas (NH_3) which is then lost into the atmosphere
Unchannelled valley-bottom wetland	**:** refers to a wetland without a river channel running through it with where diffuse flow prevails
Water quality	**:** refers to the purity of the water, determined by the combined effects of its physical attributes and its chemical constituents

LIST OF ACRONYMS AND ABREVIATIONS

DO	- dissolved oxygen
WWTW	- wastewater treatment works
MPN5 Test	- most probable number 5 test
DWS	- department of water and sanitation
E. coli	- *Escherichia coli*
mg/L	- milligrams per litre
µs/cm:	- microsiemens per centimeter
NTU	- nephelometric turbidity units

1. INTRODUCTION

1.1 BACKGROUND AND RATIONALE

Methods or systems used to purify water include Wastewater Treatment Works (WWTW) and wetlands. Even though WWTW are generally efficient for the improvement of water quality in the environment, some studies show that after secondary cleaning processes, contaminants may remain at concentrations that can affect human or environmental health (Nichols 1983, Verhoeven & Meuleman 1999). Wetlands may provide a more cost-effective means of treating wastewater, however, literature studies do not mention the use of wetlands for portable water (Verhoeven & Meuleman 1999; Fisher & Acreman. 2004; Stottmeister *et al*. 2003). Despite the fact that wastewater may not be discharged within natural wetlands intentionally, there are cases throughout South Africa where wetlands under natural conditions are receiving wastewater. Some examples of cases like these include a sewerage pump overflowing in Hout Bay - Cape Town, Western Cape – into a nearby wetland (Downie, 2006), a sewerage spill into Cape Town wetland nature reserves - Zandvlei and Zeekoevlei – caused by a power cut (Yeld, 2006) and a sewerage spill into a Blesbokspruit Ramsar Convention wetland in Gauteng (Bega, 2008).

Extensive research has been done on the use of artificial wetlands for wastewater treatment (Wu *et al*., 2015). This is because wetlands have been proven to have functions that are beneficial to the enhancement of water quality regulation (Millennium Ecosystem Assessment, 2005; Kotze *et al*., 2009). These functions are the basis of ecosystem services such as the sequestration of sediment, toxicants, nutrients and coliforms by wetlands, which contribute to the quality of water in rivers downstream and, thereby, the health and well-being of humanity and the environment (Forslund *et al*., 2009). However, despite the benefits and the policies implemented for the protection of wetlands, more than half of the world's wetlands have already been lost (Turpie *et al*., 2010) due to development of infrastructure and alteration of land (Millennium Ecosystem Assessment, 2005). More possibilities for the loss or degradation of wetlands are lack of understanding of the complex functions of wetlands and lack of communication to local communities (Millennium Ecosystem Assessment, 2005; Turpie *et al*., 2010). In fact, the Millennium Ecosystem Assessment (2005) pointed out that an increased focus in the knowledge of ecosystems under the Adapting Mosaic scenario could result in great success in the protection of wetlands. Therefore, this research focuses on the improvement of water quality by wetlands, and attempts to determine what functions within the Karsriviervlei wetland - near Bredasdorp - influence the water quality. Additionally, this study aims to evaluate the significance of this wetland as well as attempting to fill specific gaps in research. Examples of these gaps include the lack of research done on the sequestration of nutrients and coliforms by wetlands (Rogers, 1983; Fisher & Acreman, 2004; Millennium Ecosystem Assessment, 2005) and the limited research and data available on natural wetlands that improve agricultural wastewater (Zelder & Kercher, 2005).

1.2 PROBLEM STATEMENT

The Bredasdorp area is predominantly occupied by agricultural activity, which may yield diffuse, and point sources of nutrient, toxicant, and biological contaminants. The implications of contamination for the Kars River's water quality are of concern due to the presence of a Ramsar site - the De Mond Nature Reserve - as well as potential floodback from the Kars River into Soetendalsvlei downstream.

1.3 RESEARCH QUESTIONS

- How does the Karsriviervlei influence the water quality of the Kars River?
- What wetland characteristics are likely to be responsible for changes in water quality?

1.4 AIMS AND OBJECTIVES

This research aimed to evaluate the assumption that wetlands improve the quality of water in river systems, using the Karsriviervlei as a case study. Two objectives were set to achieve this aim:

- To investigate spatial and temporal variation in selected physico-chemical and biological water quality variables upstream of, within, and downstream of the Karsriviervlei.
- To investigate hydrogeomorphic characteristics and processes of the Karsriviervlei that would determine the effectiveness of a wetland at regulating water quality.

1.5 OVERVIEW OF THE RESEARCH APPROACH

The methodology of this study is comprised of a two part procedure. This includes a WET-EcoServices Assessment (Kotze *et al.*, 2009) and fieldwork with laboratory analysis.

1.5.1 WET-EcoServices Assessment

WET-EcoServices Assessment (Kotze *et al.*, 2009) was utilized as a tool to score the importance of the wetland. This was done by evaluating individual ecosystem services specifically appropriate for this study. The ecosystem services assessed using this tool included flood attenuation, sediment trapping, phosphate removal, nitrate removal, toxicant removal and erosion control.

1.5.2 Fieldwork & Laboratory Analysis

Fieldwork included the collection of water samples and measurements of specific physical water quality parameters, once during a flood in the early wet season to capture high-flow conditions, and once during the dry season to capture low-flow conditions. Continuous monitoring, although preferable, was not possible given available resources, and the high-flow / low-flow comparison was considered the best possible sampling approach to yield some insight into the temporal variability in parameter values. The physical parameters measured in the field included pH, water temperature, electrical conductivity (EC), dissolved oxygen (DO) and turbidity. The collected water samples were taken to the University of the Western Cape (UWC) for laboratory analysis. The test for the concentration of chemical parameters was determined by spectrophotometry and parameters included nitrite, nitrate, ammonia and orthophosphate. Biological parameters included total coliforms and *Escherichia coli*. A coliform count was established through the Most Probable Number 5 (MPN5) Test. Furthermore, Gram Staining and API 20E tests were done to determine the presence of *Escherichia coli*.

1.6 THESIS OVERVIEW

This thesis is comprised of seven chapters including the introductory chapter. Chapter two provides an overview of the research study area. Chapter three provides a critical review of literature. Chapter four gives a description of the specific methods used in this investigation. Chapter five describes the results of the selected water quality variables over a spatial and temporal variation as well as the results for WET-EcoServices. Chapter six interprets and discusses the results and chapter seven concludes and suggests recommendations for future research.

2. STUDY AREA

2.1 DESCRIPTION OF STUDY AREA

The study area was Karsriviervlei located in the Western Cape, specifically in a town called Bredasdorp (**Figure 2-1**).

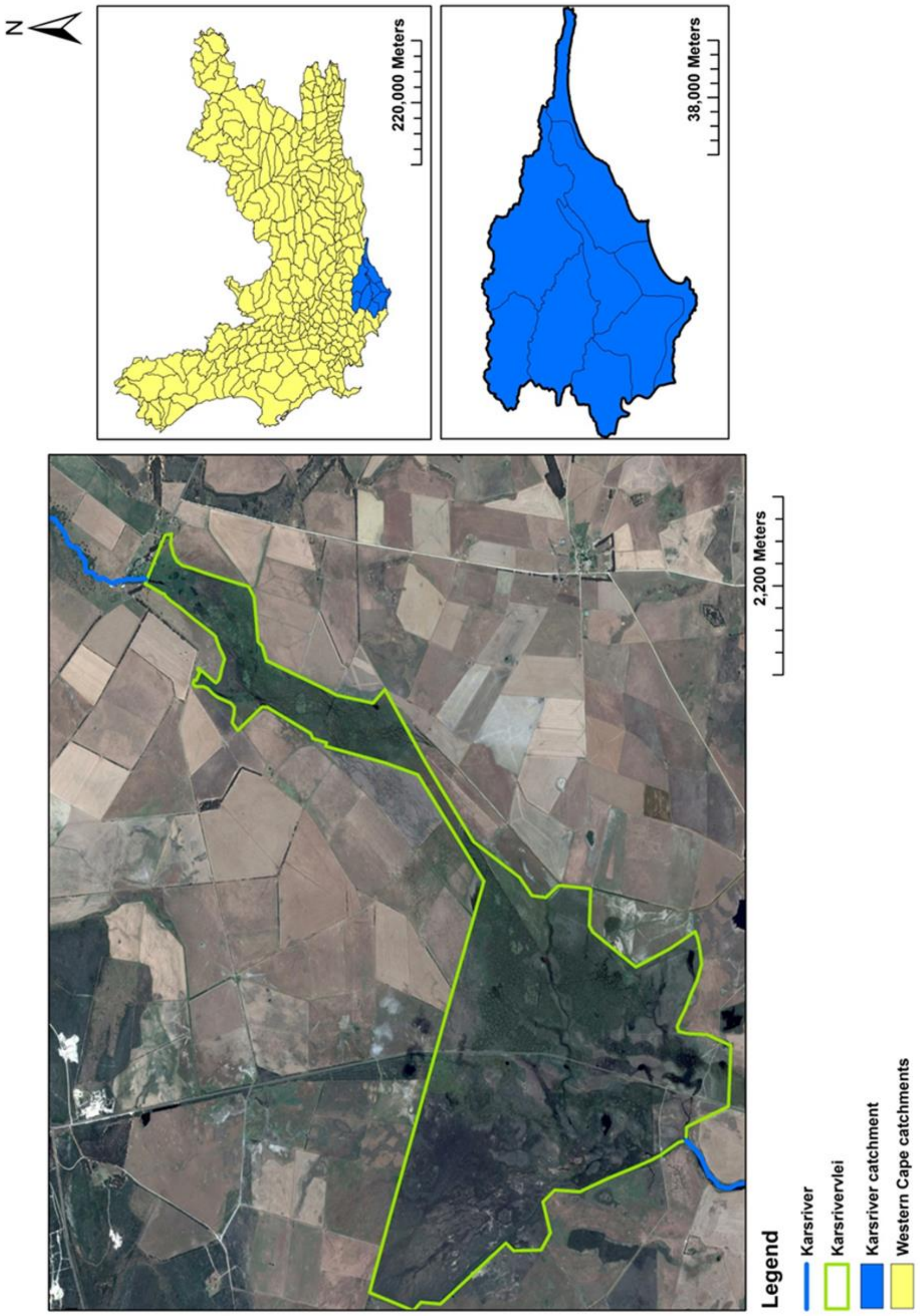

Figure 2-1: Map of Stud Area

Figure 2-2: Kars River channel feeding into Karsriviervlei

The Kars River feeds the Karsriviervlei as a single channel (**Figure 2-2**) and exits the wetland diffusively into the Heuningnes River just downstream from Soetendals Vlei. Local accounts suggest that the flow in the Heuningnes River channel near the confluence with the Karsriviervlei outlet can be towards Soetendals Vlei or towards the Ramsar De Mond estuary, although the controls on this such as tidal forcing have not been extensively studied.

Karsriviervlei is predominantly surrounded by agricultural activities (e.g. cattle grazing) and associated landscape modifications (**Figure 2-3**). Landscape modifications within the wetland included the implementation of an agricultural drainage system and the construction of a berm to prevent flooding of an adjacent field and allow for cattle grazing. The agricultural drainage system in place appears to have resulted in a loss of wetland area.

Figure 2-3: Karsriviervlei system - wetland, agricultural drainage system, berm, cattle grazing

2.2 CLIMATE AND VEGETATION

The climate of the Western Cape can be described by semi-arid cool temperatures with a low mean annual precipitation (MAP) of 579mm and a mean annual temperature (MAT) of 17.7˚C. However, these descriptions may be influenced by eddies of the Cape Agulhas current (Mucina & Rutherford, 2010).

The Karsriviervlei may consist of grassy, herbaceous and (succulent) dwarf-shrub vegetation (Muccina & Rutherford, 2010). Plant types and species observed within the Karsriviervlei may include *Phragmites australis*, *Typha capensis*, *Sarcocornia sp.*, *Cyperus sp.* and *Restionaceae*. *Sarcocornia sp* (**Figure 2-4**) is usually associated with salt marshes or coastal areas, which gives an expectation for high electrical conductivity levels in water bodies.

Figure 2-4: *Sarcocornia sp.* observed along the drainage system

2.3 SLOPE AND GEOLOGY

The Karsriviervlei was located on a flat surface with an area of approximately 1306ha and a gradient of 0.00157. Meanwhile, the area of the Kars River catchment was 393 7859ha with a gradient of 0.069.

The Karsriviervlei has formed within brackish calcareous soil with the occurrence of loam and sandy loam textured soil in certain parts of the wetland (**Figure 2-5)**. Furthermore, the underlying rock of the study area was established as quarzitic sandstone. Also, a possible fault was found cutting through the apex of the wetland, located near the river inlet near the crossing of the R316. This possible fault may influence the long-term development of the wetland, and may explain the location of channel flood out within this system.

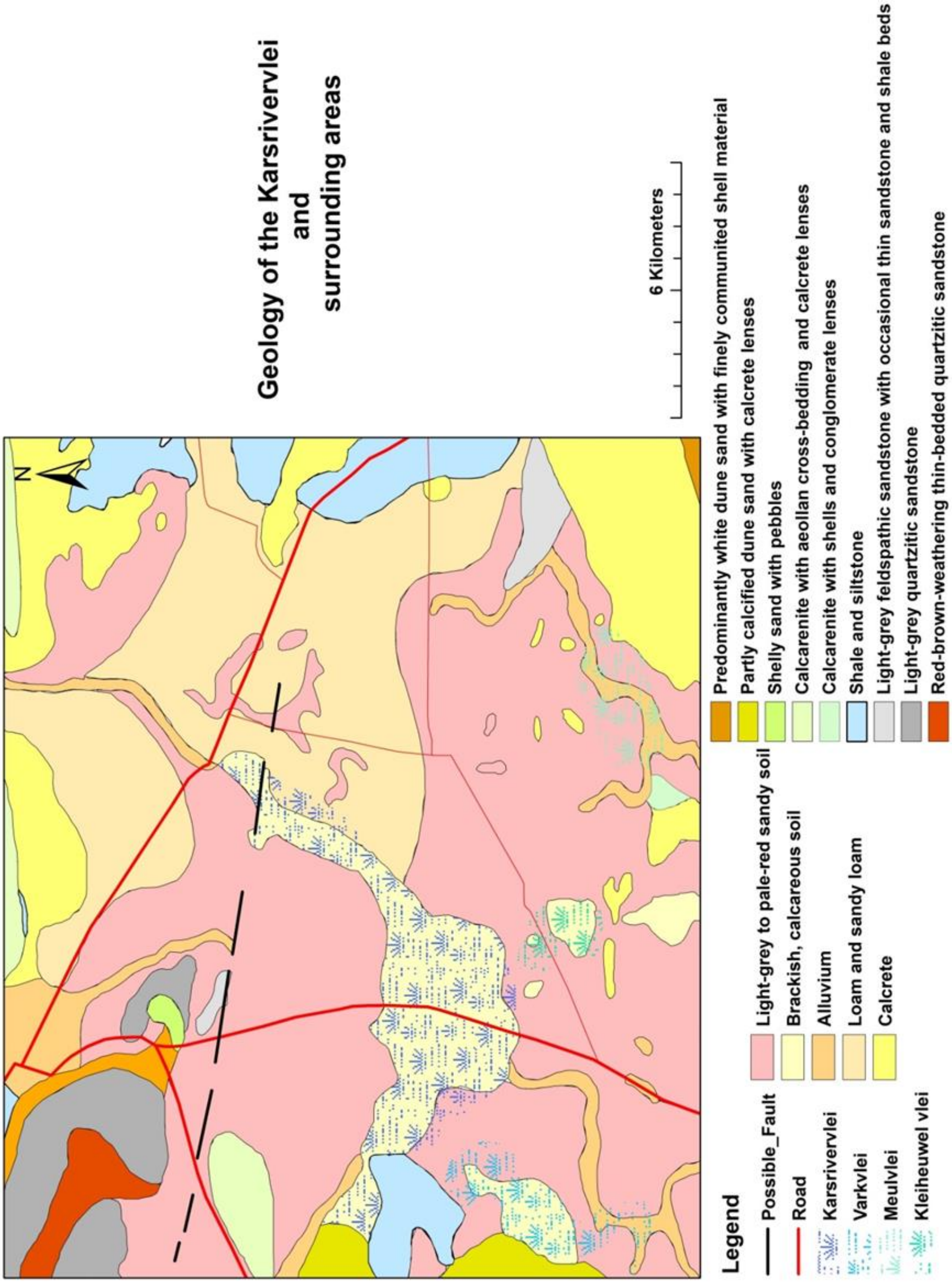

Figure 2-5: Geology of Karsriviervlei with surrounding areas

3. LITERATURE REVIEW

This chapter will provide a background on wetlands, how they are formed and their role in purifying water quality, specifically targeting selected physico-chemical and biological water quality parameters.

3.1 WETLANDS

Wetlands have been acknowledged for their extensive values and vast amount of services they provide to the benefit of humankind (Millennium Ecosystem Assessment, 2005). Some of these include: the provision of food and freshwater, the provision of recreational and educational services, supporting soil fermentation and nutrient cycling, regulation of water (hydrological flows) and water purification (Millennium Ecosystem Assessment, 2005; Kotze *et al*., 2009). However, despite national policies and legislation which are geared towards wetland protection and rehabilitation, wetlands are still being lost and degraded in South Africa (Ellery *et al*. 2009). Malan *et al*. (2015) investigated 65 wetlands within the Western Cape, South Africa over the course of 25 years. The results indicated a general deterioration of the water quality (Malan *et al*., 2015). Karsriviervlei, was however, not included in this report and to the best of my knowledge, has very limited research available.

According to the National Water Act 36 of 1998, wetlands can be defined as 'land which is transitional between terrestrial and aquatic systems where the water table is usually at or near the surface, or the land is periodically covered with shallow water which land in normal circumstances supports or would support vegetation typically adapted to life in saturated soil.'

Common terms which may be incorrectly used interchangeably, to describe various types of wetlands are marshes, swamps and vleis, which are simply attributes of a wetland (Mitsch & Gosselink, 2007). However, a marsh is characterized by shallow water and emergent herbaceous vegetation, while a swamp is characterized by trees or shrubs and a vlei is a colloquial term used in South Africa that indicates a wetland similar to a Dambo. Dambos are characterized by grassland, rushes and sedges (Mitsch & Gosselink, 2007).

South Africa has a rich abundance in wetlands; however, it is important to note that even though there is richness in wetlands, wetlands are still endangered due to major losses and degradation (Ellery *et al*., 2009). This loss and degradation occurs as a result of hydrological alterations, salinization, eutrophication, sedimentation, filling, invasions of exotic species

(Zelder & Kercher, 2005) and may be broadly driven by a lack of understanding of the value of wetlands within the landscape (Rogers, 1983; Fisher & Acreman, 2004; Malan & Day, 2012). In fact, the Millennium Ecosystem Assessment (2005) has determined bleak probable scenarios for wetlands in 2050.

3.1.1 Formation of Wetlands (South Africa)

The presence of various types of wetlands in South Africa is predicated on three components, namely hydrology, climate and geomorphology (Mitsch & Gosselink, 2007). Hydrology is the key component in the formation, size and persistence of wetlands (Mitsch & Gosselink, 2007). A wetland first and foremost requires a surplus of water at or near the surface. The surplus can be aided by the inflow exceeding the outflow to the area or by an impermeable barrier (e.g. some types of bedrock) limiting outflow (Ellery *et al*., 2009). The hydrology of the excess water is responsible for the physical and chemical conditions within the wetland and the transportation of energy, sediments and nutrients through the wetland. This transportation is achieved by various hydrological pathways, namely: precipitation, surface runoff, groundwater, tides and flooding of rivers in, through and out of wetlands (Mitsch & Gosselink, 2007; Ellery *et al*., 2009). Ultimately, these continuous processes will shape the physical parameters of the wetland (Mitsch & Gosselink, 2007; Ellery *et al*., 2009). Furthermore, hydrology is generally understood in terms of the mass balance approach (Ellery *et al*., 2009). The approach is defined by an equation, which states that the change in a wetland's storage volume equates to the relationship between inflows and outflows of a wetland (Mitsch & Gosselink, 2000). Therefore, climate and geomorphology of a wetland goes hand-in-hand with the hydrology of a wetland (Mitsch & Gosselink, 2007).

The presence of diverse wetlands on a global scale is predominantly found in humid regions; however, they may also occur in drylands (Tooth *et al*., 2012). Most of South Africa is classified as a dryland due to its semi-arid climate (Tooth *et al*., 2012). Also, South Africa is not expected to have wetlands that are primarily dependent on rainfall because of its low annual rainfall and very high evapotranspiration. Instead, the majority of the wetlands found in South Africa, especially large wetlands, are associated with rivers (Ellery *et al*., 2009). Wetlands associated with groundwater / hillslope seepage or springs are usually small or located near to the sea level where the groundwater table is near the surface (Ellery *et al*., 2009). Furthermore, the preferred geomorphology is flat or gentle slopes, but wetlands can occur on hillslopes (Zelder & Kercher, 2005; Mitsch & Gosselink, 2007). Additionally, as noted from the above statements, the conditions for the formation of wetlands may vary, and therefore require the combined information of a wetland's climate, geomorphology and hydrology as a unit, which is referred to as the hydrogeomorphic unit / wetland type (Mitsch & Gosselink, 2007).

3.1.2 Hydrogeomorphic unit or Wetland types

The six different types of hydrogeomorphic units are rivers, floodplain wetlands; valley-bottom wetlands, depressions, seeps and wetland flats (Ollis *et al*., 2013). For the purpose of this study, however, valley-bottom wetlands will be isolated; specifically the unchannelled valley-bottom wetlands as presented in **Figure 3-1**.

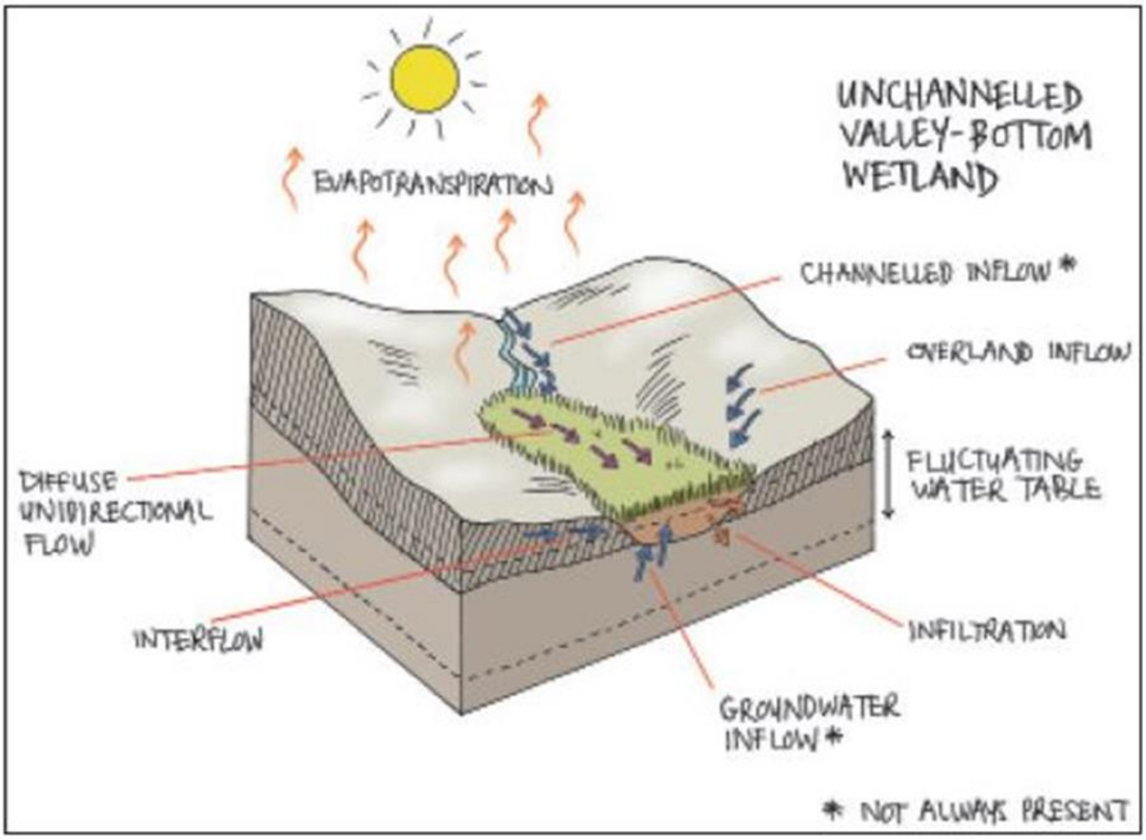

Figure 3-1: Unchannelled valley-bottom wetland (Ollis *et al*., 2013)

The unchannelled valley-bottom wetland is usually associated with valley floor located areas with the loss of channel confinement, causing the channel to spread out into diffuse flow (Ollis *et al*., 2013). This spreading of diffuse flow causes extensive areas of the wetland to remain permanently saturated and high levels of organic matter to accumulate (Kotze *et al*., 2009). Ollis *et al*. (2013), suggesting that this may be due to the change in gradient and the build-up of sediment. Examples are found in Tooth *et al*. (2012) and Grenfell *et al*. (2012) who discovered similar findings in their research. Tooth *et al*. (2012) explained that the Blood River channel decreases as it enters the wetland, thereby decreasing stream power and thus inducing sediment deposition. Tooth *et al*. (2012) then suggested that the continuous decrease in streamflow promoted sediment deposition and the development of alluvial ridges – which leads to cross valley gradients, resulting in diffuse channels. According to Grenfell *et al*. (2012) the Jackal Valley gully (in the Sneeuberg Mountains of the semi-arid Karoo, South Africa) decreases in the channel dimension and gradient and flow then loses confinement as a result of a lobate structure of sediments. Ollis *et al*. (2013) also outlined that these wetlands may receive water (inflow) by a channel upstream, overland flow, interflow and sometimes groundwater flow with a fluctuating water table. The indications of possible losses of water are by infiltration, diffuse unidirectional flow and evapotranspiration (Ollis *et al*., 2013). According to Kotze *et al*. (2009), the unchannelled valley-bottom wetland location may resemble that of a floodplain with gentle gradient and high levels of sediment deposition, which is dependent on the sources of sediments. The specific ecosystem services associated with this type of wetland that may promote the improvement of water quality are: the trapping of sediments, the removal or reduction of nitrates and phosphates and the photo-degradation of certain toxicants because of sunlight penetration occurring in shallow waters (Kotze *et al*., 2009).

3.2 WETLANDS FOR WATER PURIFICATION

The assumption that wetlands improve / purify water systems by the functions they perform has been considered more often for wastewater as a more cost-effective approach (Sim, 2003; Stottmeister *et al*, 2003; Wu *et al*, 2015). Additionally, studies have indicated that natural wetlands are indeed effective in the purification of water. An example of this was found by Fisher & Acreman (2004) who investigated 57 natural wetlands across the world. They established that 80% of the wetlands reduced nitrogen and 84% of the wetlands reduced phosphorus. However, available studies also indicate that research conducted on the improvement of water quality by natural wetlands is still quite limited and requires further investigation (Zelder & Kercher, 2005). Furthermore, natural wetlands are protected under policies, specifically by the National Water Act of 36 (1998) and can therefore not be used as a means to research this concept by intentionally adding wastewater to the systems. This accounts for recent studies being conducted predominantly in constructed wetlands, as they were meant to emulate the same processes which occur in natural wetlands, but in a more controlled environment (Vymazal, 2007).

3.3 THE RELATIONSHIP BETWEEN WETLANDS AND WATER QUALITY

Research has indicated that wetlands provide a vast range of ecosystem services, whether it is indirect or direct by physical and biological processes occurring within wetlands (Kotze *et al*., 2009; Ellery *et al*., 2009; Turpie *et al*., 2010). However, literature has also indicated that insufficient research has been done on the water purification services, specifically the sequestration of sediments, toxicants, nutrients and microorganisms such as coliforms by natural wetlands (Rogers, 1983; Fisher & Acreman, 2004; Millennium Ecosystem Assessment, 2005; Reddy *et al*., 1999).

Water quality in the context of this study refers to how useful or valuable water is and it is usually indicated by the measurements of its biological, physical and chemical constitutes (Dallas & Day, 2004; Brauman *et al*., 2007). Water quality variables are the biological, physical or chemical constituents of the water body (Dallas & Day, 2004). In a country that is struggling with water scarcity, improvement of water quality is needed (Rogers, 1983).

The underlying question is therefore: Can water quality be improved by wetlands and how would the wetlands achieve this? Wetlands are often considered to be "the kidneys of a catchment" or "sinks", as studies have shown their effectiveness in reducing nutrient loads caused by runoff of agricultural / domestic waste (Fisher & Acreman, 2004; Borin & Tocchetto, 2007; Scott *et al*., 2008). Wetlands have also been known as an alternative approach in purifying wastewater from pathogens such as *Escherichia coli* (Stottmeister *et al*., 2003; Vymazal, 2005; Masters, 2012). However, some studies have shown that wetlands can be ineffective in this capacity and in certain cases can deteriorate the water quality (Fisher & Acremen, 2004). Why is this so? Wetland specialists have advised against the speculation that "all wetlands improve water quality and provide the same functions". This is because the type, size, slope and position of the wetland in the catchment with its surrounding features / factors have to be investigated in order to determine its effectiveness or capacity in providing ecosystem services (Zelder & Kercher, 2005; Vymazal, 2007; Ollis *et al*., 2013). Possible scenarios to elaborate on this statement may include the change in climate of the wetland area, the change in systems (open / closed), variability and size of vegetation and the change in channel flow (single / diffuse) (Malan & Day, 2012).

3.3.1 Removal of Nutrients by Wetlands

Various studies have indicated that wetlands are efficient at removing nutrients (Rogers, 1983; Fisher & Acreman, 2004; Vymazal, 2007; Borin & Tocchetto, 2007; Reddy, 1999; Mitsch *et al*., 2012). However, the processes involved in wetlands that instigate the removal or reduction of nutrients vary per parameter, and thus, have to be identified and investigated separately.

3.3.1.1. Nitrogen Cycle

Nitrogen is considered bioavailable in both organic and inorganic forms (Mitsch & Gosselink, 2007). Furthermore, nitrogen undergoes various transformations within a wetland that may result in the removal of nitrogen from water systems**.** These transformations may include ammonification, denitrification, nitrification, fixation, and volatilization. The nitrogen cycle involves the conversion of organic nitrogen to inorganic nitrogen and thereafter back to organic nitrogen (Vymazal, 2007; Mitsch & Gosselink, 2007; **Figure 3-2**).

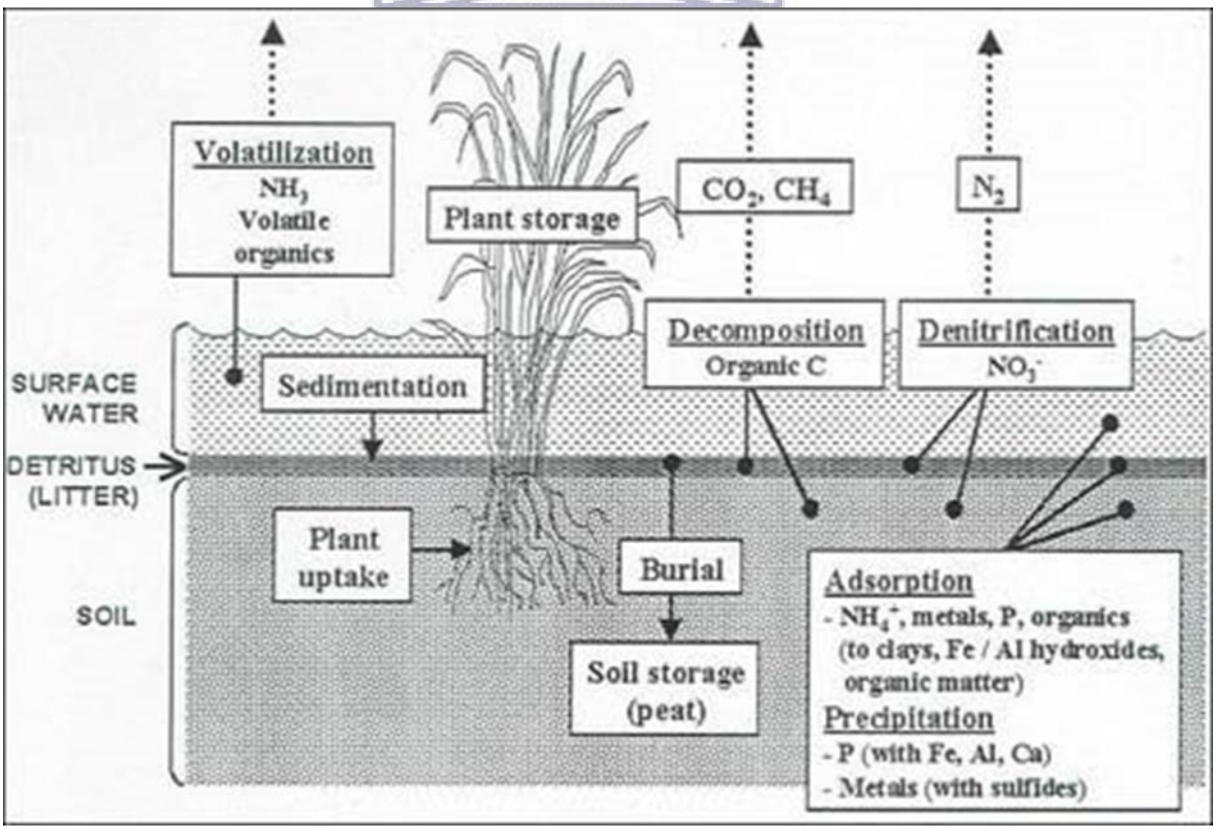

Figure 3-2: Conceptual model of the Nitrogen Cycle within wetlands (Haard, 2008)

i) Ammonification, volatilization, nitrification and denitrification

Ammonification, also known as nitrogen mineralization, is the transformation of organically bound nitrogen to ammonium-nitrogen. This process occurs through a sequence of biological transformations under anaerobic and aerobic environments. In this form, the ammonium-nitrogen can undergo various pathways of transformation which may include plant absorption by the root, transformation back to organic matter under anaerobic environment or conversion to ammonia in an environment with pH 8. The resultant ammonia can then be released into the atmosphere by means of *volatilization* (Rogers, 1983; Vyzamal, 2007; Mitsch & Gosselink, 2007). Lastly, the oxidized layer at the surface of a wetland provides an aerobic environment which allows for the nitrification process to take place (Mitsch & Gosselink, 2007). *Nitrification* is one of the primary methods to reduce nitrogen within wetlands; this is the two-step process whereby ammonium transforms to nitrite-nitrogen and thereafter, to nitrate-nitrogen by bacteria under an aerobic environment (Rogers, 1983; Vymazal, 2007; Mitsch & Gosselink, 2007). Nitrate-nitrogen has high mobility in solutions and if it is not removed by plant uptake or groundwater, it may follow the path of *denitrification*. This process results in the loss of nitrogen by means of nitrate being transformed to nitrous oxide and molecular nitrogen by bacteria under an anaerobic environment (Rogers, 1983; Vymazal, 2007; Mitsch & Gosselink, 2007). Since dinitrogen-nitrogen is a harmless gas; there is no environmental concern for it being released into the atmosphere. However, nitrous oxide-nitrogen is acknowledged as one of the greenhouse gases causing climate change and should be considered in this process (Mitsch & Gosselink, 2007). Also, dinitrogen-nitrogen can then be transformed back into organic nitrogen by means of fixation, showing that these pathways can then be repeated and recycled (Vymazal, 2007; Mitsch & Gosselink, 2007).

ii) Vegetation uptake/absorption of nitrogen

Macrophytes and algae are one of the most effective ways of removing nitrogen in wetlands (Rogers, 1983). Furthermore, literature has indicated that emergent plants are more efficient than submerged plants in the uptake of nitrogen (Rogers, 1983). Based on a five year study by Borin & Tocchetto (2007), the specific emergent plant species associated with effective removal of nitrogen are reeds, specifically *Phragmites australis* and *Typha latifolia*. It is, however, worth stating, that the nitrogen stored in the plant would be temporary as it will be lost again once the plant dies and decomposes. Alternatively, if the nitrogen is stored in peat, the nitrogen will be removed on a more permanent basis as it will be trapped and stored in the peat (Rogers, 1983). Nitrogen is generally stored in the cells and tissues of the plant; the efficiency of nitrogen removal depends on the plant's nutrient storage and the plant's ability to reach a high standing crop; like reeds (Vymazal, 2007). There are preferential nitrogen forms which are utilized for the assimilation, namely: ammonia and nitrate. However, the preferred forms of nitrogen vary by plant species and the forms that are accessible within the soil (Vymazal, 2007).

3.3.1.2. Phosphorus Cycle

Phosphorus may enter a wetland in four specific forms. These are dissolved inorganic phosphorus (DIP), dissolved organic phosphorus (DOP), particulate organic phosphorus (POP) and particulate inorganic phosphorus (PIP) (Mitsch & Gosselink, 2007; Reddy *et al*., 1999). However, phosphorus is only known for being bioavailable in the form of dissolved inorganic phosphorus (DIP) - also referred to as orthophosphates - and would have to undergo transformations in order for the other forms of phosphorus to be considered as bioavailable (Mitsch & Gosselink, 2007; Reddy *et al*., 1999). Also, there are various processes occurring within wetlands that are responsible for the removal of phosphorus in its various forms. These processes include co-precipitation and precipitation, adsorption by soil and sediments, vegetation uptake and the uptake by periphyton and microorganisms (Vymazal, 2007; Mitsch & Gosselink, 2007; Reddy *et al*., 1999; **Figure 3-3**).

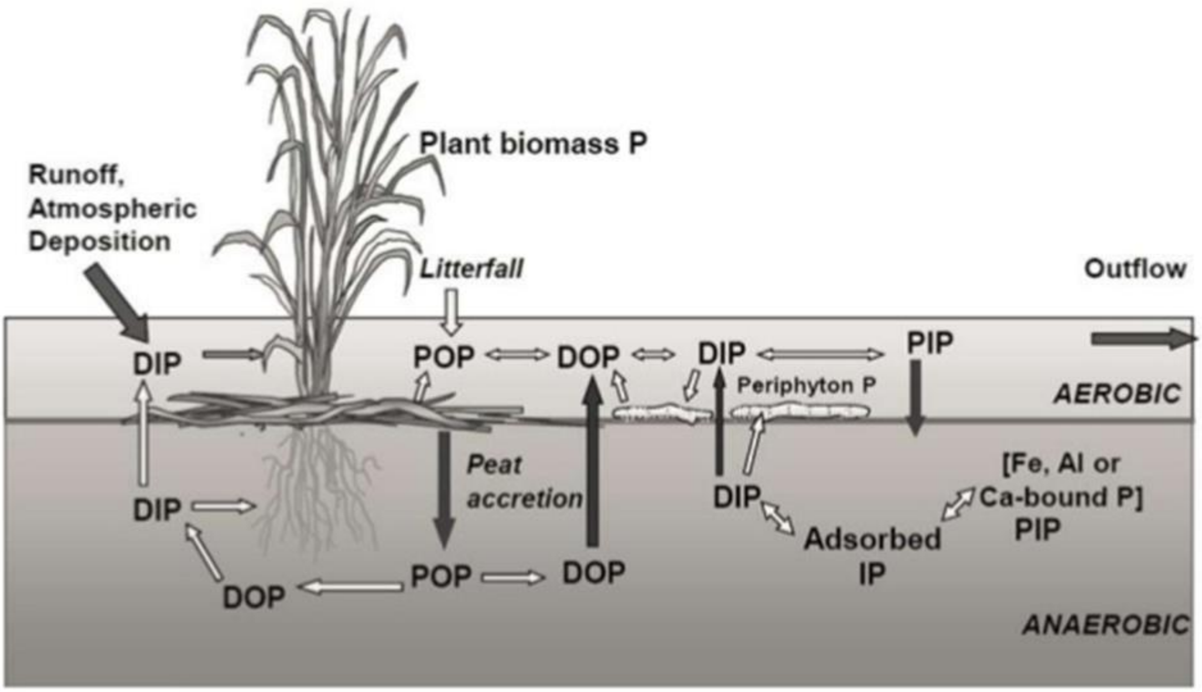

Figure 3-3: Conceptual model of the Phosphorus Cycle within wetlands (Reddy. 1999)

i) Precipitation, Co-Precipitation and Cells of algae

The high productivity of algae in a wetland entails two pathways; the first results in precipitation and the other results in the assimilation of phosphorus into the cells of algae. Precipitation of phosphorus refers to phosphate ions reacting with metallic cations such as iron, aluminium, calcium or magnesium, forming a crystalline solid (Vymazal, 2007). Co-precipitation occurs within aerobic conditions, as the high algae productivity extracts carbon dioxide from the water body. The extraction of carbon dioxide results in an increase in pH as high as 9 or 10 because the carbonate equilibrium is affected. Co-precipitation occurs as phosphorus is adsorbed onto calcite, which can be found in the geology or soil / sediment of the wetland and precipitates into calcium phosphate (Mitcsh & Gosselink, 2007, Reddy *et al*., 1999). Periphyton can initiate the process of co-precipitation, which is discussed in a later section.

ii) Adsorption of phosphorus by sediments

Fine sediments have the capacity to store and trap considerable amounts of phosphorus by **adsorption** and **precipitation** (Rogers, 1983). The production of macrophytes enhances sedimentation and therefore, enhances the removal of phosphorus (Turpie *et al*., 2010). The removal of phosphate by sediments is greater in wetlands with low water velocities and high hydraulic roughness (Turpie *et al*., 2010). This is because low velocities in water equates to longer suspension time and thus, more time for phosphorus to be adsorbed by the sediments; and higher hydraulic roughness within wetlands allows better opportunities for sedimentation (Turpie *et al*., 2010). Furthermore, the removal of phosphorus by sediment adsorption is on a temporary basis due to factors that may include biological uptake of phosphorus in the overlying water. This causes sediments to release phosphorus and the aquatic plants that are drawing phosphorus from sediments to release it back into the overlying water. One of the pathways in which phosphorus is removed permanently is if the phosphorus is trapped in sediment that is buried (Rogers, 1983).

iii) Vegetation uptake/absorption of phosphorus

Vegetation uptake only occurs with inorganic phosphorus such as orthophosphates (Mitsch & Gosselink, 2007). The efficiency of vegetation uptake depends on the type, growth and age of plants, as well as the nutrient status of the water body (Rogers, 1983; Reddy *et al*., 1999). However, similar to the nitrogen uptake of plants, the absorption of phosphorus by vegetation can be both long term as well as short term (Rogers, 1983; Reddy *et al*., 1999). Short term storage usually occurs when the vegetation decomposes and long term storage usually occurs when the phosphorus is trapped in the plant structure that is found within the soil or peat (Rogers, 1983; Vymazal, 2007; Reddy *et al*., 1999). Emergent macrophytes are found to be more effective than submerged macrophytes, due their extensive network of roots and rhizomes (Reddy *et al*., 1999).

iv) Periphyton and Microorganisms uptake of phosphorus

Periphyton and microorganisms uptake are mechanisms of removing phosphorus within a wetland (Turpie *et al*., 2010; Reddy *et al*., 1999). Periphyton is a living organism that can be found tied to macrophytes or in the form of a benthic layer. It draws phosphorus from the water column and from soil / sediments. These organisms can absorb phosphorus in its organic and inorganic forms known as the biological uptake. They also have the ability to activate changes in the pH and dissolved oxygen from a water column, as well as to activate changes in the soil floodwater interface. In a high calcium ion environment, the pH changes by the induction of periphyton, this can initiate co-precipitation of phosphorus onto calcite (Reddy *et al*., 1999). Dissolved phosphorus (DP) is absorbed into the cellular structure of microorganisms and then becomes part of its structure (Reddy *et al*., 1999). Microorganisms are significant to the removal of phosphorus as they can survive in both aerobic and anaerobic environments and the organisms germinate and reproduce at high rates (Vymazal, 2007; Reddy *et al*., 1999).

3.3.2 Removal of *Escherichia coli* Coliforms by wetlands

Available literature suggests that wetlands (especially constructed wetlands) are efficient systems used for the removal of pathogens such as *Escherichia coli* and other coliforms from wastewater (Thurston *et al*., 2000; Stottmeister *et al*., 2003; Reinoso *et al*., 2008; Boutilier *et al*., 2009). However, as mentioned before, the wetland type, size, and area play a vital role in the capacity of a wetland to perform this function. The removal mechanisms for *Escherichia coli* within wetlands may include adsorption onto particles, plant uptake and plant secretion of toxic substances, change in atmospheric temperature and predation (Rogers, 1983).

3.3.2.1. Adsorption into particles

The adsorption of *Escherichia coli* to particles depends on the size and density of the particle. The size and density also indicates the relevance of sedimentation. Sedimentation can be insignificant for the adsorption of *Escherichia coli* to small light-weight organic particles; however, sedimentation is significant in the adsorption of *Escherichia coli* to bigger, denser inorganic particles (Boutlier *et al*., 2009). Boutlier *et al*. suggests that the adsorption of

Escherichia coli is most effective for fine or cohesive particles within an environment of soil-water.

3.3.2.2. Plant uptake and plant secretion of toxic substances

Macrophytes have been acknowledged as an efficient tool in the removal of *Escherichia coli*, due to the tannic and gallic acid toxicants excreted by the roots. An example of such macrophytes may include *Phragmites australis*. The development of bacterial populations with antibiotic properties within the rhizosphere may also contribute to the removal of *Escherichia coli* (Decamp & Warren, 2000).

3.3.2.3. Predation by protozoa and nematodes

Predation of *Esherichia coli* by protozoa and nematodes is another mechanism that should be considered (Hoorman, 2011). Protozoa are single-cell animals whereas nematodes are non-segmented worms which feed on bacteria (Hoorman, 2011).

3.3.2.4. Temperature

Temperature is required for growth, and temperature is also responsible for bacteria dying off (Reinoso *et al*., 2008). *Escherichia coli* in particular has shown to prefer high temperatures ranging from approximately 35˚C and up. However, in refrigerated temperatures such as 4˚C, *Escherichia coli* is found to be below detection levels as growth is restricted under these temperature conditions (Boutlier *et al*., 2009). However, it is worth noting that the survival of bacteria tends to increase in the presence of nutrients (Boutlier *et al*., 2009), because nutrients provide resources for the metabolism of microorganisms (Boutlier *et al*., 2009).

3.4 POSSIBLE PATHWAYS OF NUTRIENTS AND *ESCHERICHIA COLI* COLIFORMS THROUGH WETLANDS

This section will discuss the different pathways in which nutrients (nitrogen, phosphorus) and *Escherichia coli* may take entering, exiting and moving through the wetland, thereby highlighting certain aspects from section 3.3.

Nutrients and *Escherichia coli* may enter wetlands via enriched groundwater or surface water. The possible sources responsible for the presence of these parameters are domestic, agricultural and animal waste (Zelder & Kercher, 2004; Morris & Reich, 2013). Within the wetland organic nitrogen from animal waste or fertilizer may be converted to ammonium by ammonification. Ammonium-nitrogen may either be absorbed by vegetation and groundwater, be converted to NH_3 gas under high pH levels (8-10) or undergo nitrification (**Figure 3-2**). Nitrification is a two-step process within the wetland converting ammonium nitrogen to nitrite and thereafter nitrate. Nitrate can then be transformed to nitrous gas (N_2 and N_2O) or be absorbed by groundwater or vegetation. Nitrous gas, however, can be converted back into organic nitrogen within the wetland or exit by volatilization. Nitrogen may therefore exit the wetland in the form of gas (N_2, NH_3, and N_2O) or by surface water or groundwater exiting the wetland.

In the presence of high algae productivity phosphorus may undergo precipitation or absorption by algae cells, vegetation, periphyton and microorganisms. Phosphorus may therefore exit the wetland through sediments (to which it is adsorbed), groundwater and surface water (in solution) (Mitsch & Gosselink, 2007; **Figure 3-3**).

Escherichia coli may undergo various pathways of uptake or within a wetland as presented in **Figure 3-4**. These include the adsorption of *Escherichia coli* onto particles (i.e. sediments) and vegetation, destruction by UV rays from the sun as well as the predation from nematodes and protozoa. Flooding may result in the distribution of *Escherichia coli* across a larger area by the transportation of water. *Escherichia coli* may leave the wetland through sediments, groundwater or surface water.

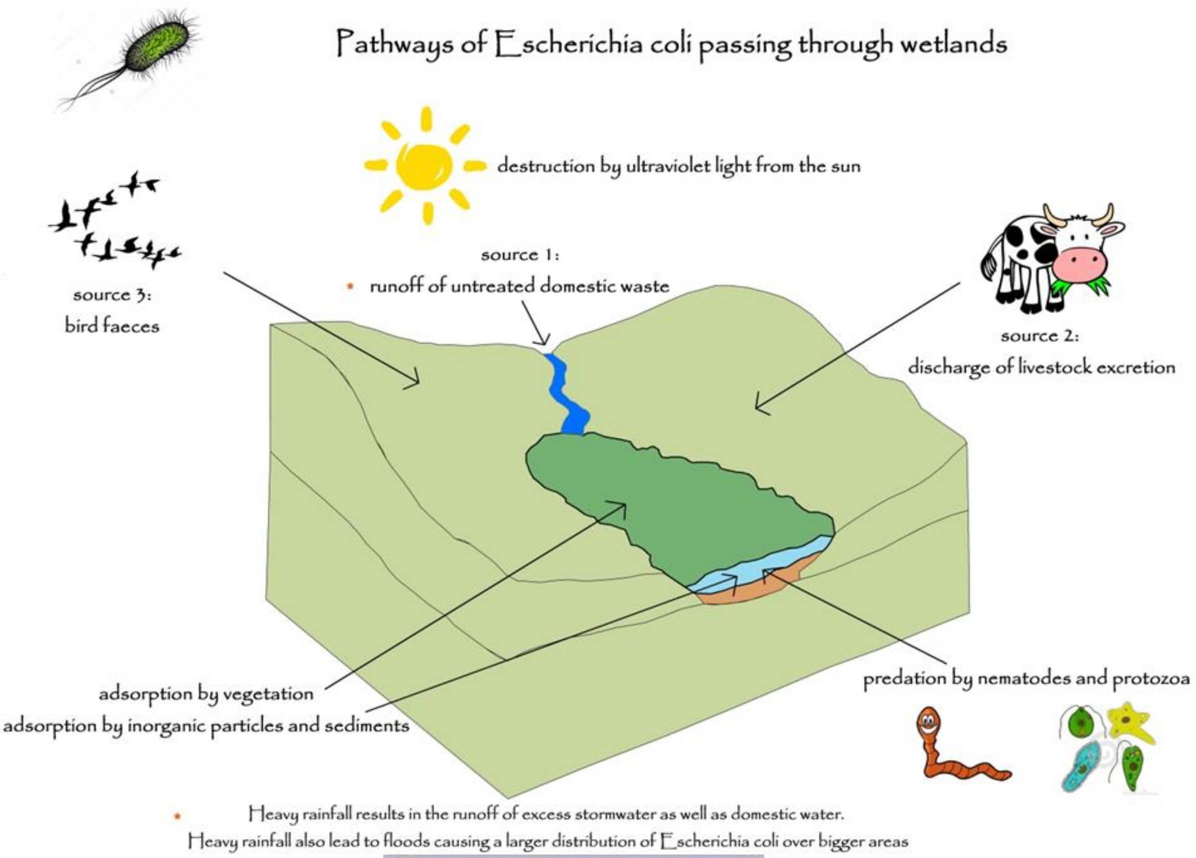

Figure 3-4: Conceptual model of *Escherichia coli* pathways passing through wetlands

3.5 IDENTIFICATION OF SCIENTIFIC GAPS ON WETLANDS AND WATER QUALITY

Based on the research conducted for this study, it was found that the research done on how wetlands remove or reduce nutrients and coliforms such as *Escherichia coli* from the environment and its effectiveness in improving water quality, is insufficient and has primarily been conducted on constructed wetlands in controlled environments. Therefore limited research is available on natural wetlands; more specifically, the hydrogeomorphic processes and characteristics involved in the effectiveness of improving water quality. Furthermore, research available for the effectiveness of wetlands in improving water quality has been conducted using sewage wastewater, limited research has focused on wastewater in natural wetlands (Zelder & Kercher, 2005), specifically agricultural wastewater. These gaps are lacking more so within the Karsriviervlei; gaps which this research aspires to close.

4. METHODS OF INVESTIGATION

The following chapter entails a detailed description of the methods used within this research project to evaluate the potential of Karsriviervlei to improve the quality of influent waters. These methods include the WET-EcoServices Assessment and fieldwork with laboratory analysis. Physical parameters are determined during fieldwork whereas chemical and biological parameters are determined during laboratory analysis. Meanwhile, the WET-EcoServices Assessment is a tool used to determine the effectiveness and opportunity of ecosystem service provision by a specific wetland or set of wetlands.

4.1 WET-ECOSERVICES ASSESSMENT

WET-EcoServices is a tool used to score the importance of a wetland, by means of evaluating the ecosystem services it provides (Kotze *et al*., 2009). Some of these ecosystem services may include: the provisioning of food, fresh water, fibre and fuel, the regulation of water quality, climate change and natural hazards (Millennium Ecosystem Assessment, 2005). It was imperative to note that the assessment only provided guidelines for the scoring of ecosystem services. These aforementioned guidelines were developed over a nine-year comprehensive research program on wetland management (Kotze *et al*., 2009).

The first step was to define the objectives of this study, in order to determine the relevance of this assessment. Thereafter, Level 1 and 2 assessments were conducted. The level 1 assessment was comprised of the interpretation of orthophotographs with a scale of 1:10 000 and Google Earth imagery, for the determination of the hydrogeomorphic setting of the Karsriviervlei. Furthermore, the level 2 assessment consisted of field verification, confirming by direct observation the discoveries on the orthophotographs and Google Earth images. Scoring also took place within the level 2 assessment, using the WET-EcoServices checksheets (Kotze *et al*., 2009). The checksheets were lists which contained several characteristics that are considered to determine ecosystem service provision. The ecosystem services that were assessed in this study were as follows: sediment trapping, phosphate assimilation, nitrate assimilation, toxicant assimilation and erosion control (**Appendix B**). The checksheet also contained a scoring column, which ranged from 0-4 as seen in the example below (**Table 4-1**). For more information on the interpretation of the scoring values refer to **Appendix B**.

Table 4-1: An example of a WET-EcoServices checksheet – Phosphate removal scoring checksheet

Characteristics Score:	0	1	2	3	4
Effectiveness					
Effectiveness in trapping sediment	Low	Moderately low	Intermediate	Moderately high	High
Pattern of low flows within the HGM unit	Strongly channelled	Moderately channelled	Intermediate	Moderately diffuse	Very diffuse
Extent of vegetation cover	Low	Moderately low	Intermediate	Moderately high	High
Extent to which fertilizers/biocides are added directly to the HGM unit	High	Moderately high	Intermediate	Moderately low	Low
Opportunity: i.e. level of phosphate input					
Level of sediment input	Low	Moderately low	Intermediate	Moderately high	High
Extent of potential sources of phosphate in the HGM unit's catchment	Low	Moderately low	Intermediate	Moderately high	High
Presence of any important wetland or aquatic system downstream	None		Intermediate Importance		High importance

4.2 FIELDWORK & LABORATORY ANALYSIS

After the establishment of the hydrogeomorphic setting by the use of maps, document analysis took place. Document analysis was based on the characteristics and processes of that specific hydrogeomorphic setting. Data collected in the field comprised of a collection of water samples and water quality variable measurements. The study area was visited twice over the course of two years; once during a flood in the late wet season (August 2015) and once during the dry season (February 2016). Eight sites were determined and marked by GPS readings as presented in **Figure 4-1**. The measurements for the water quality variables at each site were taken twice as a quality check measure. Additionally, the water samples for the laboratory were collected in quadruplicate at each site. The water quality variables that were measured on-site were: water temperature, conductivity, turbidity, pH and dissolved oxygen. HACH probes and a Hach Portable Turbidimeter were used to determine these aforesaid variables. The water samples were transported in a cooler box with ice to the laboratory at the

University of the Western Cape (UWC), where the water samples were stored in a fridge at 4˚C prior to analysis, which took place within 24 hours of collection. A short description of each site is discussed first, followed by a discussion on the water quality variables measured in the field; thereafter the water quality variables determined in the laboratories will be covered.

4.2.1 Short site descriptions

Sites (S) 1-7 were established during winter, with S8 added in summer as seen in **Figure 4-1**. S1 (Outlet) is located by the R319 road on a flat slope with a marshy grass area, S2 (Inlet) is the main river channel into the wetland at the bridge road crossing by Nacht Wacht restaurant, S3 (Agri-Road) is located in the agricultural drainage system by the road (R316) crossing, S4 (WET-Agri-Berm) is the point further downstream where the water from the wetland and field (parallel to berm) connects within the agricultural drainage system; with short restios, marshy grass, reeds (*typha capensis* and *phragmites australis*) and sedges. S5 (Agri-Drain) was approximately in the middle of S3 and S4 with the same vegetation as S4 within the agricultural drain. S6 (Inflow) was the point where the channel flowed from the inlet through the wetland and into the agricultural drainage system, with the same vegetation as S4 and S5. S7 (Wetland) was densely vegetated with reeds (*typha latifolia* and *phragmites australis*) and S8 (Agri-New) was a replacement for previously established sites that were dried up during summer. S8 was found further downstream along the agricultural drainage system with dried vegetation (reeds).

Figure 4-1: Sample Locations of Sites (S) 1 to 8

4.2.2 Field Parameters

These parameters measured in the field include pH, turbidity, conductivity, dissolved oxygen and water temperature.

4.2.2.1. pH Levels

pH is the measurement of negative $\log_{10}$ of the hydrogen ions as follows:

$$pH = -\log_{10} (H^{+})$$, which determines the acidity or alkalinity in water

Acidity (pH < 7), Alkalinity (pH > 7)

Dallas & Day (2004) suggest that as H^{+} is 10^{-7}mol. 1/L at 24˚C, pH at 24˚C would equate to 7.0; which indicates that the water is neutral. The relationship between hydrogen ions and pH thus indicates that an increase in hydrogen ions will decrease the pH, making the water more acidic and a decrease in hydrogen ions will cause the water to become more alkaline (Dallas & Day, 2004). Furthermore, the typical range for pH in South African water bodies is 6-8 (Dallas & Day, 2004).

pH within a water body (wetland) can determine the solubility and availability of biological constituents such as nutrients and heavy metals (Rao, 1989). Additionally, it has the ability to transform ammonium to a highly un-ionized toxic compound (ammonia) at pH of 8 which is another method of reducing nitrogen within a wetland (Rogers, 1983).

The temperature and geology of a water body can naturally influence pH in water. Other natural factors include the vegetation (fynbos and some forest types) and atmospheric conditions (acid rain). Human induced influences include leather industries, drainage mines and sewerage (Dallas & Day, 2004).

4.2.2.2. Turbidity

Turbidity is a visual observation of water clarity. The combined influence of turbidity and water colour, results in the impediment of light penetrating the water (Dallas & Day, 2004). The inefficient or excessive penetration of light could have ecological consequences such as death of aquatic ecosystems as they have preferences for living conditions. Furthermore, factors that influence turbidity include dissolved organic and inorganic matter and suspended organic and inorganic matter, which is responsible for the scattering and absorption of light (Dallas & Day, 2004). In addition to this, other important factors that influence turbidity are hydrology (rainfall and flow regime); which causes erosion and the possible growth of algae and the degradation of organisms in water (Rasolofomanana, 2009).

4.2.2.3. Dissolved Oxygen (DO)

Dissolved oxygen (DO) refers to the available oxygen in a water body. This parameter is particularly significant as aquatic organisms within water bodies require dissolved oxygen to survive and function (Dallas & Day, 2004). DO also affects the main processes involved in the removal of nitrogen in wetlands as these processes (nitrification and denitrification) depend on aerobic (oxygen) and anaerobic (lack of oxygen) environments (Rogers, 1983; Rasolofomanana, 2009; World Health Organization, 2011).

DO is measured in milligrams per litre (mg/L) or percentage of saturation. The fundamental factors that influence dissolved oxygen within a water body are atmospheric pressure, temperature, salinity, respiration by animals and plants, photosynthesis by plants and the decomposition of aquatic organisms (Dallas & Day, 2004).

4.2.2.4. Water Temperature

Temperature can be defined by how hot or cold the water is. Water quality implications associated with temperature are the reduction in dissolved oxygen and an increase in chemical toxicity with an increase in temperature (Quagliano & Vallarino, 1969; Dallas & Day, 2004). This usually has a greater implication on the aquatic species within the water body as well as the pathogens within the water. Temperature defines the growth or death of pathogens, for example *Escherichia coli* prefer warmer conditions for growth and survival such as 35˚C and up. A possible source that would influence the temperature variation is industrial discharges (Dallas & Day, 2004).

4.2.2.5. Conductivity

The total amount of dissolved material in the water is one of the most fundamental components of water quality. This component can be measured as total dissolved solids (TDS), conductivity or salinity due to their close correlating relationship (Dallas & Day, 2004). For the purpose of this study conductivity and TDS will only be considered, but the relationship between the three terms will be defined.

Salinity refers to how saline the water is, which is determined by a chloride cation. TDS on the other hand, is defined by the measure of the total amount of material dissolved in water;

the mass of these materials is usually inorganic ions such as sodium & potassium cations and HCO^- & carbon dioxide anions. Finally, conductivity is the measurement of the ability of water to conduct electricity, by determining the number of charged particles (ions) in a solution. Although there may be small variations between these three components due to conductivity variation in ions, the relationship between these components is close enough to utilize any one of these components individually (Dallas & Day, 2004). Furthermore, Dallas & Day (2004) reflect the close relationship between conductivity and TDS for south western-cape waters by the following equation:

$$\text{TDS (mg/L)} = \text{Conductivity (mS m/L)} * 5.5$$

4.2.3 Laboratory Parameters

The four water samples were divided equally between the Earth Sciences and Biotechnology department at UWC. In the Earth Sciences Department, a Spectrophotometer was used to determine the nutrients (nitrate, nitrite, ammonia and orthophosphate) within the water sample, while in the Biotechnology Department; the Most probable Number 5 (MPN5) Test was used to determine coliforms, specifically *Escherichia coli*. The materials that were used for these experiments are listed in **Appendix A**, while the variables used in the laboratory are explained below.

4.2.3.1. Nutrients: Nitrate, Nitrite, Ammonia and Phosphate

Phosphorus and Nitrogen are the most limiting and essential nutrients for the growth of plants (Dallas & Day, 2004). Phosphorous and nitrogen are found in both organic and inorganic forms within the Nitrogen and Phosphorus cycle, within wetlands (Mitsch & Gosselink, 2007). Furthermore, for the purpose of this study, phosphorous is measured as bio-available orthophosphate; while nitrogen will be measured as inorganic nitrate-nitrogen, nitrite-nitrogen and ammonia-nitrogen due to being the most common anthropogenic sources (Dallas & Day, 2004). Nutrients usually affect the quality of water by excessive eutrophication causing harmful algae blooms (Burgin & Hamilton, 2007), which may in turn affect the turbidity and dissolved oxygen of the water body or gives rise to the displacement of native species by providing invasive plants the competitive advantage to grow (Zelder & Kercher, 2005).

4.2.3.2. *Escherichia coli* coliforms

With poor water quality, comes the implications on the health and well-being of humans. These implications are caused by waterborne diseases such as diarrhoea and gastrointestinal diseases caused by pathogens which include enteric bacteria, viruses or protozoa (Stevens *et al*, 2003). Pathogens are defined by biological agents, which instigate illness or diseases to its host (Stevens *et al*, 2003). Furthermore, indicators have been developed for the determination of the presence or absence of pathogens, including total coliforms, faecal coliforms, enterococci and *Escherichia coli* (Ishi & Sadowsky, 2008). In order for these indicators to be effective, they have to i) exist in the intestinal tracts of warm blooded animals, ii) be present when pathogens are present, iii) be absent when pathogens are absent, iv) be in greater multitude than that of the pathogens, v) have the ability to survive equivalently to that of the pathogens and vi) have the inability to multiply in the environment. Finally, they need to be detected by easy, rapid and cost effective methods, and be non-pathogenic (Ishi & Sadowsky, 2008). This study focuses on two indicators for the presence of pathogens, namely Total Coliforms and *Escherichia coli.*

4.2.3.3. Total Coliform Indicator

Coliforms are defined by lactose fermentation and the production of acid or aldehyde within 24 hours at approximately 35˚C, as well as the production of enzyme ß-galactosidase. Coliforms are gram-negative Enterobactericeae, which includes *Escherichia coli, Entrobacter*, *Klebsiella* and *Citrobactor* genera's (Ishi & Sadowsky, 2008) (**Table 4-2**).

Table 4-2: The family, genres and species of common coliforms (Stevens *et al*., 2003)

Family	Genres	Species
Enterobacteriaceae	*Escherichia*	*Escherichia coli (E. coli)*
	Klebsiella	*Klebsiella pneumoniae (K. pneumoniae)*
	Enterobacter	*Enterobacter amnigenus (E. amnigenus)*
	Citrobacter	*Citrobacter freundii (C. freundii)*

The significance of the Total Coliforms indicator is the survival and growth of the organisms within the water (World Health Organization, 2011). The relevance of this indicator to this study is to determine the total coliforms per 100ml at specific sites.

4.2.3.4. *Escherichia coli* Coliform Indicator

Escherichia coli coliforms are rode shaped, gram-negative bacterium, it is one of the species found under the *Escherichia* genera, within the Enterobactericeae family (Steven *et al*, 2003; Ishi & Sadowsky, 2008). *Escherichia coli* were discovered in 1885 by Theodor Escherichia; however, back then, he named it *Bacterium coli* (*B. coli*). He used *Bacterium coli* as an indication for pollution in water, and used its ability to produce acid and gas from lactose to differentiate or set it apart from another bacterium (Steven *et al*. 2003).

Escherichia coli is known as thermotolerant due to its ability to ferment lactose (production of gases or acids) at approximately 45˚C, and can be distinguished from other thermotolerant coliforms by its ability to produce enzyme ß-glucuronidase and indole from tryptophan (World Health Organization, 2011). Faeces and therefore sewerage water, is a common source of *Escherichia coli*. *Escherichia coli* have therefore become a significant faecal indicator because of its rare absence in the absence of faecal pollution (World Health Organization, 2011).

4.2.4 Spectrophotometer DR 6000

The Spectrophotometer DR 6000 has UV and Visible Spectrum capabilities with integrated quality assurance software and it can store more than 250 pre-programmed methods; including the programmes for nitrite, nitrate, ammonia and orthophosphate (**Figure 4-2**). The machine also included a user friendly manual, but reagents for each parameter were not included and had to be chosen carefully before purchasing.

Figure 4-2: DR 6000 Spectrophotometer (Hach Company, 2015)

4.2.4.1. Nitrate: Method 10020 Test 'N Tube Vials

In order to measure the presence and concentration of nitrates in the water sample, the program was first set on the Spectrophotometer to 344 N, Nitrate HR, and TNT. After which 1.00ml of the field sample was added to a NitraVer X Reagent A Test 'n Tube vial in order to prepare the 'blank sample'. The 'blank sample' within the vial, was inverted approximately ten times to mix the reagents, and then inserted into the Spectrophotometer to be 'Zeroed', and then removed from the machine. Meanwhile, the 'prepared sample' was then readied by adding one NitraVer X Reagent B Powder Pillow to the vial, by means of using a funnel.

After this, the vial was then inverted approximately ten times in order to mix the contents. A five minute timer was activated on the spectrophotometer to allow for the contents within the vial to react. Once the time expired, the vial (‘prepared sample’) was whipped and inserted into the machine to be ‘Read’. The reading was displayed on the machine in mg/L and then recorded. This procedure was repeated another thirteen times, twice for each of the seven sites.

4.2.4.2. Nitrite: Method 8153 Powder Pillows

For the nitrite measurement the spectrophotometer was programmed to 373 N, Nitrite HR PP. Thereafter, a sample cell was filled with 10ml of sample water, with the addition of NitriVer 2 Nitrite Reagent Powder Pillow to ready the ‘prepared sample’. The expected colour of the reaction in the sample cell was green, only if nitrite was present. A stopper was placed on the sample cell, after which, the sample cell was then shaken to dissolve the contents. A ten minute timer was activated on the machine to allow for the reaction of the contents. Meanwhile, the ‘blank sample’ was prepared separately, by adding 10ml of sample water. The ‘blank sample’ was then whipped and inserted into the machine to be ‘Zeroed’. After the ‘blank sample’ was ‘Zeroed’, the ‘prepared sample’ was then whipped and inserted into the machine to be ‘Read’. The reading was displayed on the machine in mg/L and then recorded. This procedure, as before mentioned, was repeated another thirteen times, twice for each of the seven sites.

4.2.4.3. Ammonia: Method 8038 Reagent Solution

To determine the ammonia content, the program was set to 380 N, Ammonia, and Ness on the spectrophotometer. 25ml of the water sample was added to a plastic test tube for the preparation of the ‘prepared sample’. Furthermore, 25ml of deionized water was added to a separate plastic test tube for the concoction of the ‘blank sample’. Thereafter, three drops of Mineral Stabilizer were distributed to both plastic test tubes. Both tubes were then closed and inverted numerous times to mix the contents. Additionally, three drops of Polyvinyl Alcohol Dispersing Agent were distributed into both tubes. Hereafter the tubes were closed and inverted numerous times to mix the contents. After the contents were mixed, 1.00ml of Nessler Reagent was dispersed into both tubes using a pipette. The tubes were once again closed and inverted numerously to mix the contents. A one minute timer was activated on the spectrophotometer to allow for the reaction of the contents in the tubes. After the time expired, 10ml of the ‘blank sample’ was poured into a sample cell and 10ml of the ‘prepared sample’ was poured into a separate sample cell. The sample cell which contained the ‘blank sample' was then whipped and placed in the machine to be 'Zeroed', after which it was removed. The sample cell which consisted of the 'prepared sample' was then whipped and placed into the machine to be 'Read'. The reading was, as before said, displayed on the machine in mg/L and then recorded. This procedure was repeated another thirteen times, twice for each of the seven sites.

4.2.4.4. Orthophosphate: Method 8114 Reagent Solution

Lastly, the spectrophotometer was programmed to 480 P, React, Mo for the measurements of phosphates. The 'blank sample' was again prepared first by filling a sample cell with 10ml of deionized water. The 'prepared sample' was then readied in a separate sample cell by adding 10ml of the water sample. 0.5ml of Molybdovanadate Reagent was then added to each sample cell, after this, both cells were swirled. A seven minute reaction timer was activated on the spectrophotometer. Once again, after the time expired, the 'blank sample' was whipped and placed into the machine to be 'Zeroed' and thereafter, removed. Hereafter, the 'prepared sample' was then placed into the machine to be 'Read'. The reading was once again displayed on the machine in mg/L and then recorded. This procedure was repeated twice for each of the seven sites.

4.2.5 Test for Microorganisms

4.2.5.1. Most Probable Number 5 (MPN5) Test: Coliforms

The MPN5 test is divided into three components, namely: the Presumptive, Confirmed and Completed test as seen in **Figure 4-3** (Harley & Prescott, 2002).

i) Presumptive Test

This presumptive test was done to determine whether Coliforms were present or absent by the presence of gas production.

In total fifteen lab tubes were used and each tube contained a Durham tube, within which the gas production took place. Ten of the fifteen tubes contained single-strength lactose broth (SSLB) and five tubes contained double-strength lactose broth (DSLB). The first five SSLB tubes were inoculated with 0.1ml of the sample water, whereas the other five SSLB tubes were inoculated with 1ml of the sample water. The DSLB tubes were each inoculated with 10ml of sample water and incubated at 35˚C for 24 to 48 hours. The Durham tubes were **positive** for Coliforms when gas bubbles were observed. The quantity of Coliforms was determined by comparing the positive tubes with the MPN Index, which gave an estimation of

Coliforms per 100ml. This procedure was repeated as a result of the two water samples collected at each site.

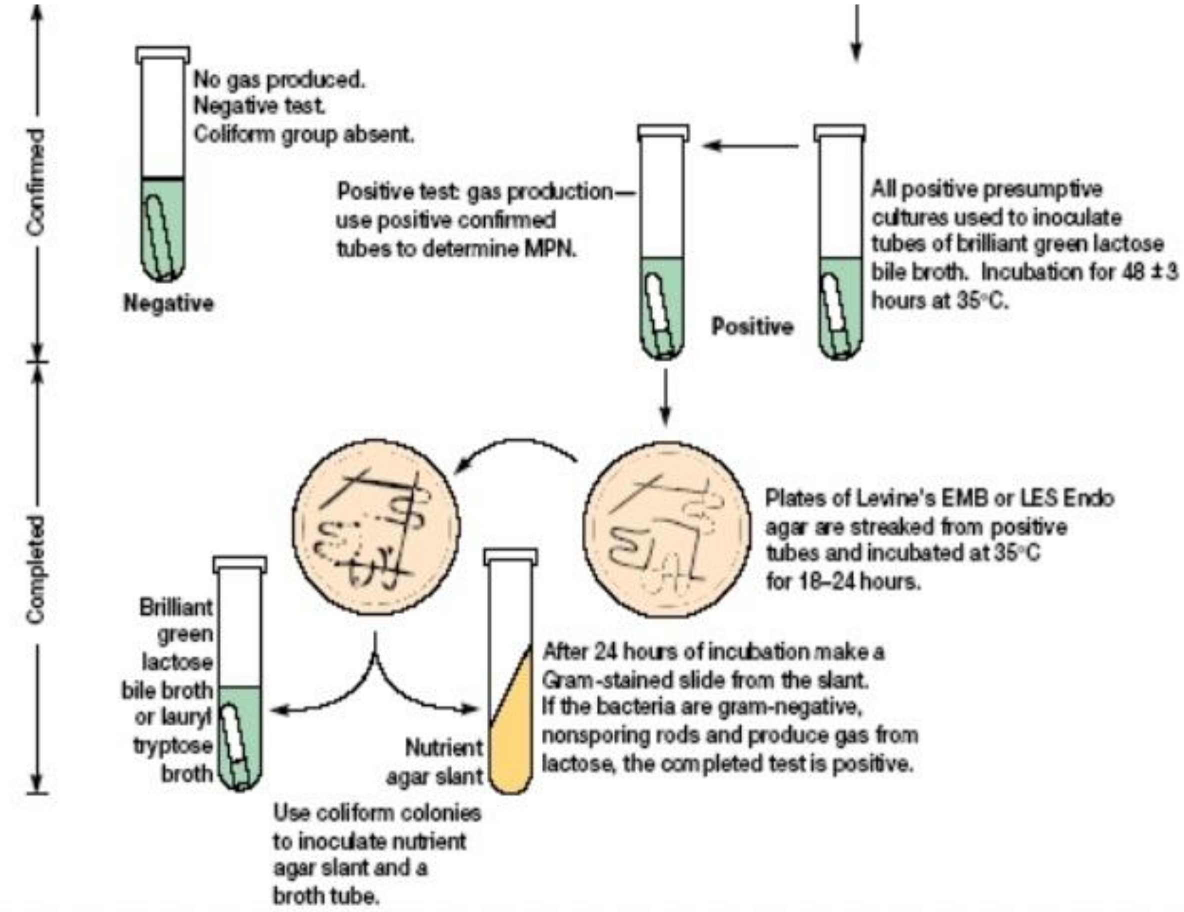

Figure 4-3: Schematic Diagram of the Most Probable Number Test (Harley & Prescott, 2002)

ii) Consumptive Test

The consumptive test confirms the presence or absence of Coliforms by the production of gas by using brilliant green bile broth (BGBB).

A sterile inoculating loop was used to inoculate the BGBB tubes with the **positive** 0.1ml SSLB tube. This step was then repeated for each site and for the duplicates. The tubes were then incubated at approximately 35˚C for 24 to 48 hours. The presence of gas production indicated a positive confirmed test, confirming that Coliforms were present.

iii) Completed Test

The purpose of the completed test was to identify the types of Coliforms present. The positive BGBB was streaked on Eosin-Methylene Blue (EMB) plates using a sterile inoculating loop. The EMB plate came with an index sheet where the variation in the colour of the agar (caused by the coliform metabolizing the agar), represented its type. For example, according to the research metallic green and blue/black colonies produced on an EMB Agar plate would indicate *Escherichia coli* (Zimbro *et al*., 2009). The EMB streaked plates were incubated at 35˚C for 24 hours, after which, the identifications of coliforms was accomplished. Isolated colonies from the EMB plates were streaked onto the Nutrient Agar plates with a sterile inoculating loop for gram-staining and thereafter incubated for 24 hours.

iv) Gram-Staining

The gram-staining test was utilized in order to determine whether the coliforms were gram-positive or gram-negative since *Escherichia coli* is gram-negative.

Gram-staining was predicated on the capability of microorganisms to retain colour. The process included the inoculation of one isolated colony from the nutrient agar plates onto microbial slides, and staining with crystal violet dye, safranin, iodine, distilled water and alcohol. Gram-positive microorganisms retain the crystal violet stain and are seen as purple microorganisms when viewed under the microscope, while gram negative microorganisms do not retain the stain and are therefore pink in colour.

The next test discussed were tests to confirm the MPN5 test.

4.2.5.2. API 20E Test

The API 20E test was used for the confirmation or identification of coliforms that could not be identified by the EMB Agar plates and gram-staining.

A single isolated colony was transferred from the nutrient agar plate (used after the completed test) to 5ml of saline with a sterile pipette, to prepare the suspension. After preparation, the suspension was distributed into the tubes of the API strip with a sterile pipette according to the manufactures instructions. The incubation procedure was also carried out according to the same instructions. The results were then compared to a provided Chart Index to indicate positivity or negativity. This was recorded to an API sheet where the positive tubes' values were calculated. The sequence of the calculated numbers was then associated to an index provided by a textbook to identify the type of coliform the colony represented.

4.3 AN INTEGRATED APPROACH

The overall theme for this project falls in line with 'water quality and wetlands', specifically whether or not the Karsriviervlei alters / improves the water quality of the Kars River downstream. This is evaluated using both the WET-EcoServices Assessment as well as doing fieldwork with laboratory analysis.

The WET-EcoServices Assessment is a rational descriptive approach to determine whether or not the Karsriviervlei improves the quality of water (see section 4.1). This is done by assessing the importance of the Karsriviervlei based on how well the wetland can provide significant water quality services such as nitrate and phosphate removal. Meanwhile, the fieldwork and laboratory analysis is a quantitative approach to answer this research question (see section 4.2). In this approach, physical evidence is provided on spatial and temporal variation in the quality of water.

Step-by-step instructions on how to apply the Integrated Approach for this study *(best used side-by-side)*

Step 1: WET-EcoServices Assessment sets a foundation for the researcher before going into the field as it relies mainly on a desktop analysis of available data.
This will not suffice for the project alone, as it will not cover all of the constituents covered in the study (e.g. physical parameters and Escherichia coli).

Step 2: The WET-EcoServices includes observation made in the field, which are then compared to the results of the desktop analysis. Any additional observations will contribute to the initial desktop assessment.
This will only be effective if the researcher has worked through the assessment itself. Alternatively, observations can be made by Google Earth, but it will not be as effective without actually working through the assessment first.

Step 3: Fieldwork includes recording observations made and measurements taken in the field as well as collecting sampled water for laboratory analysis.
Some of the observations made in the field include high vegetation cover, presence of an agricultural drainage system.

Field parameters for this study include water temperature, pH, conductivity as well as turbidity. Chemical and biological parameters tested in the laboratories include nitrate, nitrite, ammonia, orthophosphate, coliform count and the presence of Escherichia coli.

For further detailed information on the methodology and results of all the above mentioned parameters see Chapters 4 and 5

Step 4: (*The assessment can be completed while step 4 is taking place or after*) Compare the findings of the fieldwork and laboratory analysis with the WET-EcoServices Assessment's results to determine if they complement each other or differ.

The comparison of these findings observed whilst using this approach will be discussed in Chapter 6.

5. RESULTS

The following chapter describes the spatial variation in results of the physico-chemical and biological water quality parameters, for the two sampling dates. Furthermore, the chapter also presents the results of the WET-EcoServices Assessment for Karsriviervlei.

5.1 DESCRIPTION OF THE ENVIRONMENTAL CONDITIONS

The environmental conditions varied with each trip due to the different seasons. The first trip was taken during a flood in the late wet season (winter) and the second trip was taken during the dry season (summer).

Figure 5-1: Flooded agricultural drainage system

Figure 5-2: Dried-up agricultural drainage system

During the winter sampling trip the Kars River was at bankfull level and the unchannelled part of the wetland was inundated by a shallow flood. Floodwaters had spilled towards the agricultural drain, and the flow in this drain was of sufficiently high velocity to produce runs (**Figure 5-1**) and rapids in parts of the channel with abrupt drops in elevation (small headcuts). In contrast, during the summer sampling trip, flow in the Kars River was barely perceptible and water in the agricultural drain had largely dried up, leaving a chain of disconnected pools (**Figure 5-2**). The hot and dry conditions also meant that several of the winter sampling sites were no longer available, and site 8 was added at a point of available surface water located as close as possible to the nearest winter sampling location. The vegetation cover across the wetland was moderately high and the identified vegetation types and species included: *Phragmites australis*, *Typha capensis*, *Sarcocornia sp.*, *Cyperus sp.* and *Restionaceae and*. *Sarcocornia sp*.

5.2 PHYSICAL PARAMETERS

5.2.1 Water Temperature

The water temperature in summer was at an average of 28.7˚C as compared to an average of 15.7˚C during winter.

a) Winter

During winter the temperature of water varied from 16.2 °C at the inlet to 14.9 °C at the outlet (**Figure 5-3**). However, the water temperature increased rapidly at site 5 and thereafter, slight fluctuations in the temperature of water were observed along the agricultural drainage system to the outlet.

b) Summer

The temperature of water increased greatly from the inlet (25.4˚C) to the agricultural drainage system (30.3˚C) by 4.4˚C (**Figure 5-4**). After which, the temperature remained constant throughout the area sampled.

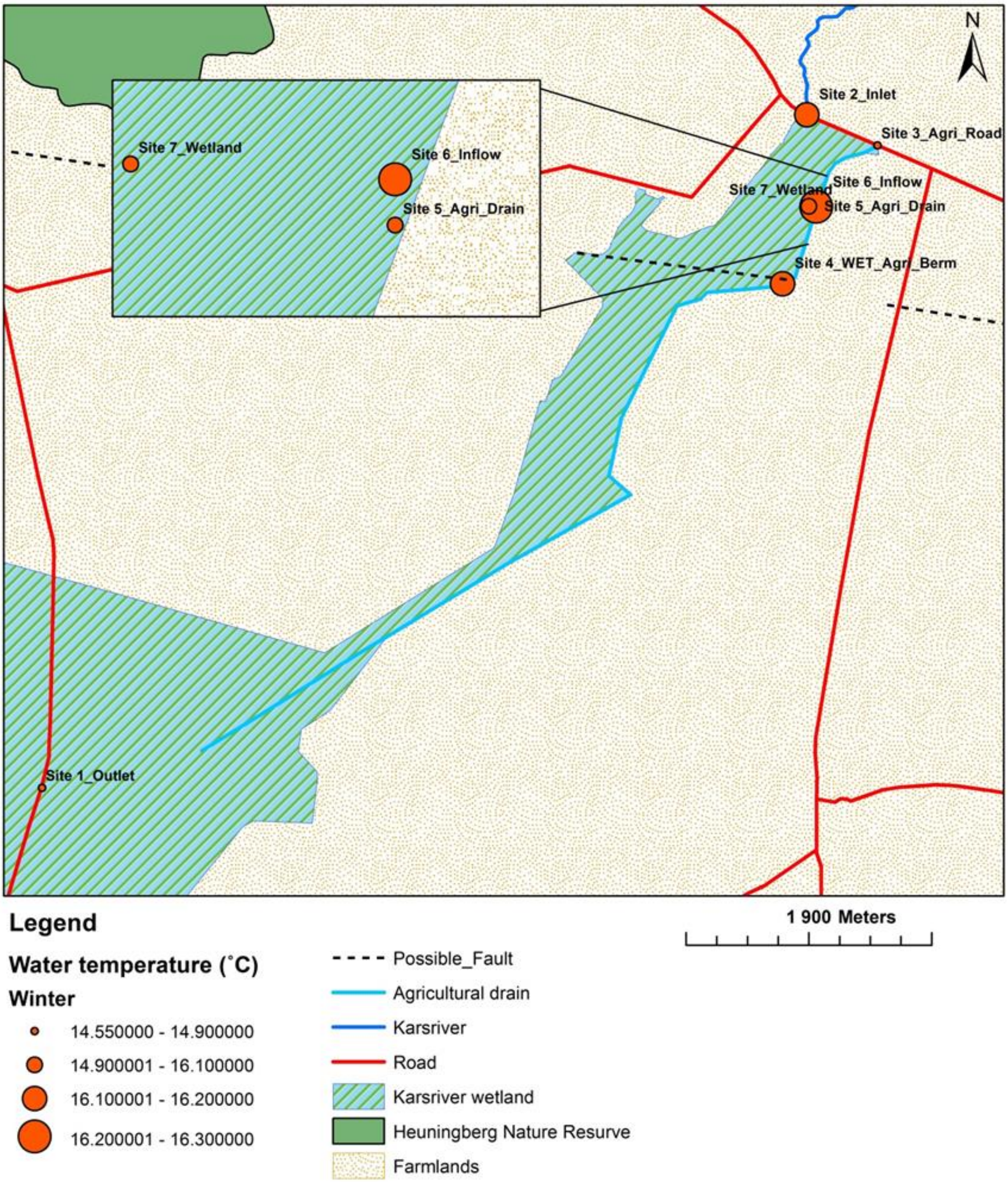

Figure 5-3: Water temperature of the Karsriviervlei during winter

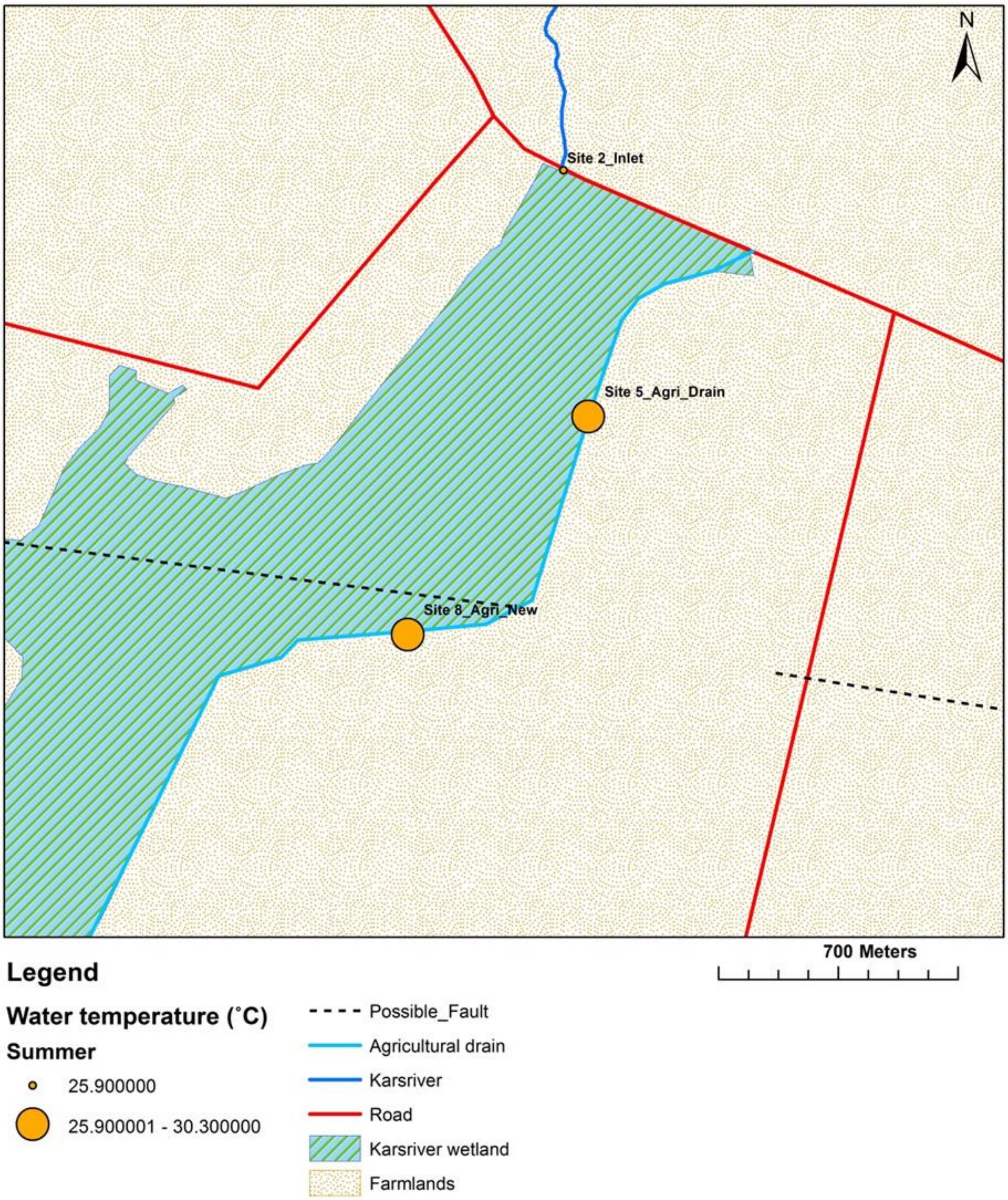

Figure 5-4: Water temperature of the Karsriviervlei during summer

5.2.2 pH Levels

The results indicated that Karsriviervlei was more alkaline during summer as compared to winter (**Figure 5-5** & **Figure 5-6**). Additionally, pH levels remained relatively constant during winter, whereas the pH levels increased across sites 2, 5 and 8.

a) Winter

The results as seen in **Figure 5-5** indicated that the Karsriviervlei was slightly alkaline with a relatively constant pH level in the range of 7.3 to 7.6, despite the marginal variation in the pH level throughout the spatial distribution.

b) Summer

Even though the pH levels indicated Karsriviervlei was still alkaline during summer, it was still significantly higher with further increases. These increases were observed at all the selected sites (2. 5 and 8), with pH levels of 8.22, 8.45 and 9.105.

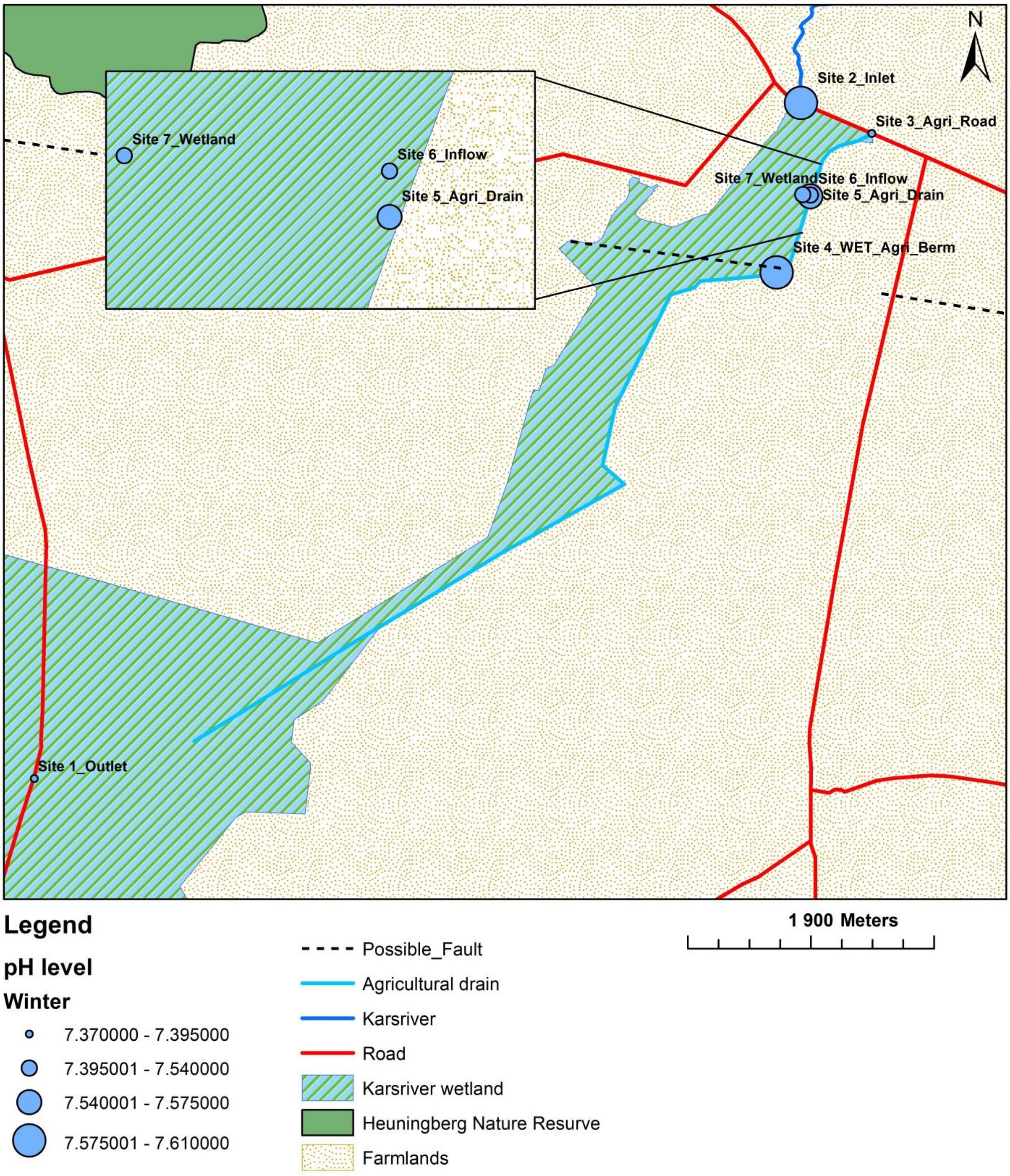

Figure 5-5: pH levels within the Karsriviervlei during winter

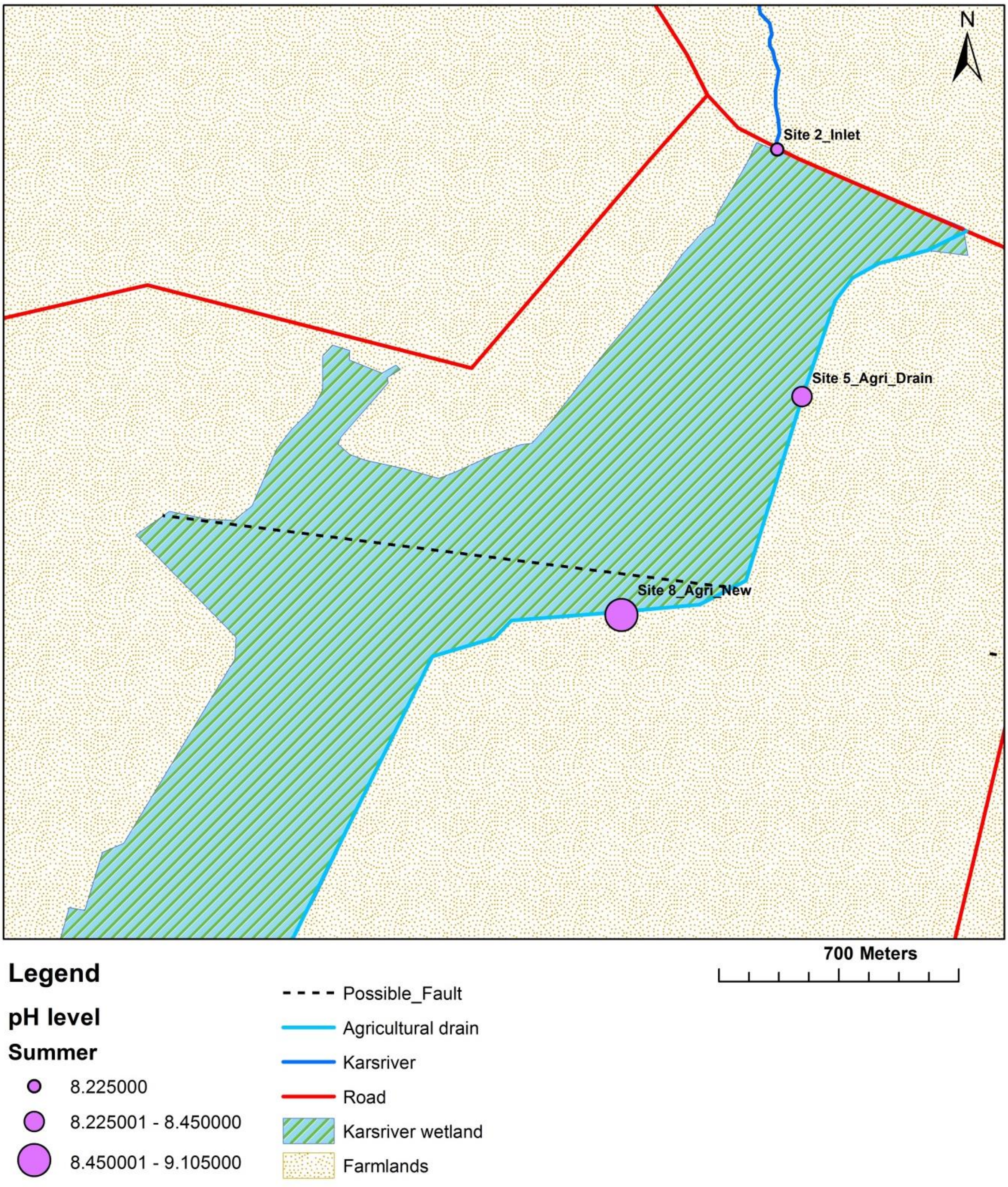

Figure 5-6: pH levels within the Karsriviervlei during summer

5.2.3 Conductivity

The average levels of conductivity measured during summer (8078µs/cm) were almost double the average measurements taken during winter (4149µs/cm).

a) Winter

The conductivity levels within the study area decreased continuously from the inlet and all along the agricultural drainage system (including sites 2, 3 and 5) with measurements of 4439µs/cm, 4194µs/cm and 3970µs/cm (**Figure 5-7**). These results also indicated a significant increase in conductivity levels within the channel flowing from the wetland into the agricultural drainage system at site 6 (5163µs/cm). However, the conductivity levels continued to decline within the wetland at site 4 and declined even more at the outlet with a measurement of 2794.5µs/cm.

b) Summer

During summer, the levels of conductivity rose greatly from the river inlet (5495µs/cm) to site 5 (10295µs/cm) as seen in **Figure 5-8**. However, the levels of conductivity decreased at site 8 - further down the agricultural drainage system to 8445µs/cm.

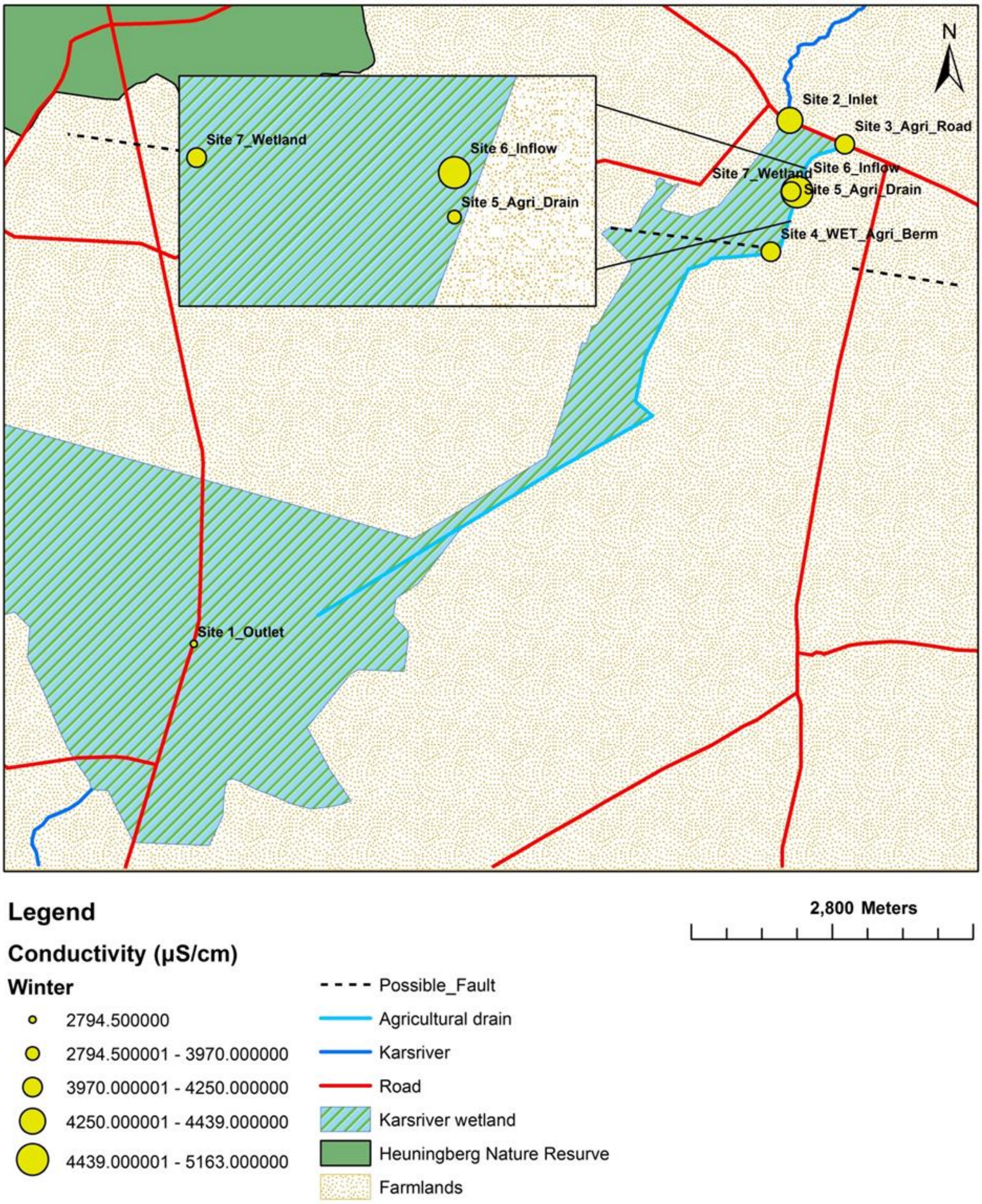

Figure 5-7: Levels of Conductivity within the Karsriviervlei during winter

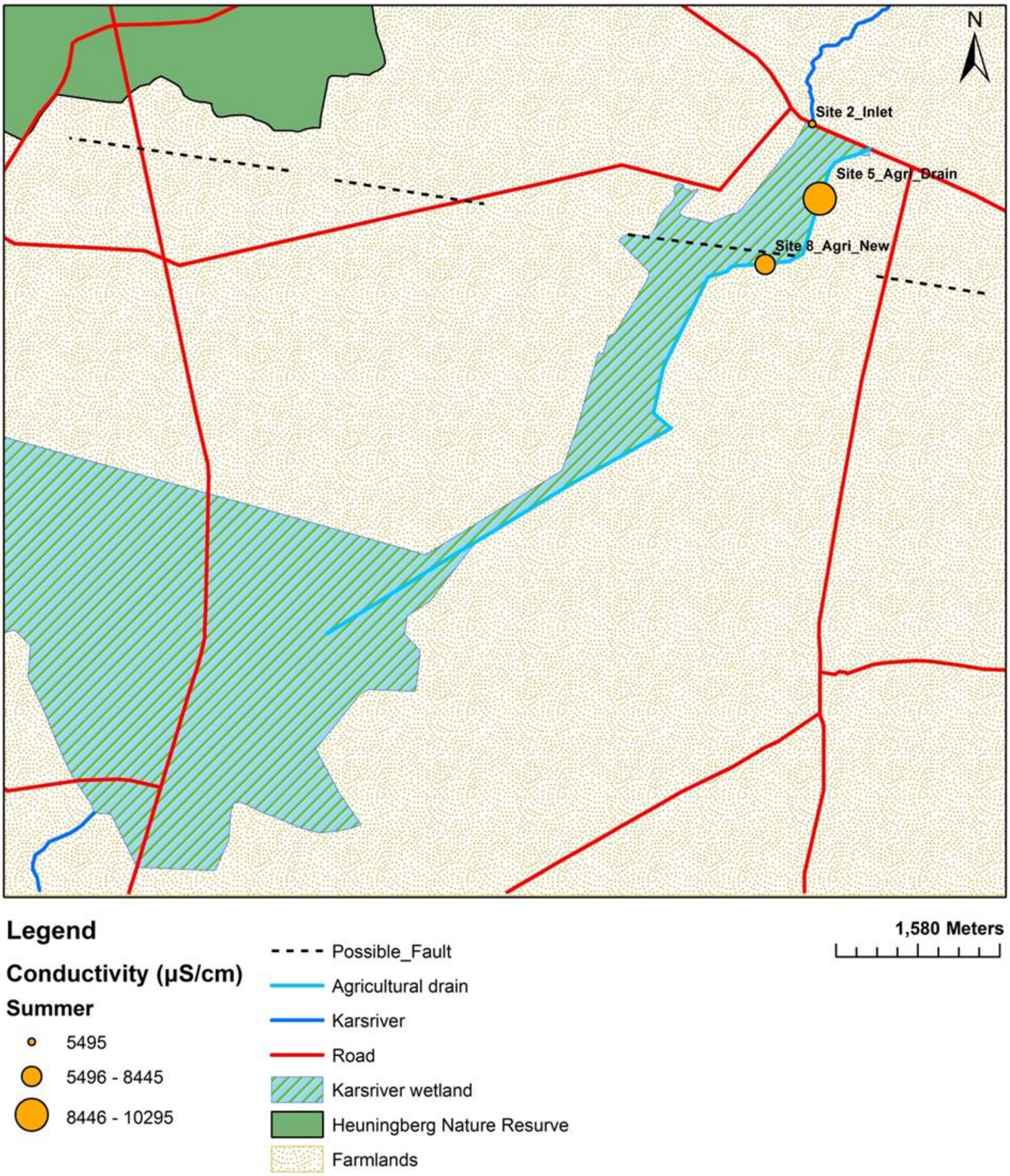

Figure 5-8: Levels of Conductivity within the Karsriviervlei during summer

5.2.4 Turbidity

The levels of turbidity decreased from the inlet (34.45NTU) to the outlet (14.95NTU) during winter, whereas the levels of turbidity increased during summer from the inlet (20.4NTU) to site 8 (27.3NTU).

a) Winter

A large increase in turbidity was observed at site 3, with a level of 69.15NTU as compared to 34.45NTU at the river inlet (**Figure 5-9**). Thereafter, the levels of turbidity decreased steadily from site 5, 6 and 7 with 28.4 NTU, 20.4 NTU and 19.1 NTU respectively. Despite the continuous decrease, a significant increase was observed from 19.1NTU to 45.1 NTU at site 4. This increase was followed by a rapid drop to 14.95NTU at the outlet.

b) Summer

The clarity of water was found to have degraded from the river inlet to site 5 as shown in **Figure 5-10**. This was due to the increase in turbidity from 20.4NTU to 27.85NTU. However, it was also observed that the levels of turbidity dropped slightly to 27.3NTU further downstream at site 8.

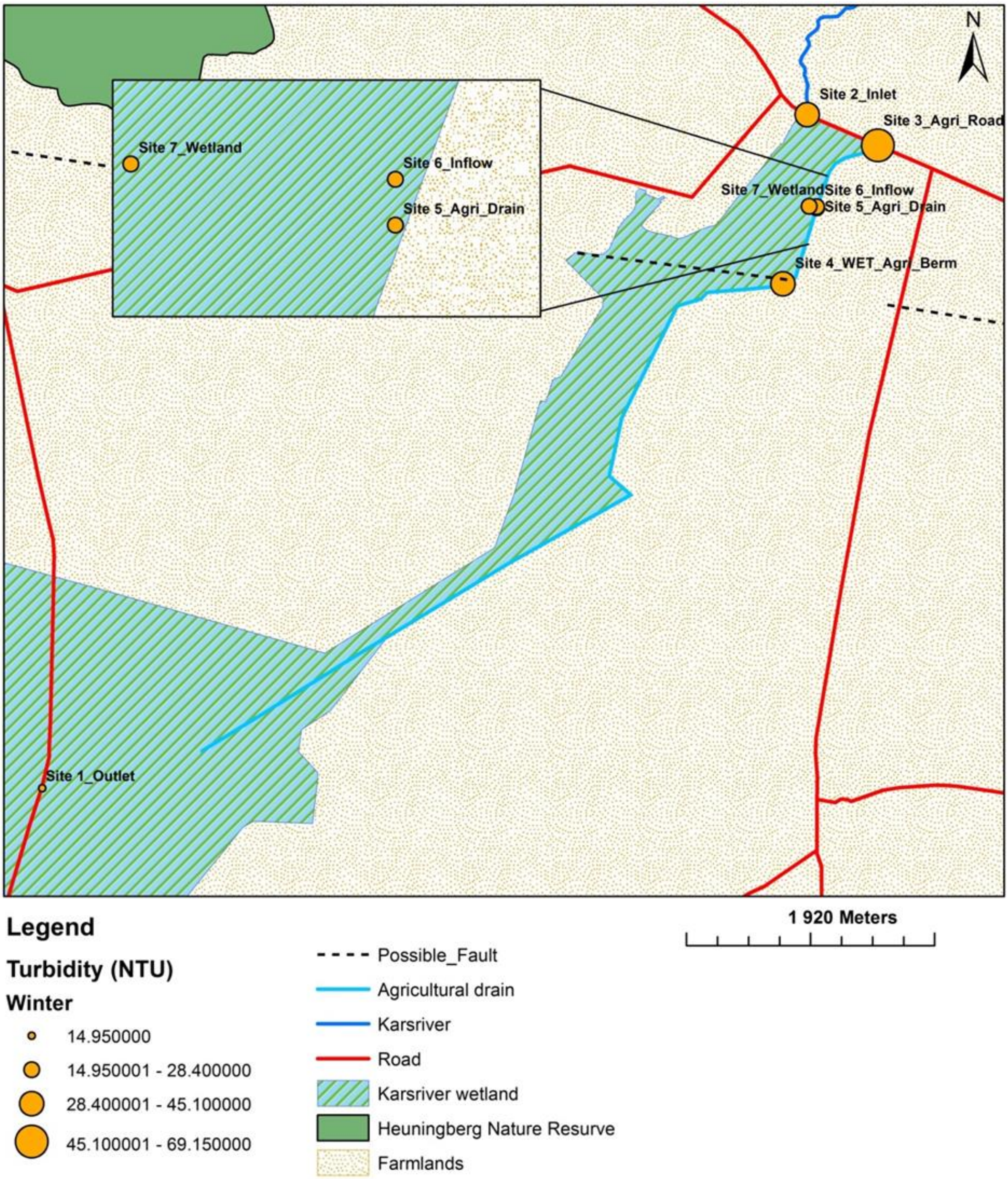

Figure 5-9: Level of turbidity within the Karsriviervlei during winter

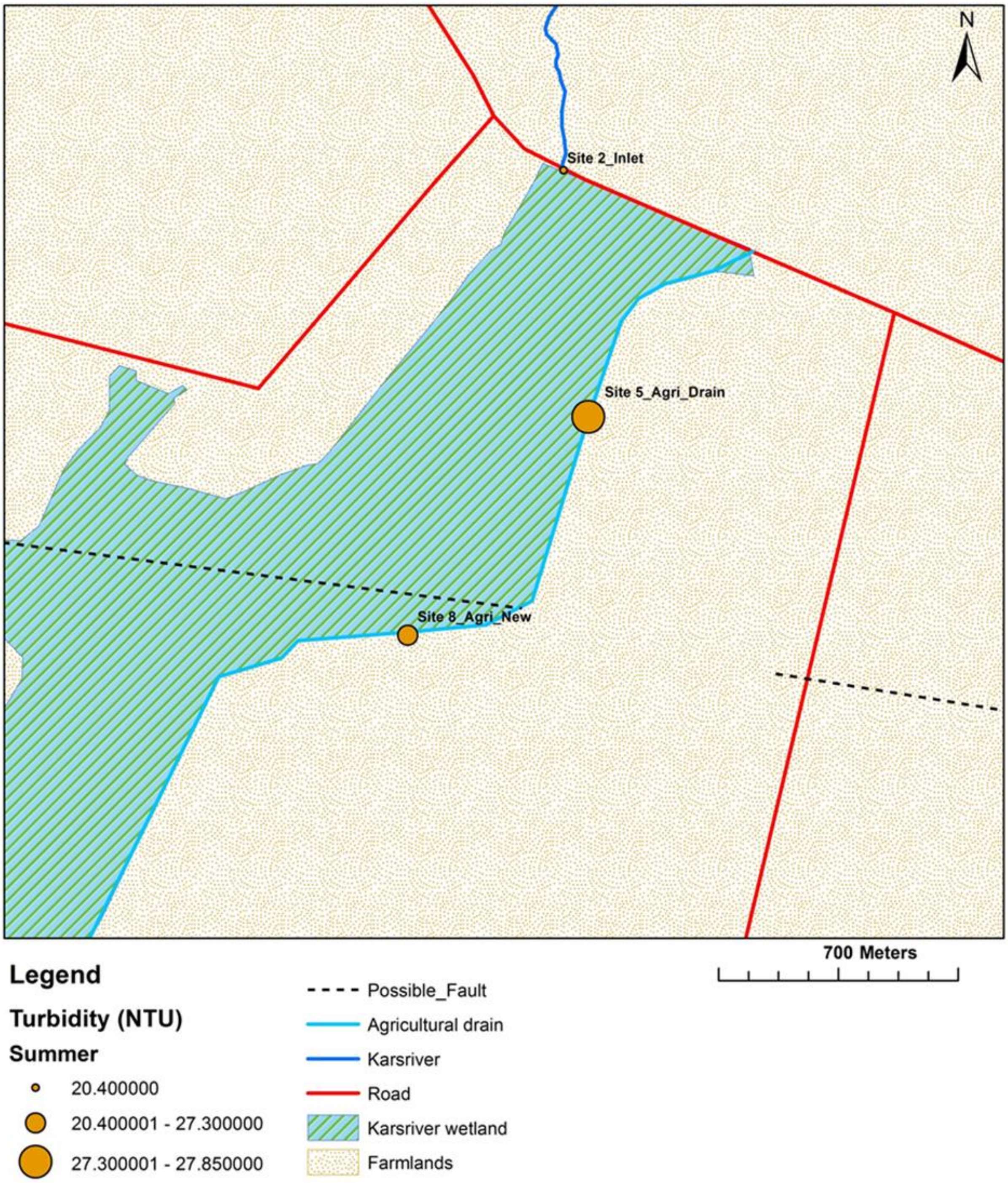

Figure 5-10: Level of turbidity within the Karsriviervlei during summer

5.2.5 Dissolved Oxygen (DO)

The average concentrations of dissolved oxygen were found to be much higher during summer (8.54mg/L) than in winter (6.9mg/L).

a) Winter

In winter continuous fluctuations in the dissolved oxygen (DO) were observed throughout the spatial distribution (**Figure 5-11**). The concentration of DO was less at site 3 within the agricultural drainage system (6.13 mg/L) compared to the concentration of DO at the river inlet (7.88 mg/L). Furthermore, noticeable variations were observed at site 3, 5 and 6 with concentrations of 6.13mg/L, 6.86mg/L and 6.63mg/L respectively. The concentration of DO then dropped within the wetland (5.63mg/L) at site 7, followed by a rapid increase at site 4 with a concentration of 8.09mg/L. Finally, the outlet was observed to have a decrease in the concentration of DO to 7.37mg/L.

b) Summer

The concentration of dissolved oxygen perpetually increased throughout the spatial distribution (**Figure 5-12**). However, there was a substantial increase from the river inlet (6.01mg/L) to the agricultural drainage system. DO concentrations were measured to be 9.26mg/L and 10.35mg/L at sites 5 and 8 respectively.

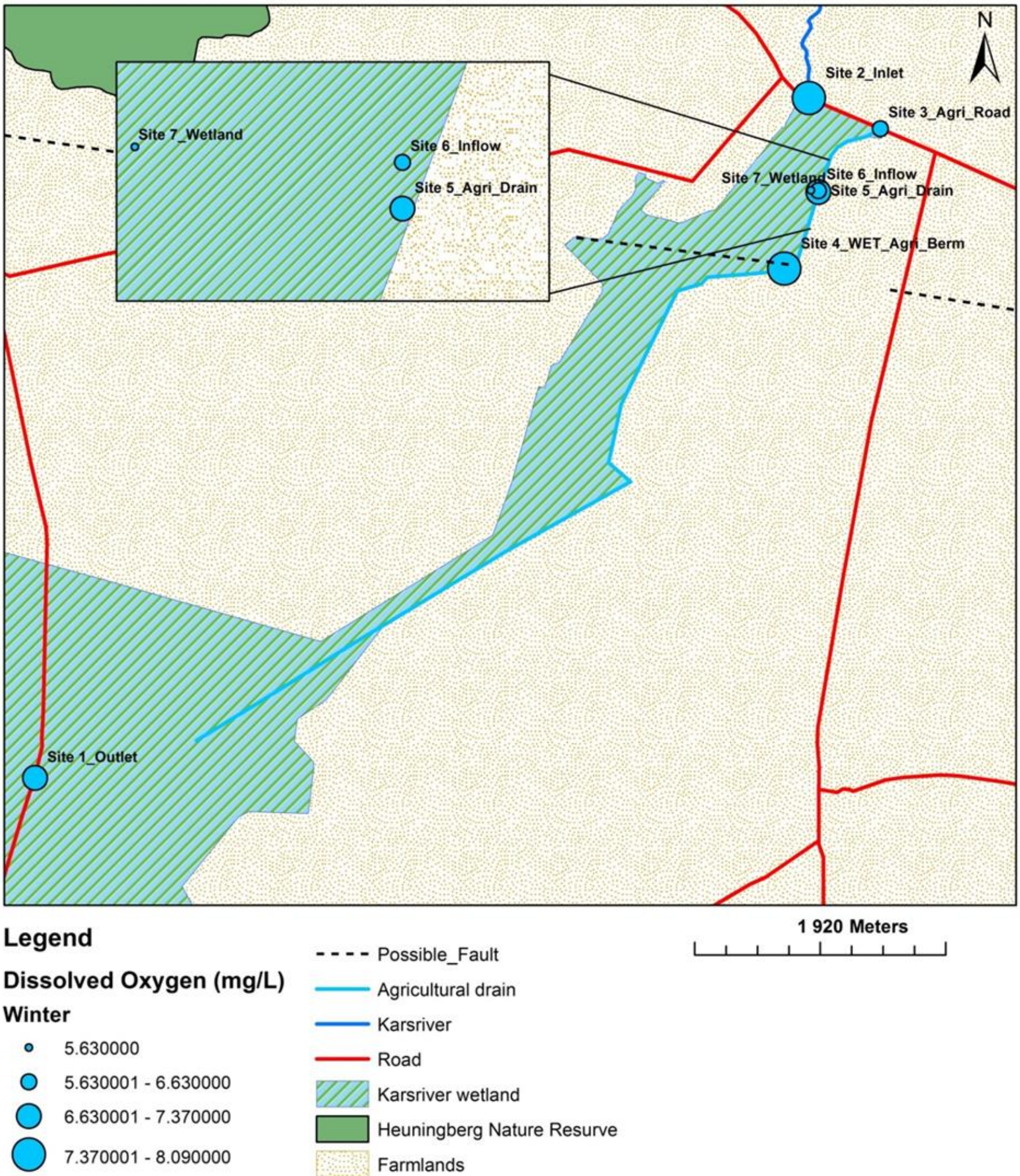

Figure 5-11: Concentration of DO within the Karsriviervlei during winter

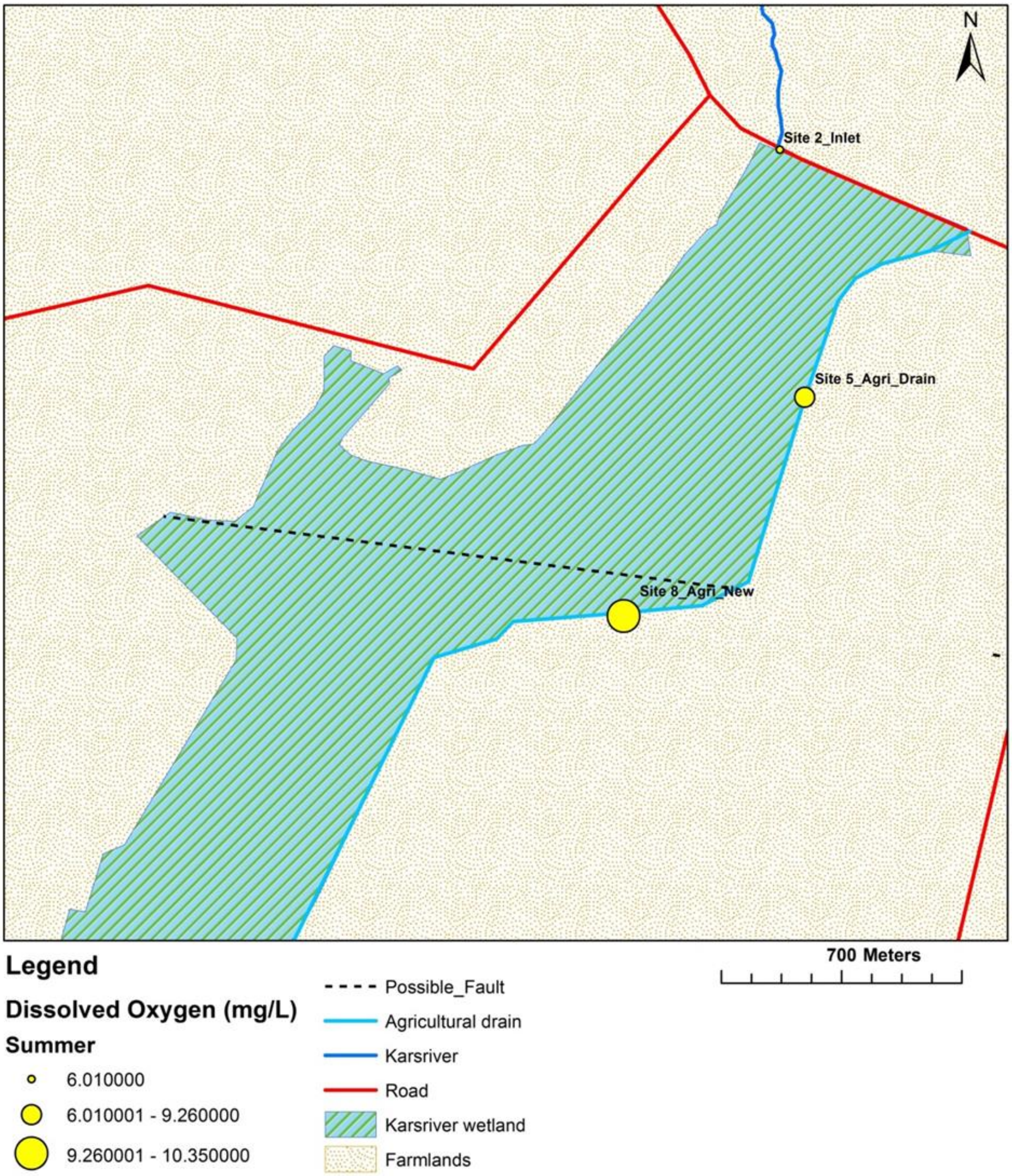

Figure 5-12: Concentration of DO within the Karsriviervlei during summer

5.3 CHEMICAL AND BIOLOGICAL PARAMETERS

This section describes the results of the chemical and biological water quality parameters; especially their established concentrations and/or presence throughout the spatial distribution. The chemical parameters included nitrites, nitrates, ammonia and orthophosphates which were determined using a Spectrophotometer. Additionally, the biological parameters included Coliforms and *Escherichia coli* which were established using the Most Probable Number and API 20E tests.

5.3.1 Nitrates

a) Winter

Based on the laboratory results, fluctuations in the concentration of nitrates were observed from the river inlet to the outlet as presented in **Figure 5-13**. These positions included sites 3 , 5 and 6 with concentrations of 1.25mg/L, 0.9mg/L, 1.3mg/L and 0.95mg/L respectively. There was no change in the concentration of nitrates at site 7 as compared to site 6. Hereafter, there was a steady reduction from site 7 (within the wetland) to site 4, followed by a rapid decrease at the outlet from 0.75mg/L to 0.4mg/L. Additionally, the concentration of nitrates at the outlet (0.4mg/L) decreased more than half its original amount at the inlet (1.25mg/L).

b) Summer

During summer, the concentration of nitrates continuously increased from the river inlet (0.3mg/L) to site 5 (0.35mg/L), then increased even more at site 8 (4mg/L) (**Figure 5-14**).

5.3.2 Nitrites

The results showed that the concentration of nitrites increased from the inlet to the outlet during winter and increased from the inlet to site 8 during summer.

a) Winter

In **Figure 5-13**, the concentration of nitrites showed a continuous variation throughout the spatial distribution. Also, the concentration of nitrites was found to have increased from the river inlet (6.5mg/L) to the outlet (9.5mg/L). The nitrites initially dropped at site 3 (4mg/L) in relation to the river inlet (6.5mg/L). After which, it increased by two fold at site 5 from 4mg/L to 8mg/L, which was then followed by an additional increase at site 6 with a concentration of 9mg/L. A gradual decrease in the concentration of nitrites was observed all along the drainage system from site 6 to site 4. This decrease was then followed by the final increase at the outlet (9.5mg/L).

b) Summer

The concentration of nitrites doubled from the inlet (3.25mg/L) to site 5 (9.5mg/L) as seen in **Figure 5-14**. This increase was followed by a rapid decrease to more than half its initial concentration of 9.5mg/L at site 8 (4mg/L).

5.3.3 Ammonia

A decrease in the concentration of ammonia was observed for both seasons from the river inlet to its final sites (outlet and site 8). However, summer had a very high concentration of ammonia at site 5 as compared to all the sites for both winter and summer.

a) Winter

The amount of ammonia continuously fluctuated throughout the spatial distribution with concentrations of 0.67mg/L, 0.84mg/L, 0.515mg/L, 0.62mg/L, 0.47mg/L, 0.68mg/L and 0.655mg/L respectively. However, despite the fluctuations, the results also showed that the concentration of ammonia decreased at the outlet (0.655mg/L) compared to the river inlet (0.67mg/L).

b) Summer

In **Figure 5-14**, it was observed that the concentration of ammonia was 0.775mg/L at the river inlet. However, a significant increase occurred at site 5 with a concentration of 1.215mg/L. Despite this rapid increase, site 8 (0.525mg/L) showed a rapid decrease in ammonia of more than half the concentration observed at site 5.

5.3.4 Orthophosphate

The results showed that the concentration of orthophosphate fluctuated throughout the study area for both sampling seasons. However, the results also showed a decrease in the concentration of orthophosphates from the river inlet to the river outlet in winter and from the inlet to site 8 in summer.

a) Winter

During winter the concentration of orthophosphates decreased slightly from the river inlet (5.95mg/L) to the outlet (5.3mg/L). Additionally, the concentration of orthophosphates was found to have continuously fluctuated across the study area as seen in **Figure 5-13**. The increases in the concentration of orthophosphates at site 3, 5 and 4 was 9.55mg/L, 6.85mg/L and 8.4mg/L respectively. On the other hand, the decreases at 6, 7 and 1 (outlet) were 4.85mg/L, 5.35mg/L and 5.3mg/L respectively.

b) Summer

In **Figure 5-14**, it was found that the concentration of orthophosphates increased steeply from 4.8mg/L at the river inlet, to 10.4mg/L at site 5. After which, the concentration of orthophosphates then decreased greatly at site 8 (4.4mg/L) to more than half its concentration at site 5.

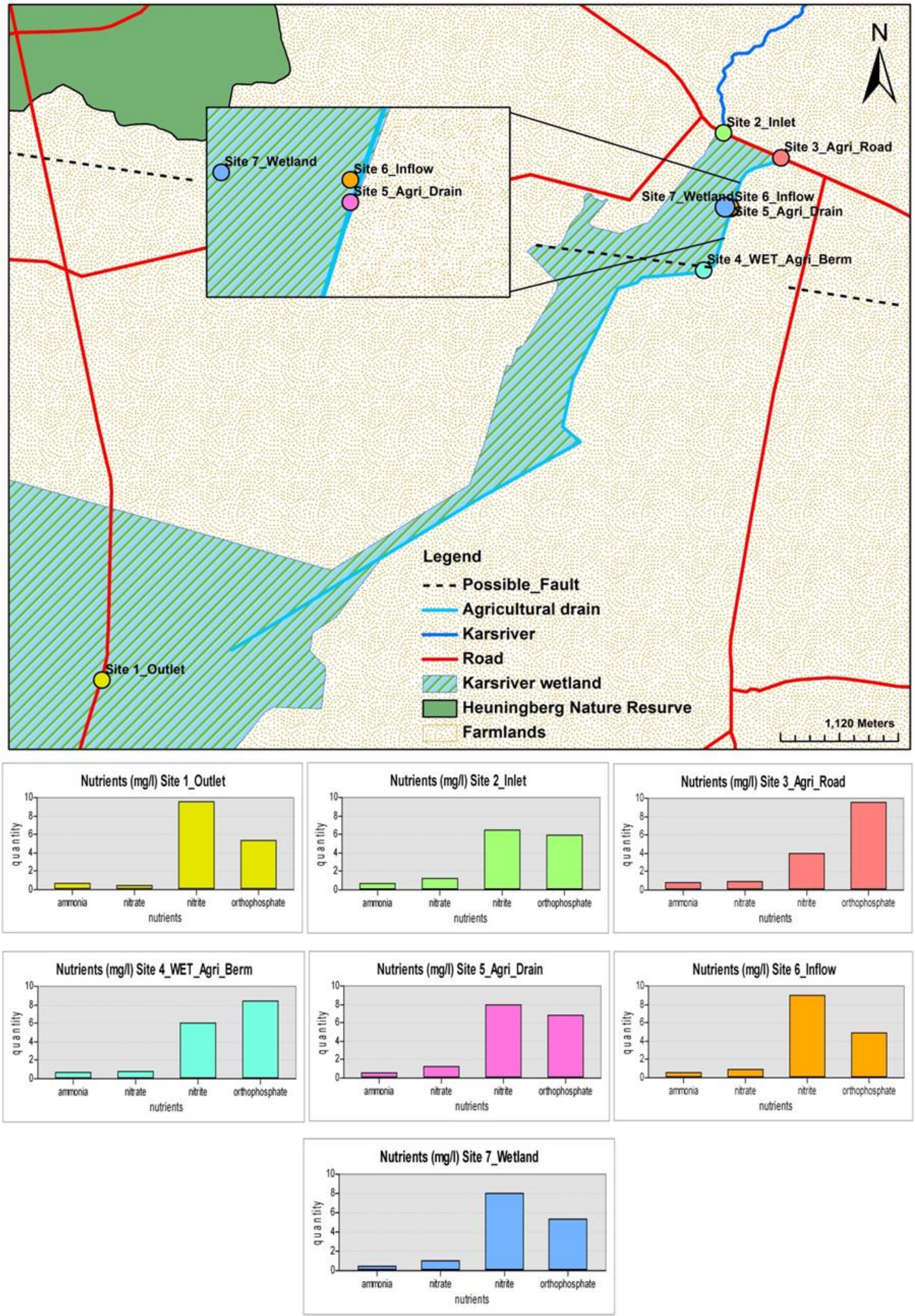

Figure 5-13: Concentrations of nutrients within the Karsriviervlei during winter

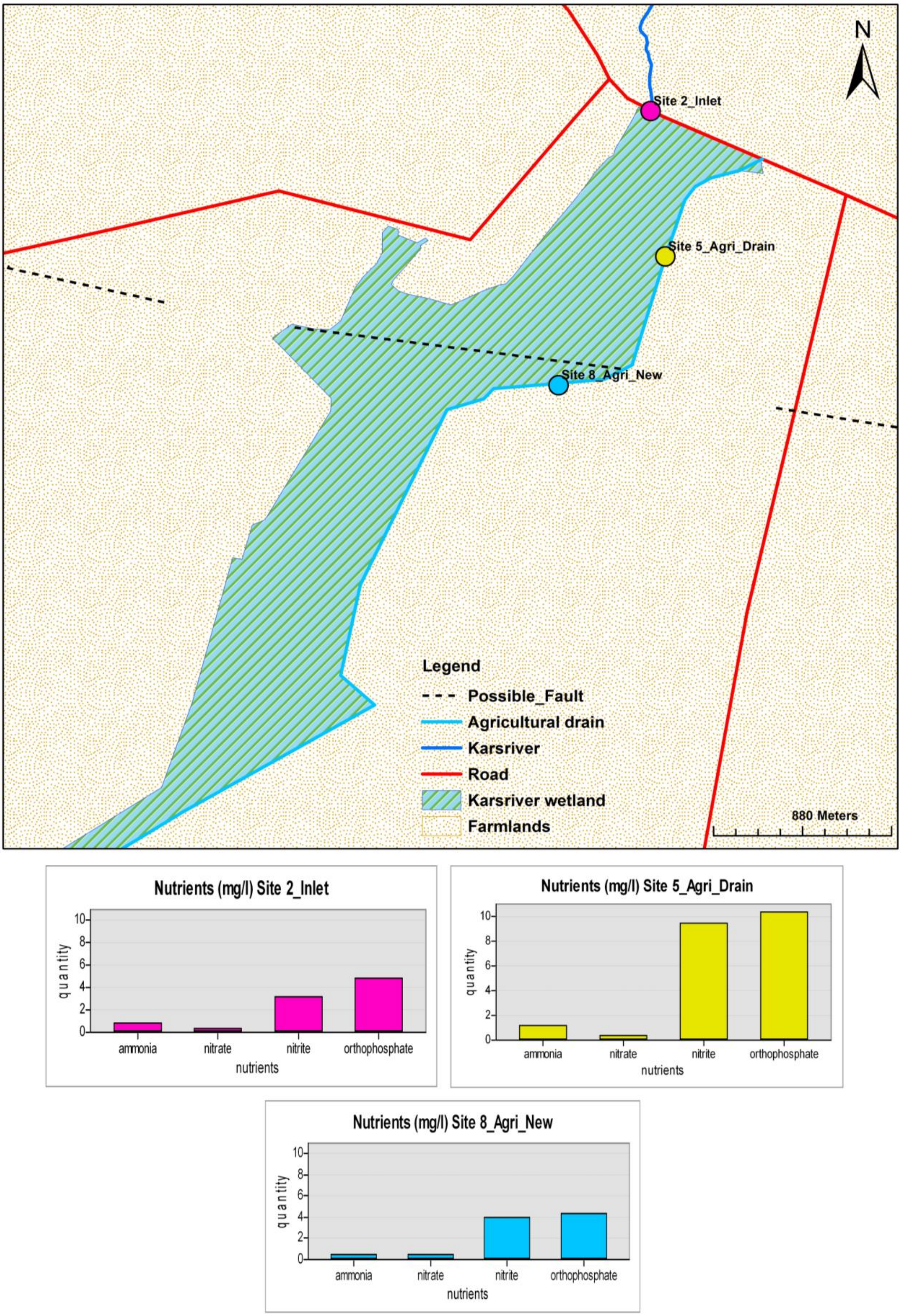

Figure 5-14: Concentrations of nutrients within the Karsriviervlei during summer

5.3.5 Coliform Count

The presence of *Escherichia coli* was detected throughout the entire spatial distribution during both winter and summer field trips. Additionally, the coliform count showed significant decreases in coliforms during summer as compared to winter. **Figure 5-15** & **Figure 5-16** illustrates the presence of *Escherichia coli* in a water sample taken in the field using two different tests (see Chapter 4).

a) Winter

The amount of coliforms was fairly constant throughout the study area with quantities of ≥1600 coliforms per 100ml (**Figure 5-17**). However, a rapid decrease within the wetland at site 7 (445 coliforms per 100ml) was observed. The results also pointed out an anomaly between the two water samples collected within the wetland with coliform counts of 350 and 540 coliforms per 100ml averaged to 445 coliforms per 100ml.

b) Summer

The resultant analysis for the river inlet indicated another anomaly of a two fold increase in the amount of coliforms between the two inlet samples at site 2. Sample 2C had a count of 350 coliforms per 100ml whereas sample 2D had a count of 930 coliforms per 100ml. Also, there was an exponential increase in the quantity of coliforms at site 5 to ≥1600 coliforms per 100ml as seen in **Figure 5-18**. However, further down the drainage system at site 8, the quantity of coliforms decreased considerably to 63 coliforms per 100ml.

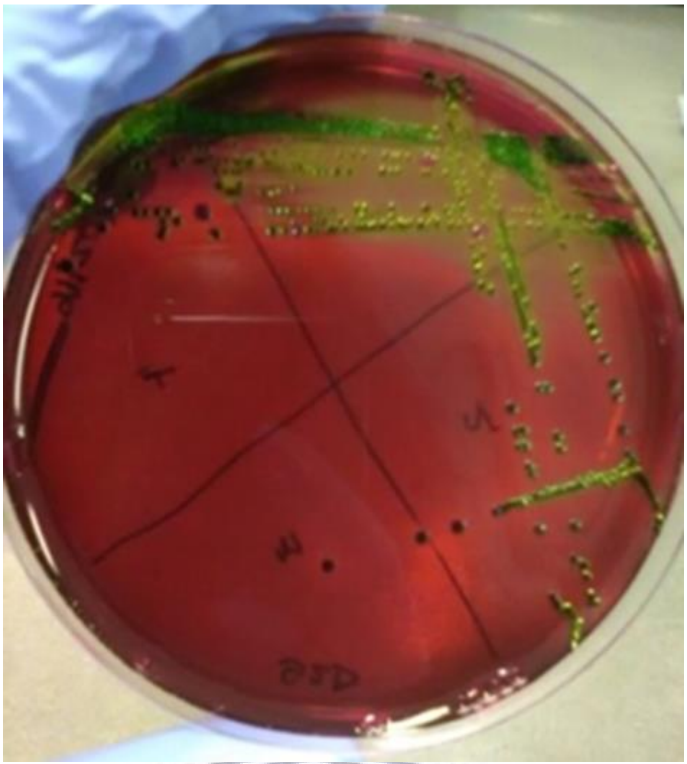

Figure 5-15: The above image was an example of the results of a water sample taken from site 2 during summer. The petri dish contains a dark purple medium called eosin methylene blue agar (EMB) – *Escherichia coli* indicated by the blue / black and metallic green colour - each circle representing an individual colony

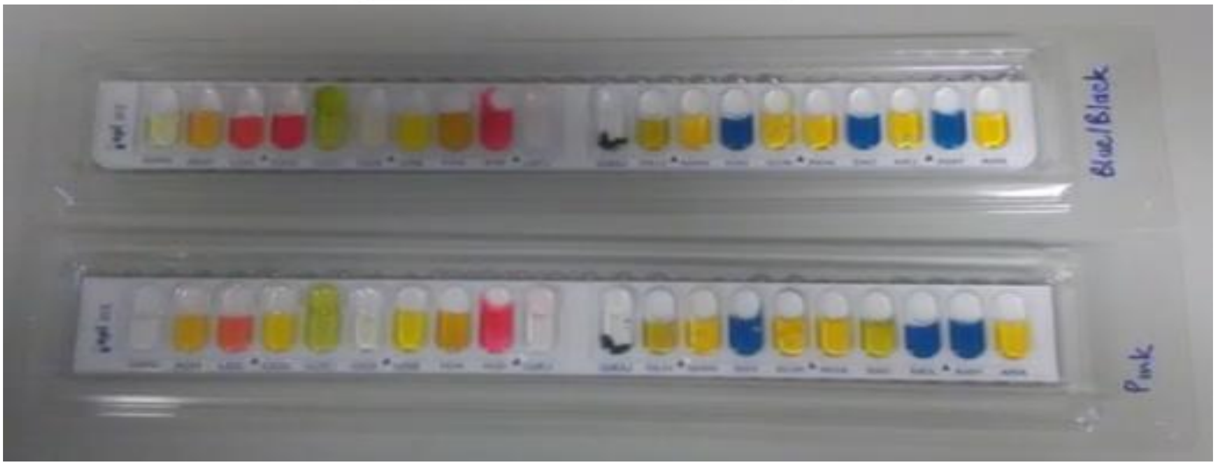

Figure 5-16: The above image was a result of an API 20E test which also demonstrated *Escherichia coli* according to an API 20E code book

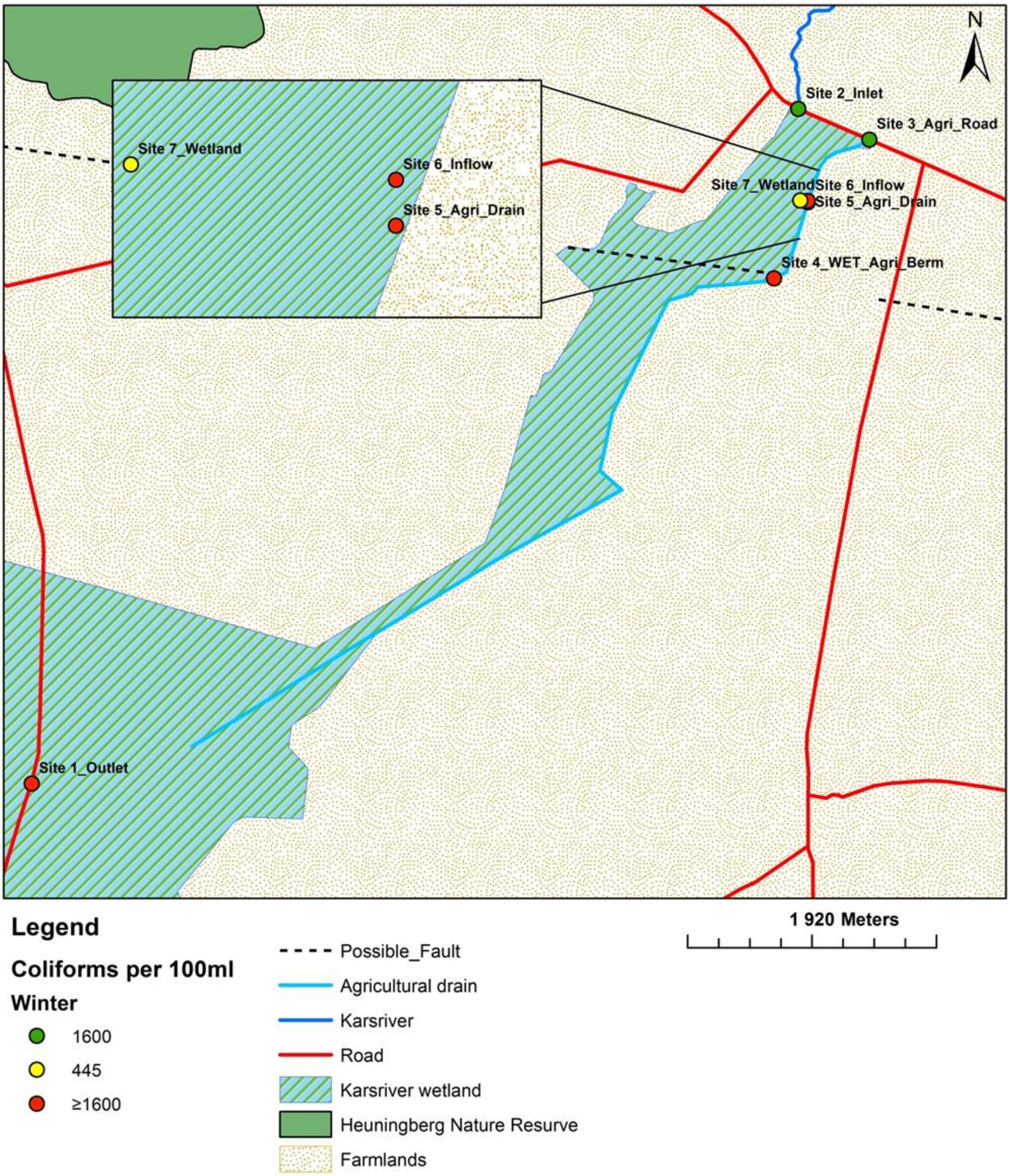

Figure 5-17: Coliform Count within the Karsriviervlei during winter

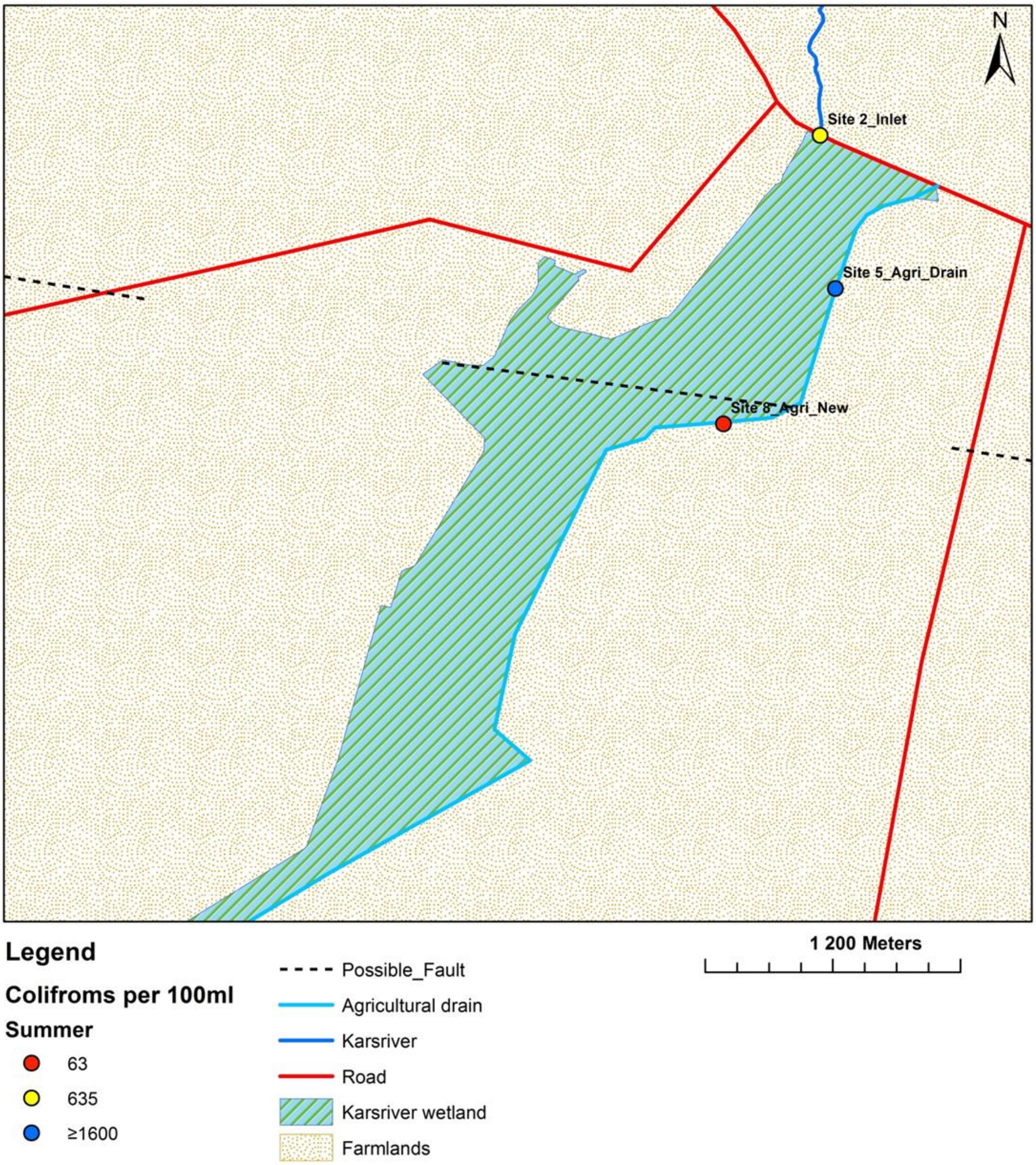

Figure 5-18: Coliform Count within the Karsriviervlei during summer

5.4 WET-EcoServices Assessment

The ecosystem services within this assessment were flood attenuation, sediment trapping, nitrate removal, nitrite removal, toxicant removal and erosion control services. The total scores determined within this assessment were based on a combined analysis of both the effectiveness and opportunity services by the wetland. This was established by means of assessing individual characteristics for the effectiveness and opportunity for each of the services.

5.4.1 Karsriviervlei Ecosystem Services Scores

5.4.1.1. Flood attenuation

Overall, the results from the radar diagram as seen in **Figure 5-19** showed that the Karsriviervlei scored intermediate (1.67) for the flood attenuation ecosystem service, with reference to the WET-EcoServices Assessment guidelines (Kotze *et al.*, 2009). The characteristics which brought about this moderate score were primarily the 'opportunity' characteristics of the wetland (**Figure 5-20**). These include: the low inherent run-off potential of soils in the Kars River catchment, the negligible contribution of the Kars River catchment's land-uses in changing the runoff intensity from its natural condition, the moderately low rainfall intensity and negligible floodable infrastructure/property downstream (**Appendix C**). On the other hand, the 'effectiveness' characteristics of the Karsrivierlei had a much higher score. This was as a result of the following: the size of the wetland is more than 10% relative to the Kars River catchment, the flat gradient of the wetland slope - specifically less than 0.5% -, the moderately high surface roughness and the 1 to 5 year frequency of storm flows spreading across the wetland. However, the characteristics that reduced the effectiveness of the wetland were the indication that seasonal and temporal zones were collectively present, as well as the absence of depressions and the moderately low sinuosity of the channel.

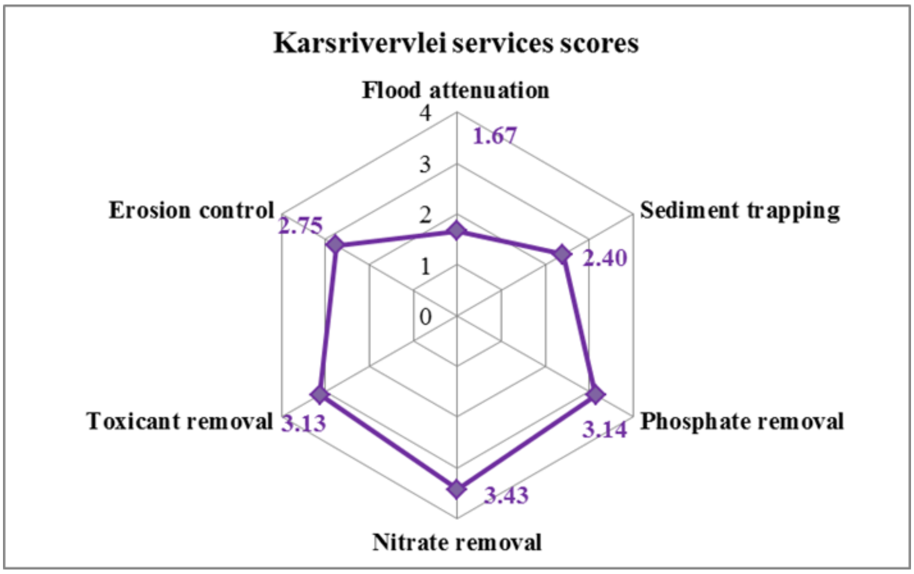

Figure 5-19: Total ecosystem services scores for the Karsriviervlei

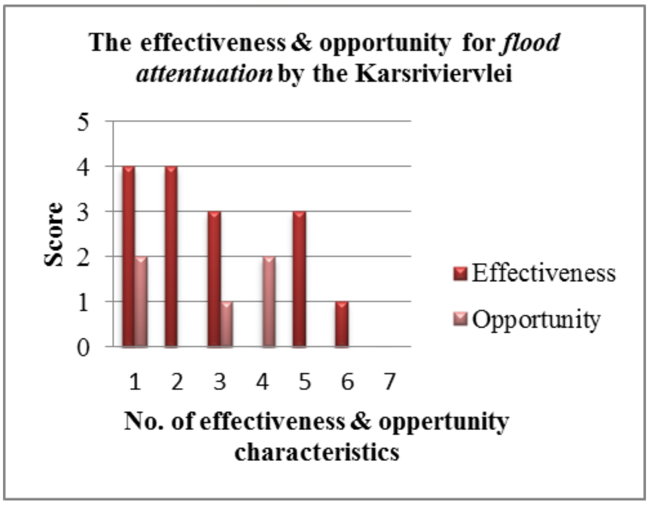

Figure 5-20: Flood attenuation effectiveness & opportunity scores for the Karsriviervlei

5.4.1.2. Sediment trapping

In **Figure 5-19**, the sediment trapping had a sum score of 2.40. This score showed that the Karsriviervlei had a moderately high service in trapping sediments (Kotze *et al*., 2009). Furthermore, the Karsriviervlei was found to have more opportunities for sediment trapping than effectiveness in trapping sediments (**Figure 5-21**). The greatest 'opportunity' the wetland has is the presence of the De Mond Nature Reserve downstream, which is also a Ramsar site (**Appendix C**). However, the 'opportunity' with the lowest score was the extent to which the dam upstream could possibly decrease the input of sediment into the Karsriviervlei. The results also indicated that the effectiveness of the wetland to reduce floods and to deposit sediments were intermediate.

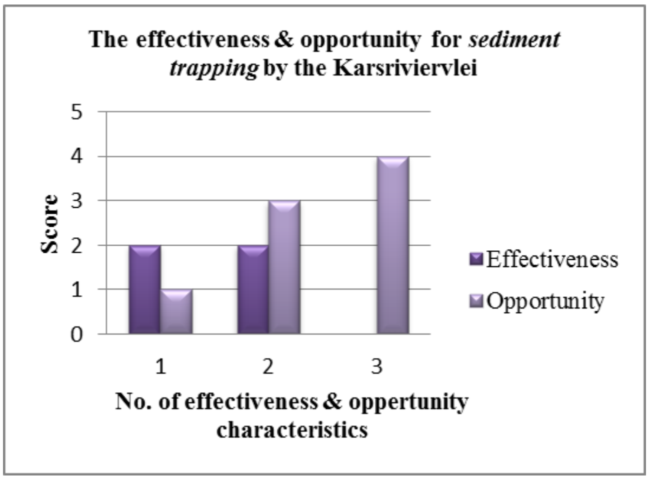

Figure 5-21: Sediment trapping effectiveness & opportunity scores for the Karsriviervlei

5.4.1.3. Phosphate removal

Figure 5-19 showed that the Karsriviervlei achieved a high score (3.20) for the phosphate removal services (Kotze *et al.*, 2009). It was found that the Karsriviervlei had fewer opportunities to remove phosphate, but appeared more effective in the removal of phosphates (**Figure 5-22**). The characteristic of Karsriviervlei with the highest score for it's 'effectiveness', was the low degree to which fertilizers/biocides are added directly to the wetland (Appendix C). Whereas the characteristic of the wetland that was least effective was the trapping of sediments. On the other hand, one of the characteristics with the lowest opportunity score was the opportunity for sediment input and the possible sources of phosphate. However, the wetland also had one good opportunity which resulted in a high opportunity score of 3.3. This opportunity was the De Mond Nature Reserve Ramsar site.

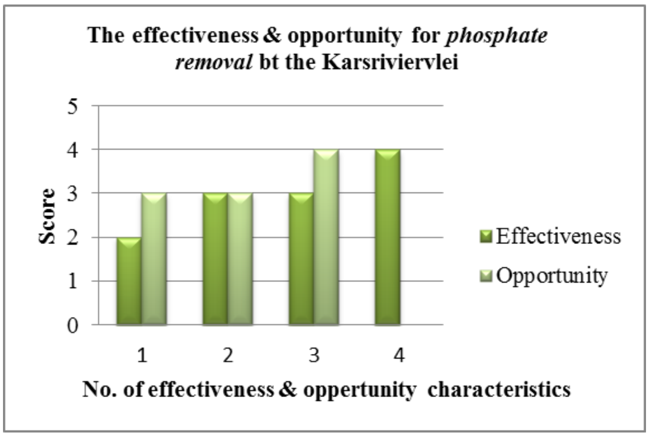

Figure 5-22: Phosphate removal effectiveness & opportunity scores for the Karsriviervlei

5.4.1.4. Nitrate removal

The nitrate removal service provided by Karsriviervlei had a high score of 3.43 (Kotze *et al*., 2009; **Figure 5-19**). The results showed that the wetland had the highest score for the removal of nitrates, compared to all the other ecosystem services assessed in this study. This high score was as a result of the relatively high effectiveness scores (**Figure 5-23**). Furthermore, the characteristics responsible for the high effectiveness scores include: the ability of the Karsriviervlei to have a low addition of direct fertilizers/biocides, the presence of both seasonal and temporary zones collectively and moderately diffuse patterns of low flows within the wetland. In addition to this are the moderately high vegetation cover and the moderately high influence of sub-surface water inputs in relation to surface water inputs (**Appendix C**). However, the wetland had restrictions in the number of opportunities to remove nitrate. Even though the wetland was restricted, it still maintained relatively high opportunities such as the Ramsar wetland downstream and the moderately high sources of nitrate within the Kars River catchment.

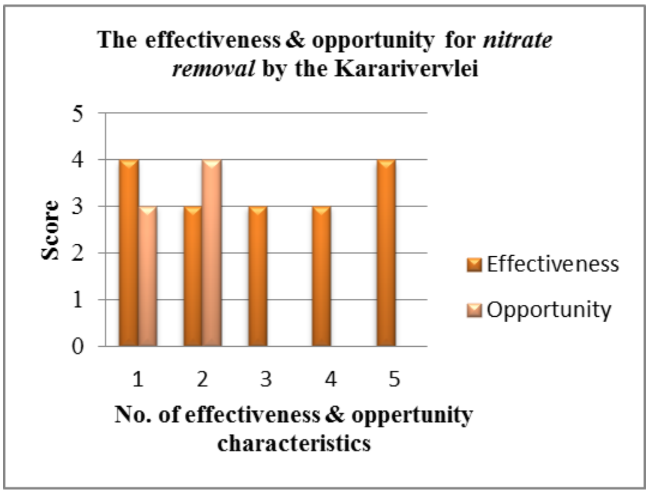

Figure 5-23: Nitrate removal effectiveness & opportunity scores for the Karsriviervlei

5.4.1.5. Toxicant removal

Toxicant removal service had a total score of 3.13, indicating a high level of service provided by the Karsriviervlei (Kotze *et al*., 2009; **Figure 5-19**). The results of the assessment indicated that the characteristics of the wetland had high effectiveness in the removal of toxicants (**Figure 5-24**). This was as a result of the low addition of direct fertilizers/biocides to the wetland; the wetland has both seasonal and temporary zones collectively with moderately high vegetation cover and an intermediary effectiveness in reducing sediment by trapping (**Appendix C**). The high opportunities in the wetland were once again due to the Ramsar site downstream and the moderately high level of sediment input. On the other hand, the intermediary toxicant sources reduced the opportunity scores of the wetland.

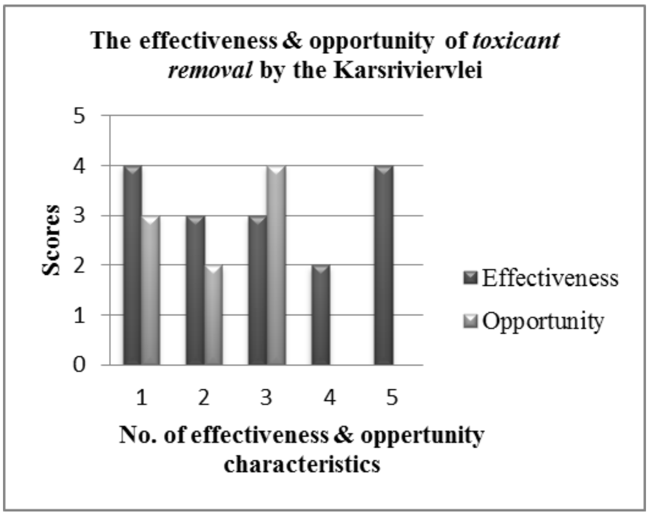

Figure 5-24: Toxicant removal effectiveness & opportunity scores for the Karsriviervlei

5.4.1.6. Erosion control

The erosion control services for Karsriviervlei achieved 2.75 presented in **Figure 5-19**, which showed a high score according to Kotze *et al.* (2009). Also, the number of 'effectiveness' and 'opportunity' characteristics for the Karsriviervlei are the same. Despite the same number, the flat slope of the site reduced the opportunity scores (**Appendix C**; **Figure 5-25**). However, the presence of the De Mond Ramsar site and the high erodibility of the soil lead to a rise in the opportunity scores. The effectiveness of the wetland did, however, surpass that of the opportunity scores due to its relatively high scores for the characteristics. The relatively high scores are a result of the: moderately low evidence of active erosion in the wetland, moderately high vegetation cover, moderately high surface roughness of the wetland and negligible level of physical disturbances of the soil within the wetland – excluding the drain which occupies a relatively small proportion of the total surface area.

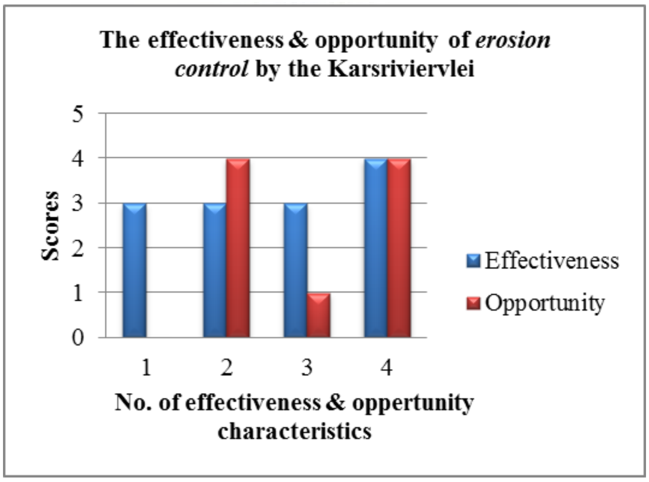

Figure 5-25: Erosion control effectiveness & opportunity scores for the Karsriviervlei

5.4.2 Karsriviervlei Confidence Scores

Collectively the confidence scores for the specified ecosystem services indicated moderately high to high confidence scores (**Figure 5-26**; Kotze *et al.*, 2009). Flood attenuation had the highest scores followed by erosion control services, nitrate removal, phosphate removal, toxicant removal and sediment trapping (**Appendix C**).

Confidence in the flood attenuation score was high due to: the availability of high quality spatial data to calculate slope of both the wetland and its catchment, to determine surface roughness, sinuosity, floodable infrastructure; and communication with the landowner

(Albertyn. M, 20 July 2016), as well as to determine the frequency with which stormflows were spread across the Karsriviervlei and due to the guidance and methods of the WET-EcoServices Assessment (Kotze *et al*., 2009). However, despite the Google imagery the wetland was still diffuse making it challenging when calculating the slope. Furthermore, the Kars River has a DWS gauging station that has hydrological data for the 1950's; however, the records are still short and inconsistent. Additionally, results were solely based on the information from the farm owner and observation by means of Google imagery and visitation to the site.

On the other hand, confidence in the score for erosion control was high because the information for the characteristics of this service was fairly achievable and accurate. For example, Google earth imagery was suffice because most of the characteristics did not require detailed information. Furthermore, ArcMap was accessible for the calculation of the slope and raw data was also accessible on the erodibility of the soil found at the Karsriviervlei.
Sediment trapping appeared to have the lowest confidence score as a result of certain restrictions. These restrictions were the inability to visit the surrounding areas and Google earth imagery. Google earth imagery limitations included the inability to determine: the actual direct evidence of sediment deposition, the extent to which the dam upstream reduced the input of sediment in the Karsriviervlei and the moderately high extent of sediment sources delivering sediment to the Karsriviervlei from its catchment. The inability to visit surrounding areas and the lack of research done on the study area made it challenging to draw accurate conclusions.

Phosphate removal and toxicant removal services had moderately high confidence scores. The lack of hydrological data resulted in the reduction of the confidence scores for accurate determination of the pattern of low flows. Additionally, the limitations to Google earth imagery and the insufficient research done on the study area produced low confidence scores. The limitations included the incapability to determine the accurate: extent to which fertilizers/biocides are added directly to the vlei, level of sediment input and extent of potential phosphate/toxicant sources in the Kars River catchment.

The confidence score for nitrate removal was 2.86, indicating moderately high to high confidence scores according to Kotze *et al*. (2009). Similarly to the phosphate removal services, the lack of hydrological data resulted in a low confidence score for the establishment for the pattern of low flows. Furthermore the same Google earth imagery limitations were found where an accurate extent to which fertilizers/biocides is added directly to the Karsriviervlei.

Despite the assessment serving as guidance for all the ecosystem services, possible inaccuracies could be detected as a result of human perception of the methods recommended and the information available. Also, some characteristics are the same throughout the six services; therefore, if the confidence of one of the characteristics were low, it would have affected all the others, including the calculations.

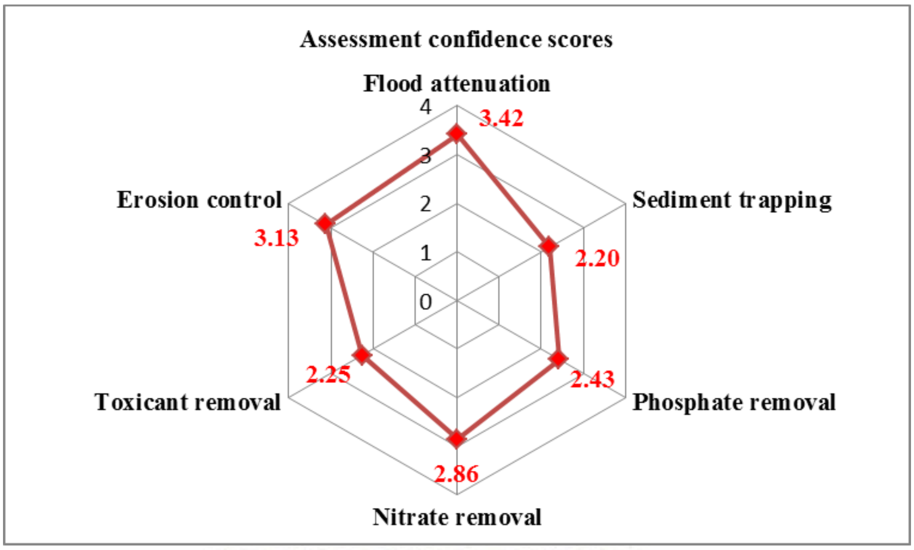

Figure 5-26: Overall confidence scores of the Karsriviervlei Ecosystem Services Scores

6. DISCUSSION

This chapter will discuss and interpret the results presented in the previous chapter. Additionally, this chapter will include a comparative discussion of the WET-EcoServices Assessment results with the results of the fieldwork and laboratory analysis.

The results indicated that the Karsriviervlei influences the quality of water of the Kars River. This influence was evident by the change in water quality parameters across the spatial distribution (i.e. fluctuations / changes in concentration of parameters). The results also illustrated a decrease in the concentration of certain water quality parameters (i.e. nitrate, ammonia, phosphate, turbidity and conductivity) from the inlet to the outlet during winter, suggesting an improvement in the water quality downstream.

Turbidity

The average level of turbidity during winter (33NTU) was higher than the average level of turbidity recorded during summer (25NTU). During winter the levels of turbidity had extensive fluctuations throughout the entire spatial distribution with rapid increases to 45.1NTU and 69.15NTU at sites 3 and 4. This is due to the relatively high flow rates (that are related to heavy rainfall) and cattle treading; as evidenced by hoof prints observed within the drainage system. The treading causes sediments to become suspended in the water column. These findings are consistent with the findings of Morris & Reich (2013); who illustrated that turbidity increases due to trampling by livestock and a published report by Dallas & Day (2004); who confirmed that the flow rate and seasonality influences turbidity levels. Additionally, Behar (1997) also found that turbidity increases during a season of heavy rainfall. The lowering of the berm coupled with the muddy runoff from the adjacent field into the drainage system that has an unconsolidated sediment bed (**Figure 2-5**), resulted in the high turbidity at site 4. On the other hand, rapid decreases were found at sites 6, 7 and 1 during winter, with turbidity levels of 20.4NTU, 19.1NTU and 14.95NTU respectively. The rapid decrease from the inlet (34.45NTU) to the outlet (14.95NTU) and from site 3 (69.15NTU) to site 6 (20.14NTU), shows that the upper part of the Karsriviervlei is trapping sediment. The low turbidity level at site 7 (19.1NTU), together with the low coliform count suggests that the water sampled at this site was predominantly rainwater. Despite the fluctuations during summer, there was a significant increase in the levels of turbidity at site 5. Relatively high turbidity levels in the disconnected pools in summer are also indicative of trampling as cattle entered the pools to drink water. The suspended sediments caused by the cattle were likely trapped, resulting in even higher levels of turbidity.

Coliforms and *Escherichia coli*

Total coliforms during winter were predominantly at 1600 coliforms per 100ml throughout the spatial variation. Also, laboratory results show that *Escherichia coli* was present throughout the spatial distribution during both winter and summer during. This is concerning as WHO (2011) guidelines state that no detection of *Escherichia coli* or thermotolerant coliform (known as total coliform) must be present in any 100ml sample within treated water entering the distributional system. Moreover, the presence of *Escherichia coli* was not surprising as excrements of cattle were observed but it could suggest possible pathogens, especially based on the high levels of coliforms. For temperatures as low as 14˚C, there was an expectation of less coliforms during winter, however, the high coliform count recorded at sites 4 and 5 were found near the excrements of cattle. Also, flooding caused by heavy rainfall worsened the water quality of the study area by distributing high counts of coliform across the entire spatial variation. Site 7 had a low coliform count on average but also showed a big difference between the two samples taken at this site. This anomaly can likely be explained by technical errors occurring in the laboratory. Since turbidity levels are low, sediment concentrations are expected to be low for the adsorption of coliforms. Thus, indicating that the low coliform count may be as a result of dilution by heavy rainfall.

During summer water temperatures were as high as 33˚C but the total coliform count on average were as low as 635 coliforms per 100ml and 63 coliforms per 100ml at sites 2 and 8 respectively. The low coliforms can be explained by the moderate turbidity levels (20NTU & 27NTU) that indicated fairly moderate levels of sediments. This coupled with the low flow rate gives sufficient time for the suspension of sediments and thereby adsorption of coliforms onto sediments. Another contributing factor to the reduction of coliforms at sites 2 and 8 is the destruction of coliforms by the penetration of the ultraviolet rays from the sun, as it was a hot and dry day. Site 5, however, had a high coliform count (≥1600 coliforms per 100ml) that can be related to the water temperature - which gives rise to the growth of coliforms - and the presence of cattle that may result in faecal discharge. The possible adsorption of coliforms onto sediments and the increase of coliforms within high temperatures are consistent with the findings of Boutlier *et al*., 2009.

Orthophosphate (Inorganic Phosphorus)

Based on the three Ecological Categories found in Malan & Day (2012), orthophosphate concentrations indicated a severely impacted Ecological Category with values exceeding 0.04mg/L.

During winter the average concentration of orthophosphate ranged from 4.85mg/L to 9.55mg/L, while during summer the concentration ranged from 4.4mg/L to as high as 10.4mg/L. Additionally, orthophosphate concentrations fluctuated during winter and summer across the spatial variation. The fluctuations during winter may be as a result of heavy rainfall, agricultural waste, cattle excretion and turbidity. These above mentioned findings were in agreement with studies conducted by DWS (1996), Reddy *et al*. (1999), Turpie *et al*. (2010) and Morris & Reich (2013). The heavy rainfall is responsible for increased discharge of agricultural waste from farmlands upstream and relatively high flow rates, dilution of sampled water (at site 7) and flooding. The high flow rate provides less time for suspension and thereby, less time for the adsorption of orthophosphates onto sediments throughout the system. Also, faecal contaminants from cattle found along sites 4 and 5 may be washed into the wetland as a result of flooding. The turbidity results showed that the Karsriviervlei is indeed trapping sediments, suggesting that the wetland may trap orthophosphate through adsorption by sediments already trapped within the wetland. Thus, explaining the decrease from the inlet (5.95mg/L) to the outlet (5.3mg/L) as well as at site 6 (4.85mg/L).

During summer, there was a sharp increase at site 5, due to the release of excrements from the cattle coupled with the use of fertilizers upstream. The application of fertilizers upstream with the cattle found along the agricultural drainage system may have affected the capability of wetlands to buffer nutrients. Despite the rapid increase at site 5, there was a decrease at site 8 likely due to adsorption by sediments.

Conductivity

The average levels of conductivity were found to be above the standard range of 50µs/cm to 1500µs/cm within freshwater streams (Behar, 1997) for both winter and summer with levels ranging from 2794.5µs/cm to 10295µs/cm. The average high levels of conductivity during winter can be explained by the high concentration of orthophosphate ions and carbonate ions. Whereas the even higher levels of conductivity during summer is likely as a result of the high evaporation rates together with the increase in orthophosphate and carbonate conductive ions.

As evaporation occurs the water level drops as a result of water (liquid phase) converting to water vapour (gas phase), escaping into the atmosphere. The ions within the water body become concentrated, resulting in higher conductivity levels. The low flow rate during summer may result in the dissolution of calcium carbonates from calcareous soil. Also, the results showed that when orthophosphate concentration was as high as 10.4mg/L, conductivity levels was found to be 10295µs/cm. An overall decrease in conductivity was observed during winter from the inlet (4439µs/cm) to the outlet (2794.5µs/cm). This decrease was likely due to the decrease in orthophosphate ions from the inlet (5.95mg/L) to the outlet (5.3mg/L) coupled with the low temperature.

Inorganic nitrogen (nitrate, nitrite, ammonia)

Nitrate and nitrite were considered together due to similar influential factors. The results indicated a severely impacted Ecological Category as both nitrate and nitrite concentrations exceeded 0.07mg/L with alarming concentrations that went as high as 1.3mg/L (nitrate) and 9.5mg/L (nitrite). Laboratory results also showed that the concentration of nitrate and nitrite fluctuated across the spatial distribution during winter and summer. However, the concentration of nitrate decreased from the inlet (1.25mg/L) to the outlet (0.4mg/L) during winter, while concentrations of nitrite increased from the inlet (6.5mg/L) to the outlet (9.5mg/L) during winter. Furthermore, nitrate concentrations continued to increase during summer whereas nitrite concentrations fluctuated with a noticeable spike at site 5 (9.5mg/L). The excessively high nitrate and nitrite concentrations during winter can be explained by the discharge of agricultural wastewater as a result of heavy rainfall and flooding. Also, the excessive amounts of cattle excretions found along sites 5 and 4 may contribute to the increase in nitrate and nitrite. Alternatively, the extreme decrease in nitrate concentration during winter might be related to dilution in nitrate and nitrite concentration as a result of groundwater discharge coupled with rain water from heavy rainfall. The above interpretations for the fluctuations were supported by studies conducted by DWS: Aquatic Ecosystems (1996), Rogers (1983), Vymazal (2007), Mitsch & Gosselink (2007), Morris & Reich (2013) and Mitsch *et al*. (2014).

Ammonia concentrations fluctuated throughout the spatial distribution during both seasons but, ultimately decreased from the inlet to the outlet during winter. This could be related to the pH levels and the water temperature. Results indicated that the pH levels and water temperature decreased with ammonia, indicating a directly proportional relationship between the three variables. Despite the decrease, ammonia concentrations found in this study on average are excessively high as compared to the Target Water Quality Range (TWQR) of 0.007mg/L (DWS, 1996) with averages of 0.64mg/L and 0.84mg/L during winter and summer respectively. These values are of concern as ammonia is toxic to living organisms (Dallas & Day, 2004). The average high concentrations can be as a result of the heavy rainfall draining agricultural waste into the water, increasing nutrients during winter. Furthermore, significantly higher ammonia levels were recorded during summer. This could be associated with the high pH levels (ranging from 8.225 to 9.105) as a directly proportional relationship between these variables was observed in earlier discussions. This relationship is supported by DWS (1996) who observed the same relationship.

Levels of pH

The levels of pH throughout the spatial variation during winter (pH: 7) and summer (pH: 8-9) were alkaline. This is as a result of the underlying geology (**Figure 2-5**), agricultural wastewater from fertilizers upstream, the change in flow rate as well as photosynthesis and respiration. The underlying geology of the area is calcareous soil also known as 'alkaline soils' due to its high pH levels. Furthermore, the high pH levels within calcareous soils are due to the presence of carbonates (Kishchuck, 2000). However, in summer the levels of pH were much higher due to the slow flow rate of water. The slower the flow rate, the more time is allowed for the dilution of the calcareous soil with the water. Additionally, the relatively high concentration of dissolved oxygen is related to the extreme rates of photosynthesis and respiration. This implies that at one point there was high level of carbon dioxide for photosynthesis and respiration to occur, thereby resulting in high levels of pH during summer. DWS (1996) established the primary factor influencing pH to be carbonate species, in addition to this; they also supported the findings of photosynthesis and respiration that causes high alkalinity. Additional studies conducted in support of these factors are Dallas & Day (2004).

Dissolved Oxygen (DO)

During winter the concentration of dissolved oxygen ranged from 5.63mg/L to 8.06mg/L, while during summer, it ranged from 6.01mg/L to 10.35mg/L. Moreover, the overall levels of dissolved oxygen for both seasons were not too low or detrimental. This statement was supported by Behar (1997), who established concentrations of dissolved oxygen ranging from 4mg/L to 7mg/L were good for several animals and concentrations ranging from 7mg/L to 11mg/L were very good for most stream fish.

The levels of dissolved oxygen during winter gradually fluctuated throughout the spatial distribution. The decrease in dissolved oxygen as seen at sites 3, 7 and 1 during winter were potentially due to dilution by heavy rainfall, an increase in turbidity - reducing penetration of sunlight for photosynthesis and respiration - and the change in flow rate. Alternatively, the increase in the concentration of dissolved oxygen as seen at site 4 during winter can be explained through the increase in nutrients due to agricultural discharge by heavy rainfall and cattle excretion. The continuous increase in the concentration of dissolved at sites 5 & 8 during summer are also indicative of cattle excretion but with the addition of increased water temperatures.

The increase in water temperature effects organism life (such as plants) and the increase in nutrients results in the increase in plant growth and thereby, the increase in photosynthesis and respiration. This relationship was confirmed through research conducted by DWS (1996), Behar (1997) and Dallas & Day (2004)

WET-EcoServices Assessment

This study covered six ecosystem services within the WET-EcoServices Assessment. These ecosystem services included flood control, nitrate removal, phosphate removal, toxicant removal, erosion control and sediment trapping. The results indicated that the WET-EcoServices Assessment showed similar trends to the field and laboratory results. However, it is important to note that the assessment is a qualitative method that scores the importance of a wetland long term based on its ecosystem services, whereas the field and laboratory procedures is a quantitative method based on once off measurements and sampling of specific physico-chemical and biological parameters during winter and summer.

Nitrate removal services had the highest score in the assessment, implying that based on its characteristics, the wetland favours the removal of nitrate compared to the other five ecosystem services included in this study. According to the laboratory results, the removal of nitrate was favoured during winter; however, summer had no outlet to make deductions from as most of the study area was dried up. Additionally, the assessment also gave relatively high scores for the wetland in the phosphate removal service. Yet the results from the fieldwork and laboratory tests indicate that the wetland may not remove phosphates as highly as the assessment implied. It is also worth considering that most of the confidence scores for this service were fairly low (**Figure 5-26**). In addition to this, the wetland may be lacking in its ability to provide its functions due to modification (agricultural drainage system) and cattle excrements. Also, flooding during winter and a dry outlet during summer, may also affect measurements and water samples taken in the field. Flooding may cause the distribution of contaminants across the spatial distribution and it may also cause dilution as the results have showed. The dry outlet on the other hand, makes it difficult to conclude whether there was a change in water quality from the inlet to the outlet during summer.

The Assessment however, provided extra information on parameters that were not covered in this study including toxicant control and sediment trapping. This provided a broader understanding of the entire area whereas the measurements and water samples taken were localised to specific points. Nevertheless, the WET-EcoServices Assessment is still a ‘rapid’ assessment which scores the importance of specific wetlands based predominantly on observation, therefore human error is possible. This is where the field data becomes essential, because it provides physical evidence by taking actual measurements on-site using field equipment and taking relevant samples back to the laboratory for analysis. There should be coherence in the results provided by the field data and the WET-EcoServices Assessment, if there were no discrepancies. However, there are occasions where there is incoherence, but the assessment and field data are both correct. In those cases, a possible recommendation would be to do more document analysis on the project itself (e.g. wetlands or water quality parameters) if limited information is available on the study area.

In conclusion, based on the results, fieldwork and laboratory analysis with the WET-EcoServices Assessment produced satisfactory results for this project. However, there might be some discrepancies due to the amount of visitations. The WET-EcoServices is beneficial in a field study but also quite effective when visitations to the site are limited. However, the WET-EcoServices Assessment may not be as effective when used alone, but this statement depends on the research questions of the project itself. Additionally, it would also depend on whether or not the entire assessment was being considered or only a small portion. In the case of this study, the use of the Assessment tool alone would not provide the relevant quantitative data required to build this project despite its benefits.

7. CONCLUSION & RECOMMENDATIONS

In conclusion the objectives were addressed by implementing the two part procedure discussed in Chapter 4. Fieldwork and laboratory analysis addressed the first objective to investigate spatial and temporal variation in selected physico-chemical and biological water quality variables upstream of, within, and downstream of the Karsriviervlei. Fieldwork included the selection of eight sites across the study area - specifically at the river inlet (upstream), river outlet (downstream) and along the agricultural drainage system. At each site selected physical water quality parameters were measured and water samples were collected. Water samples were than taken to the laboratory for testing of selected chemical and biological water quality parameters. This was done over the course of two field trips. The second objective was addressed by using the WET-EcoServices Assessment and applying it to the Karsriviervlei in order to investigate the hydrogeomorphic characteristics and processes that would cause the wetland to be effective in regulating water quality. However, both procedures were used in-line with one another as an integrated approach (see Chapter 4).

The results of this investigation concluded that the Karsriviervlei is indeed improving the water quality of the Kars River. This conclusion was based on values of specific parameters determined from the inlet to the outlet and from the changes in the values of parameters throughout the spatial distribution. The primary functions of the Karsriviervlei that may have improved the quality of water were adsorption by sediments and vegetation cover - affecting the flow rate. Additional factors that influenced the fluctuations of the physico-chemical and biological constituents - as evidence from literature and the results - include seasonality, wetland modification (agricultural system) and the application of fertilizers upstream with cattle excretion; which may affect the wetland's ability to buffer nutrients (DWS, 1996; Behar, 1997, Dallas & Day, 2004; Morris & Reich, 2013). Results also indicated the presence of *Escherichia coli* throughout the spatial distribution during both winter and summer. The presence of *Escherichia coli* indicates the presence of pathogens in the water. This could be a health risk for people downstream using the water for recreation or consumption.

Despite the addition of the WET-EcoServices Assessment, there was still a lack in data as the outlet was dried up during summer. More collections of samples over a longer period would be preferable or possible visitation in the early summer season instead of near the end (if more visitations cannot be achieved). Also, a recommendation of three to four samples of water would be more accurate instead of two when testing microbes, to prevent discrepancies such as the coliform anomalies found in the results. In addition to this, further analysis on the type of pathogens that could be present as well as a quantitative analysis would also be recommended. Another recommendation would be to identify whether the cattle are the only source of *Escherichia coli* by monitoring the movements of the cattle, in relation to the presence of *Escherichia coli*. All of the above mentioned in relation to coliforms could be added to serve as a guide to the WET-EcoServices tool as a service provided by wetlands for coliforms.

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APPENDIX A

Materials and Equipment

Nitrate: Method 10020 Test 'N Tube Vials

Materials needed:

- NitraVer X Reagent A Test 'n Tube vials
- NitraVer X Reagent B Powder Pillow
- Water sample
- Sample cells with stoppers
- Funnel
- Disposable 1Lt glass bottle

Nitrite: Method 8153 Powder Pillows

Materials needed:

- NitriVer 2 Nitrite Reagent Powder Pillow
- Water sample
- Sample cells with stoppers
- Mineral Stabilizer
- Polyvinyl Alcohol Dispersing Agent
- Nessler Reagent
- Deionized water
- Water sample
- Sample cells with stoppers
- Plastic test tubes
- Pipette

Phosphate: Method 8114 Reagent Solution

Materials needed:

- Molybdovanadate reagent
- Hydrochloric acid
- Deionized water
- Water sample
- Sample cells with stoppers

Most Probable Number (MPN) Test: Coliforms

Materials needed:

- Single-strength lactose broth (SSLB)
- Double-strength lactose broth (DSLB)
- Brilliant green lactose bile broth
- Eosin-Methylene Blue (EMB) Agar and Nutrient Agar plates

- Durham and lab Tubes
- Gram staining reagent
- Petri dishes
- Pippets (0.1ml, 1ml, 10ml)
- 35˚C incubator
- Bunsen burner
- Incubating loop
- Autoclave
- Water sample

Gram-staining

Materials needed:

- Crystal violet dye
- Safranine
- Alcohol
- Iodine
- Distilled water
- Microbial slides

API 20E test

Materials needed:

- Nutrient Agar plate with visible colonies
- 5ml of saline water (NaCl) per test
- Distilled water
- API strips
- Incubation boxes
- Mineral oil
- Sterile pipettes

APPENDIX B

WET-EcoServices Assessment

Table 1: WET-EcoServices Assessment- Sediment trapping

Characteristics Score:	**0**	**1**	**2**	**3**	**4**
			Effectiveness		
Effectiveness of HGM unit in attenuating floods	Low	Moderately low	Intermediate	Moderately high	High
Direct evidence of sediment deposition in the HGM unit	Low	Moderately low	Intermediate	Moderately high	High
			Opportunity		
Extent to which dams are reducing the input of sediment in HGM unit	Low	Moderately low	Intermediate	Moderately high	High
Extent of sediment source delivering sediment to the HGM unit from its catchment	Low	Moderately low	Intermediate	Moderately high	High
Presence of any important wetland or aquatic system downstream	None		Intermediate importance		High importance
Note: if sediment input is very high, then the effectiveness of the HGM unit in contributing to sediment trapping may be reduced where (1) vegetation is 'smothered' by recent excessive deposition or (2) the gradient and morphometric of the HGM unit is altered owing the accumulation of sediment, resulting in flow becoming more concentrated and the HGM unit therefore being more susceptible to erosion.					

Table 2: WET-EcoServices Assessment- Phosphate removal

Characteristics score:	**0**	**1**	**2**	**3**	**4**
Effectiveness					
Effectiveness in trapping sediment	low	Moderately low	intermediate	Moderately high	High
Pattern of low flows within the HGM unit	Strongly channelled	Moderately channelled	intermediate	Moderately diffuse	Very diffuse
Extent of vegetation cover	low	Moderately low	intermediate	Moderately high	High
Extent to which fertilizers/biocides are added directly to the HGM unit	high	Moderately high	intermediate	Moderately low	Low
Opportunity: i.e. level of phosphate input					
Level of sediment input	low	Moderately low	intermediate	Moderately high	High
Extent of potential sources of phosphate in the HGM unit's catchment	low	Moderately low	intermediate	Moderately high	High
Presence of any important wetland or aquatic system downstream	None		Intermediate Importance		High importance

Table 3: WET-EcoServices Assessment –Nitrate removal

Characteristics score:	**0**	**1**	**2**	**3**	**4**
Effectiveness					
Representation of different hydrological zones (temporary/seasonal and permanent)	Permanent & seasonal zoning zones lacking (I.e. only the temporary zone present)	Seasonal zone present but permanent zone absent	Permanent & seasonal zones both present but collectively <30% of total area	Permanent & seasonal zones both present but collectively 30-60%	Permanent & seasonal zones both present but collectively >60%
Pattern of low flows within the HGM unit	Strongly channelled	Moderately channelled	intermediate	Moderately diffuse	Very diffuse
Extent of vegetation cover	low	Moderately low	intermediate	Moderately high	High
Contribution of sub-surface water inputs relative to surface water inputs	Low (<10%)	Moderately low (10-20%)	Intermediate (20-35%)	Moderately high (36-50%)	High (>50%)
Extent to which fertilizers/biocides are added directly to the HGM unit	high	Moderately high	intermediate	Moderately low	Low
Opportunity					
Extent of nitrate sources in the HGM unit's catchment	low	Moderately low	intermediate	Moderately high	High
Presence of any important wetland or aquatic system downstream	None		Intermediate Importance		High importance

Table 4: WET-EcoServices Assessment – Toxicant removal

Characteristics score:	**0**	**1**	**2**	**3**	**4**
			Effectiveness		
Representation of different hydrological zones (temporary/seasonal and permanent)	Permanent & seasonal zoning zones lacking (I.e. only the temporary zone present)	Seasonal zone present but permanent zone absent	Permanent & seasonal zones both present but collectively <30% of total area	Permanent & seasonal zones both present but collectively 30-60%	Permanent & seasonal zones both present but collectively >60%
Pattern of low flows within the HGM unit	Strongly channelled	Moderately channelled	intermediate	Moderately diffuse	Very diffuse
Extent of vegetation cover	low	Moderately low	intermediate	Moderately high	High
Effectiveness in trapping sediment	Low	Moderately low	Intermediate	Moderately high	High
Extent to which fertilizers/biocides are added directly to the HGM unit	high	Moderately high	intermediate	Moderately low	Low
			Opportunity		
Level of sediment input	Low	Moderately low	Intermediate	Moderately high	High
Extent of toxicant sources in the HGM unit's catchment	low	Moderately low	intermediate	Moderately high	High
Presence of any important wetland or aquatic system downstream	None		Intermediate Importance		High importance

Table 5: WET-EcoServices Assessment- Erosion control (in HGM unit)

Characteristics score:	**0**	**1**	**2**	**3**	**4**
Effectiveness					
Direct evidence of active erosion in the HGM unit	High	Moderately high	Intermediate	Moderately low	Low/negligible
Note: if direct evidence of sediment loss is high the STOP HERE because this is direct evidence that the wetland is performing poorly in terms of erosion control, and the score for erosion control would be 0.					
Vegetation cover	Low	Moderately low	intermediate	Moderately high	High
Surface roughness of the HGM unit	Low	Moderately low		Moderately high	High
Current level of physical disturbances of the soil in HGM unit	High	Moderately high	intermediate	Moderately low	Low/negligible
Opportunity					
Slope of the site	<0.2%	0.2-0.9%	1-1.9%	2.5%	>5%
Erodibility of the soil	Low-very low	Moderately low	Moderate	Moderately high	High
Runoff intensity from the HGM unit's catchment	Low	Moderately low		Moderately high	High
Presence of any important wetland or aquatic system downstream	None		Intermediate Importance		High importance
Note: If the runoff intensity is increased considerably (through poor catchment conservation practices and/or extensive hardened surfaces) this may significantly reduce the HGM units effectiveness in controlling erosion, ultimately leading to increased gully erosion or channel incision in the HGM unit.					

Table 6: WET-EcoServices Assessment - Flood attenuation by HGM unit

Characteristics Score:	**0**	**1**	**2**	**3**	**4**
Effectiveness of the HGM unit					
Size of HGM unit relative to the HGM unit's catchment.	<1%	1-2%	3-5%	6-10%	>10%
Slope of HGM unit.	>5%	2-5%	1-1.9%	0.5-0.9%	0.5%
Surface roughness of HGM unit.	Low	Moderately low		Moderately high	High
Presence of depressions.	None	Present but few or remain permanently filled close to capacity.	Intermediate	Moderately abundant	Abundant, entire HGM unit is a depression.
Frequency with which stormflows are spread out across HGM unit.	Never	Occasionally, but less frequently than every 5 years		1 to 5 year frequency	More than once a year
Sinuosity of the stream channel	Low	Moderately low	Intermediate	Moderately high	High
Permanent & seasonal zoning zones lacking (I.e. only the temporary zone present)	Permanent & seasonal zones both present but collectively >60%	Permanent & seasonal zones both present but collectively 30-60%	Permanent & seasonal zones both present but collectively <30% of total area	Seasonal zone present but permanent zone absent	Permanent & seasonal zoning zones lacking (I.e. only the temporary zone present)
Opportunity of attenuating floods and reducing flood damage					
Average of slope o HGM unit's catchment	<3%	3-5%	6-8%	9-11%	>11%
Inherent run-off potential of soils in the HGM unit's catchment.	Low	Moderately low		Moderately high	High
Contribution of catchment land-uses to changing runoff intensity from the natural condition.	Decrease	Negligible effect	Slight increase	Moderate increase	Marked increase
Rainfall intensity	Low (Zone 1)	Moderately low (Zone 2)		Moderately high (Zone 3)	High (Zone 4)
Extent of floodable infrastructure/property downstream.	Low/ Negligible	Moderately low		Moderately high	High

APPENDIX C

WET- EcoSevices Assessment Results

Table 7: Flood attenuation

Characteristics	Score	Confidence Score
Effectiveness of the HGM unit		
Size of HGM unit relative to the HGM unit's catchment	>10%	
	4	3
Slope of HGM unit	<0.5%	
	4	4
Surface roughness of HGM unit	Moderately high	
	3	4
Presence of depressions	None	
	0	4
Frequency with which stormflows are spread out across HGM unit.	1 to 5 year frequency	
	3	1
Sinuosity of the stream channel	Moderately low	
	1	3
Permanent & seasonal zoning zones lacking (I.e. only the temporary zone present)	Seasonal & permanent zone both presents & collectively >60% of the total HGM unit	
	0	3
Opportunity of attenuating floods and reducing flood damage		
Average of slope o HGM unit's catchment	6-8%	
	2	4
Inherent run-off potential of soils in the HGM unit's catchment.	Low	
	0	3
Contribution of catchment land-uses to changing runoff intensity from the natural condition.	Negligible	
	1	2
Rainfall intensity	Moderately low (Zone 2)	
	2	4
Extent of floodable infrastructure/property downstream.	Low/Negligible	
	0	3
	[4+4+3+0+3+1+0]+[2+0+1+2]+0=	**38/12=**
Average	**20/12= 1.7**	**3.17**

Table 8: Sediment trapping

Characteristics	**Score**	**Confidence score**
Effectiveness		
Effectiveness of HGM unit in attenuating floods	Intermediate	
	2	3
Direct evidence of sediment deposition in the HGM unit	Intermediate	
	2	1
Opportunity		
Extent to which dams are reducing the input of sediment in HGM unit	Moderately high	
	1	1
Extent of sediment source delivering sediment to the HGM unit from its catchment	Moderately high	
	3	2
Presence of any important wetland or aquatic system downstream	High importance	
	4	4
	[2+2]+1+3+4=12	**11/5=**
Average	**12/5=2.4**	**2.2**

Table 9: Phosphate removal

Characteristics	**Score**	**Confidence score**
Effectiveness		
Effectiveness in trapping sediment	Intermediate	
	2	2
Pattern of low flows within the HGM unit	Moderately diffuse	
	3	1
Extent of vegetation cover	Moderately high	
	3	4
Extent to which fertilizers/biocides are added directly to the HGM unit	Low	
	4	2
Opportunity		
Level of sediment input	Moderately high	
	3	2
Extent of potential sources of phosphate in the HGM unit's catchment	Moderately high	
	3	2
Presence of any important wetland or aquatic system downstream	High	
	4	4
	2+3+3+4+3+3+4=22	**17/7=**
Average	**22/7=3.14**	**2.43**

Table 10: Nitrate removal

Characteristics	**Score**	**Confidence score**
Effectiveness		
Representation of different hydrological zones (temporary/seasonal and permanent)	Seasonal & permanent zone both presents & collectively >60% of the total HGM unit	
	4	3
Pattern of low flows within the HGM unit	Moderately diffuse	
	3	1
Extent of vegetation cover	Moderately high	
	3	4
Contribution of sub-surface water inputs relative to surface water inputs	Moderately high (36-50%)	
	3	3
Extent to which fertilizers/biocides are added directly to the HGM unit	Low	
	4	2
Opportunity		
Extent of nitrate sources in the HGM unit's catchment	Moderately high	
	3	3
Presence of any important wetland or aquatic system downstream	High importance	
	4	4
	4+3+3+3+4+3+4=24	**20/7=**
Average	**24/7=3.43**	**2.86**

Table 11: Toxicant removal

Characteristics	Score	Confidence score
Effectiveness		
Representation of different hydrological zones (temporary/seasonal and permanent)	Seasonal & permanent zone both presents & collectively >60% of the total HGM unit	
	4	3
Pattern of low flows within the HGM unit	Moderately diffuse	
	3	1
Extent of vegetation cover	Moderately high	
	3	4
Effectiveness in trapping sediment	Intermediate	
	2	2
Extent to which fertilizers/biocides are added directly to the HGM unit	Low	
	4	2
Opportunity		
Level of sediment input	Moderately high	
	3	1
Extent of toxicant sources in the HGM unit's catchment	Intermediate	
	2	1
Presence of any important wetland or aquatic system downstream	High importance	
	4	4
	4+3+3+2+4+3+2+4=25	**18/8=**
Average	**25/8=3.13**	**2.25**

Table 12: Erosion control (in HGM unit)

Characteristics	Score	Confidence score
Effectiveness		
Direct evidence of active erosion in the HGM unit	Moderately low	
	3	3
Vegetation cover	Moderately high	
	3	4
Surface roughness of the HGM unit	Moderately high	
	3	4
Current level of physical disturbances of the soil in HGM unit	Low/Negligible	
	4	2
Opportunity		
Slope of the site	<0.2%	
	0	4
Erodibility of the soil.	High	
	4	2
Runoff intensity from the HGM unit's catchment	Moderately low	
	1	2
Presence of any important wetland or aquatic system downstream	High importance	
	4	4
	3+3+3+4+0+4+1+4=22	**25/8=**
Average	**22/8=2.75**	**3.125**

Ecosystem services	Effectiveness score	Opportunity score	Average score
Flood attenuation	2.14	1	1.7 **(Intermediate)**
Sediment trapping	2	2.7	2.4 **(Mod. High)**
Phosphate removal	3	3.3	3.14 **(High)**
Nitrate removal	3.4	3.5	3.43 **(High)**
Toxicant removal	3.2	3	3.13 **(High)**
Erosion control	3.25	2.25	2.75 **(Mod. High)**

Score	**<0.5**	**0.5 – 1.2**	**1.3 – 2.0**	**2.1 – 2.8**	**>2.8**
Rating of the likely extent to which a benefit is being supplied	**Low**	**Moderately low**	**Intermediate**	**Moderately high**	**High**

Confidence score	**1**	**2**	**3**	**4**
	Marginal/low confidence	**Moderate confidence**	**High confidence**	**Very high confidence**