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Macro-nutrient and hydrological trends in some streams of the Waterberg, Limpopo: investigating the effects of land-use change on catchment water quality



Craig Burne

A dissertation submitted to the Faculty of Science in fulfilment of the requirements for the degree of Master of Science

November 2015, Johannesburg

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



Mr Craig Burne

3rd day of November 2015 in Johannesburg

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ABSTRACT

South Africa is faced with water quantity and quality issues in most catchments. Intensification of coal-based industrial activity in the Waterberg, Limpopo and the concomitant emissions of oxides of nitrogen (NO_X) and sulphur (SO_X) pose potential ecological impacts to regional freshwater systems. Some research indicates that a significant proportion of minerals in the Waterberg parent rock may be prone to acid generation with catchment soils being potentially susceptible to acidification via NO_X and SO_X deposition. Cultural and recurrent nutrient loading of freshwater bodies also impacts on primary production and can ultimately alter the natural structure and functioning of these ecosystems.

Trend analyses on historical hydrological data from 1982 to 2013 were carried out for several response water quality variables from six quaternary Waterberg catchments. Results were assessed for possible changes attributable to increased NO_X and SO_X loading post commencement of large-scale coal combustion. Historical inorganic N:P ratios were calculated in conjunction with a series of nutrient (N and P) bioassay experiments to predict which nutrient may be limiting growth of stream periphyton.

Although trends were identified in most catchments for several of the water quality variables, the notion that the onset of large scale coal combustion has led to noticeable downward trends in pH and upward trends in either inorganic N or sulphate is not unequivocal. Patterns in trends were not distinct for catchments situated in close proximity to the primary emission source and those further away. Nor were there any distinct differences in trends between upwind and downwind catchments. Climate and geo-hydrological factors are likely to still function as the primary drivers of spatial and temporal variation in past and present catchment water quality.

Contrary to the view that stream primary production is limited largely by the availability of P, predictions based on N:P ratios calculated in this study suggest N to be the limiting nutrient. This was shown to be the case in four of the five study-site rivers. N-limitation increased by 18% (67% to 85%) in the Matlabas River post-commencement of large-scale coal combustion. A greater increase of 24% (60% to 84%) was observed in the Middle Mokolo. Although cultural eutrophication levels in the Waterberg do not yet exceed management-set targets, the cumulative effect of industrial-derived nutrient inputs remains a threat to the nearshore marine ecosystem and human communities living downstream.

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List of symbols

A4H004 – Matlabas gauging station

A4H002 – Upper Mokolo gauging station

A4H005 – Middle Mokolo gauging station

A5H004 – Palala gauging station

A4H008 – Sterkstroom gauging station

A4H007 – Tambotie gauging station

H_0 – Null hypothesis

H_A – Alternative hypothesis

mg/l – milligrams per litre

$ms.m^{-1}$ – millisiemens per metre

mu – average over time

mu1 – average before detection of change

mu2 – average after detection of change

R^2 – Coefficient of determination

S_1 – Mann Kendall trend test statistic

S_2 – Seasonal Mann-Kendall test statistic

T_1 – '*before*' time period (Jan 1982 – Dec 1987)

T_2 – '*after*' time period (Jan 1994 – July 2013)

X – Explanatory variable

Y – Response variable

4°ry – quaternary

α – alpha

↑ – increasing (positive) trend

↓ – decreasing (negative) trend

≠ 0 – non-null trend

0 – no trend

Nomenclature

ANC – Acid Neutralisation Capacity

ANOVA – Analysis of Variance

AR [1] – First-Order Autocorrelation

ARC – Agricultural Research Council

B-P – Breusch-Pagan

Chl-*a* – Chlorophyll-*a*

DBSA – Development Bank of Southern Africa

DE – Department of Energy

DEA – Department of Environmental Affairs

DF – Degrees of Freedom

DIN – Dissolved Inorganic Nitrogen (NO_3^-+NO_2+NH_4^+)

DST – Department of Science and Technology

D-W – Durbin-Watson

DWA – Department of Water Affairs

DWAF - Department of Water Affairs and Forestry

DWS - Department of Water and Sanitation

EC – Electrical Conductivity

EDA – Exploratory Data Analysis

GN – Government Notice

GDP – Gross Domestic Product

HSD – Honestly Significant Difference

IPCC – Intergovernmental Panel on Climate Change

ISRIC – International Soil Reference and Information System

IWR – Institute for Water Research

IWRM – Integrated Water Resource Management

LOWESS – Locally Weighted Scatterplot Smoothing

MDL – Minimum Detection Limit

M-K – Mann-Kendall

N – Nitrogen

NDS – Nutrient Diffusing Substrate

NLC – National Land Cover

NO_X – Oxides of Nitrogen

NSBA – National Spatial Biodiversity Assessment

P –Phosphorus

PVC – Polyvinyl Chloride

Q – Discharge or Flow (used interchangeably throughout)

R – Residual

RQS – Resource Quality Services

S – Sulphur

SARVA – South African Risk and Vulnerability Atlas

SAWS – South African Weather Service

SD – Standard Deviation

SIPs – Strategic Integrated Projects

SO_X – Oxides of Sulphur

SRP – Soluble Reactive Phosphate

T – Time

UV – Ultra Violet

USEPA – United States Environmental Protection Agency

W – White test

WDM – Waterberg District Municipality

WMA – Water Management Area

WMS – Water Management System

WQ – Water quality

1 Introduction

Multiple, concurrent and altering land use activities occurring at different spatial and temporal scales have a significant influence on the natural ecological functioning of the planet's ecosystems (Chapin et al., 2002). Aquatic ecosystem pollution is largely attributable to anthropogenic activities operating simultaneously and often combining cumulatively, having adverse effects on both aquatic and terrestrial biotic communities, as well as human health (Steffen et al., 2005). The elevation of nutrient levels in rivers and their catchments is one form of pollution, a process which occurs as a natural and cyclical phenomenon but which can be exacerbated and amplified by human-induced actions (Lang et al., 2000).

The progressive acidification and eutrophication of surface water resources, occurring either separately or in combination, are of global ecological concern (Schindler 1988, Vitousek et al., 1997) which includes the South African context (Oberholster and Ashton 2008, Josipovic et al., 2011). Large extents of the sub-continent are classified as semi-arid to arid and are characterised by a high degree of climatic variability, as predicted by various modelling approaches (IPCC 2013). Increasing and competing demands for limited water supplies and the consequential deterioration in catchment water quality (WQ) resulting from development activities and growing populations continues to exert pressure on and further compromise the natural ecological functioning of freshwater ecosystems (Andersson and Arheimer 2003, DWA 2011).

Considering South Africa in the context of this research, it is necessary to highlight the overarching bio-physical and socio-economic conditions of the country and how the aim of this research project has relevance pertaining to the state of the national water situation. South Africa is not only a water-stressed country facing a potential freshwater deficit by 2025 (DWAF 2004, Oberholster and Ashton 2008), but is also highly dependent on natural resources for its economic health and prosperity (Hill et al., 2011). With respect to predictions associated with future global climate change and some of the climatic effects currently being observed, the country is situated in a region that is ranked to be amongst the most vulnerable in the world (IPCC 2013).

A descriptive overview of the biophysical, ecological and socio-economic environment entitled 'South African Risk and Vulnerability Atlas' (SARVA) is available in a relatively recent report published by the Department of Science and Technology (DST 2010). Specifically in terms of the biodiversity of freshwater systems, the most recent 'National Spatial Biodiversity Assessment' (NSBA) found that 55% of South Africa's river ecosystems are threatened by pollution and over-abstraction and that only 21% of these systems are moderately or well protected. Of the seven classified ecoregions in the

Waterberg, one is critically endangered, two are endangered and all except one are either not or poorly protected (Nel and Driver 2012). There are potential knock-on effects to the ecological health and biodiversity of the Waterberg foothills and associated streams if excessive nutrient pollution contributes to any future deterioration in catchment water quality.

Whilst it is important to consider the SARVA and NSBA reports in conjunction with recently coined concepts, such as the global *Water-Energy-Food Security Nexus* (Hoff 2011), it is equally important to consider the Gross Domestic Product (GDP) and developmental needs of the country. With respect to economic growth demanding factors on a national scale, a number of strategic integrated projects (SIPs) have recently been initiated by the national government. These SIPs are intended to address the ubiquitous socio-economic problems of unemployment and poverty in the country and to stimulate and promote further economic growth into the future (DBSA 2012).

In terms of the required energy needed to support existing infrastructure and land use, and to facilitate the recent SIPs pertaining to further infrastructure and economic development, the combustion of coal remains the primary short-term option in achieving the nation's energy objectives (DBSA 2012). In spite of widespread environmental concerns regarding persisting industrial reliance on fossil fuels such as coal, the economic cost, relative abundance and local availability of coal as a strategic energy source renders it the most viable economic option. According to recent sources, an estimated 77% of the country's primary energy production is coal-based where approximately 62% of total electricity generation originates from coal combustion (DE 2015). Industrial coal liquefaction for use as liquid fuels accounts for the 15% difference (http://www.energy.gov.za/).

With coal supplies in the Highveld region of Mpumalanga gradually becoming depleted, coal mining and power generation activities in the Waterberg coalfields west of Lephalale, Limpopo has intensified over recent decades. The associated cumulative emissions of oxides of nitrogen (NO_X) and sulphur (SO_X) pose potentially significant threats to freshwater ecosystems in the region, with future land-use development plans including more of the same activities and consequently, more emissions (Vermeulen et al., 2011).

By examining historical trends and patterns in regional rainfall records, hydrological (flow) data and selected streamwater chemistry, in conjunction with regional land-use information, this study aimed to acquire a better understanding as to if and when changing land use practises and/or the inception of NO_X and SO_X emissions may have influenced stream water quality in six quaternary catchments of the Limpopo Water Management Area (WMA). Selective analyses and quantification of the

aforementioned historical data records coupled with nutrient bioassay stream experiments were performed and subsequently evaluated within an overarching, theoretical framework of stream hydrodynamics and nutrient limitation in freshwater systems.

1.1 Rationale for the study

It is estimated that human activity has doubled the amount of bioavailable N on the Earth's surface (Galloway et al., 1994, Galloway et al., 1995, Vitousek et al., 1997) and increased riverine P-fluxes to the oceans by between 50% and 300% above that of pre-industrial times (Melack 1995, Howarth et al., 1995, Filippelli 2008). Further to this, numerous research programmes on an international scale have and continue to substantiate the effects of elevated nutrient levels in various catchments and aquatic systems around the world (Lawrence et al., 1999, Smith et al., 1999, Hohls et al., 2002, Likens et al., 2002, Likens 2004, Diaz and Rosenberg 2008, O´Driscoll et al., 2010, Burns et al., 2011, DWA 2011).

Owing to the gradual depletion of major recoverable coal reserves in the Witbank and Highveld coalfields of Mpumalanga over the last 40 years, mining in the Waterberg coalfields of the Limpopo Province was first initiated in the 1970s (De Jager, 1983). Power generation activities commenced thereafter when the largest dry-cooled coal-fired powerstation in the world was commissioned between 1988 and 1993 (Water Wheel 2009). In geological terms the Waterberg coalfields are underlain by Karoo sequence sediments which form a graben structure characterised by numerous faults exerting a major influence on surface and subsurface hydrology (McCarthy and Rubidge, 2005). The UNESCO-declared Waterberg Biosphere lies to the south east of the coalfields and is comprised largely of arenaceous sandstone and conglomerates of the Waterberg Group, with many of the soil types exhibiting acidic properties (Waterberg EMF 2010, Vermeulen et al., 2011).

Recent modelling approaches such as Acid-Base Accounting and X-Ray Diffraction also indicate that a significant proportion of minerals in the Waterberg parent rock may be prone to acid generation with sandy and infertile soils in the area being potentially susceptible to both S and N saturation and acidification. In simple terms, Acid-Base Accounting is a procedure for measuring the potential for undisturbed soil and rock to produce acid (Bester and Vermeulen 2010). It is the combined effects of coal mining and deposition of industrially-derived SO_X and NO_X emissions that pose potential ecological threats to freshwater systems in the Waterberg. In theory, the cumulative input of anthropogenic oxides of N and S into soils and rivers adds acidic anions into catchments above what is naturally variable. These (anthropogenic) additions of NO_X and SO_X coupled with the naturally dry climatic conditions and base-poor geologies in several areas (Waterberg EMF 2010, Bester and

Vermeulen 2010, Vermeulen et al., 2011), may lead future to imbalances in natural buffering capacities in some or many of the sub-catchments.

In this research I investigated the significance of associations between stream water quality (*i.e.* surface water chemical trends in pH and selected nutrients and ions) and development activities occurring in the study site catchments, represented by several quaternary catchments in the Limpopo WMA. I aimed to create a better understanding of nutrient limitation in several Waterberg streams and whether cumulative emission and deposition of NO_X may have had any observable effect on inorganic N:P ratios, where general predictions from similar type research are that P-limitation tends to prevail in rivers. In addition to calculating and examining historical N:P ratios, nutrient bioassay experiments were conducted to determine whether N or P is the main limiting nutrient to PP in the study site streams.

1.1.1 Research aim

To quantify and explain water quality trends and inorganic nutrient limitation patterns in some streams of the Waterberg by analysing the changes in conjunction with catchment land use and industrial developments over a period of ~30 years.

1.1.2 Research hypotheses

The research hypotheses proposed prior to any analyses were based on (i) the inherent characteristics of the water quality variables in question, (ii) the proximity of stream gauging stations to primary sources of SO_X and NO_X emissions; and (iii) the position of stream gauging stations in the catchments relative to other anthropogenic land-use activities upstream in the respective catchments (*i.e.* agriculture and human population settlements). The hypotheses are:

I. For the T_1 analysis (1982-1987), there would be no significant water quality trends in all streams.

II. For streams either downwind of and/or closer to SO_X and NO_X emission sources and for the T_2 analysis (1994-2013), there would be a decrease in pH and increases in electrical conductivity (EC), dissolved inorganic nitrogen (DIN) and sulphate (SO_4^{2-}).

III. Instream nutrient bioassays will conform to predictions of nutrient limitation based on inorganic stream N:P ratios.

1.1.3 Research objectives

i) Selectively compile, explore and conduct trend analyses for historical meteorological and hydrological data (chemical concentration and discharge) in the study site catchments.
ii) Contribute knowledge on regional catchment surface water quality by enhancing our understanding on the dynamics of stream N and P limitation.
iii) Inform discussions about best management practice for protecting, conserving and improving surface water quality.

1.1.4 Key questions

Key questions which guided the overall research, including which hypothesis (as stated in section 1.1.2) applied to each question, are as follows:

1) Is there any indication that the onset of large-scale coal combustion (cumulative and additive emissions of NO_X ad SO_X) has contributed to significant changes in the WQ variables under investigation?
   Hypothesis II.
2) Do streams upwind/further from emission sources of SO_X and NO_X show similar pH, nutrient and ion patterns to streams downwind/closer to such sources?
   Hypothesis I and II.
3) If there are any changes in catchment WQ, are such changes attributable primarily to physical factors (discharge and rainfall) or other concurrent anthropogenic activities (industry, agriculture and human settlement) acting in the region?
   Hypothesis I, II and III.
4) Which nutrient, if any, is limiting stream periphyton growth and what are the potential (eutrophication) implications of cumulative emissions of NO_X?
   Hypothesis III.

1.1.5 Logical framework for dissertation

To investigate if inorganic N and S concentrations have trended upwards in rivers owing to inputs from NO_X and SO_X emissions and whether deposition of N has affected stream primary production (PP) and inorganic N:P ratios:

1) Data (historical rainfall, stream chemistry and discharge) were mined, compiled and summarised.

2) Trend analyses were conducted and compared to other data records of land-use change. Trends in several other WQ variables (in addition to S and N) were included as part of the analyses.
3) Historical inorganic N:P ratios were calculated. Any changes in the ratios due to cumulative inputs of N from NO_X emissions were examined.
4) N:P nutrient bioassay field experiments were conducted to determine whether the results (borne out in the real-world) conformed to the prediction of N:P ratios, where it is generally accepted that P-limitation prevails in freshwater systems.

Chapter one provides contextual background on the relevant subject matter and associated problems, including the aims, objectives and hypotheses specific to the study. Study site catchment maps and other regional land-use maps are presented to provide the reader with ancillary and spatial information on the regional climate and geology as well as anthropogenic changes over time in terms of human populations, agriculture and industrial activity.

Several maps display the location of the associated study sites in relation to each catchment and for clarification purposes, the stream gauging stations, rainfall weather stations and nutrient bioassays all represented the respective sample sites. The inclusion of accessory and somewhat qualitative information in this chapter provides an initial and rudimentary understanding of the overall study area and also to refer back to for purposes of value judgements or to make comparisons based on quantitative findings in Chapter two. To a certain extent and due to layout constraints, Chapter three also covers appropriate and relevant theoretical information pertaining to that specific chapter, not all of which is included in Chapter one.

The first two study hypotheses are based on testing for significant trends between two periods of time, before and after the onset of large scale industrial emissions, which were related specifically to the findings in Chapter two. As a precursor to addressing study hypotheses I and II in Chapter two, a description of the exploratory methods conducted on the data are provided (Appendix A). Given the concept of *water quality* (and associated trends) being the main emphasis of the study, the Exploratory Data Analysis (EDA) phase was empirically focussed and fundamentally necessary in determining the type of data (parametric or non-parametric) prior to more advanced analyses. Included are the techniques and tests that were applied to adapt and refine the data in preparation for the trend analyses.

Based on the findings of the EDA (Appendix A), Chapter two describes in more detail the non-parametric trend analysis approach which was followed, including the various tests which were carried out. The homogeneity tests searched for trends with minimal adjustments to the original data, where

the intention was simply and objectively to identify the existence of step trends in the respective hydrological and rainfall time series. The Mann-Kendall seasonal and trend tests looked more specifically at whether trends existed for several scenarios (*i.e.* for two different time periods), and were therefore carried out iteratively in several stages. By ultimately detrending the effects of flow and seasonality (*i.e.* reducing the background noise or variation caused by these factors) and minimising the effects of autocorrelation, the ability of the test to identify which of the parameters had discernable trend 'signals' was increased.

Chapter three examines nutrient limitation patterns in the study-site streams, where the approach was mostly experimental in design and based on previous scientific inquires of a similar nature. Study hypothesis III relates to whether human-induced land-use changes may have influenced inorganic stream N:P ratios and if nutrient limitation in the study-site streams conformed to the general notion that periphyton growth (primary production) is predominantly limited by the availability of P rather than N. Stream nutrient bioassays were carried out to compare with generalised predictions of freshwater N:P ratios. Mention must be made that this (the experimental) component of the study constituted approximately 20% of the overall research dissertation with the remaining 80% assigned to the trend analysis component.

The overall purpose of the central chapters was to collectively address the original hypotheses and key questions raised in Chapter one in a systematic and step-by-step manner. By so doing, additional, baseline water quality trend information has been contributed at a quaternary catchment scale. By design, the study went some way in explaining whether NO_X and SO_X emissions have contributed to changes in catchment water quality. The trends identified in this research could be modelled with additional data and utilised in further studies, ultimately building upon and further contributing to regional knowledge. This may aid in the understanding and management of catchment water resources, particularly in a context where freshwater is becoming increasingly limited in both quantity and quality. With particular reference to the nutrient experiments conducted across several sub-catchments, the systematic approach and associated findings, to some degree, extend and enhance our regional understanding of nutrient limitation dynamics in the streams in the study area.

1.2 Conceptual framework for water quality at a catchment scale

Natural variations (spatial and temporal) occur in catchment WQ owing to biological, physical and chemical factors (Krug 1991, Biggs 2000). It is therefore necessary to explain some of the important and relevant conceptual processes for each of these factors at the onset, and to interpret any study-

specific WQ trends in the context of these factors. The interlinking between the biosphere, atmosphere and hydrological cycle is non-linear and in all cases bi- or multi-directional, where one individual variable of the system is influenced by and affects other system variables (Hutjes et al., 1998). The interlinking of hydro-biogeochemical interactions operating in a typical ecosystem setting, which includes that of a catchment albeit at a smaller scale, is illustrated (Figure 1.1). The different timescales (as orders of magnitude) and the associated process flows are represented by the arrows.

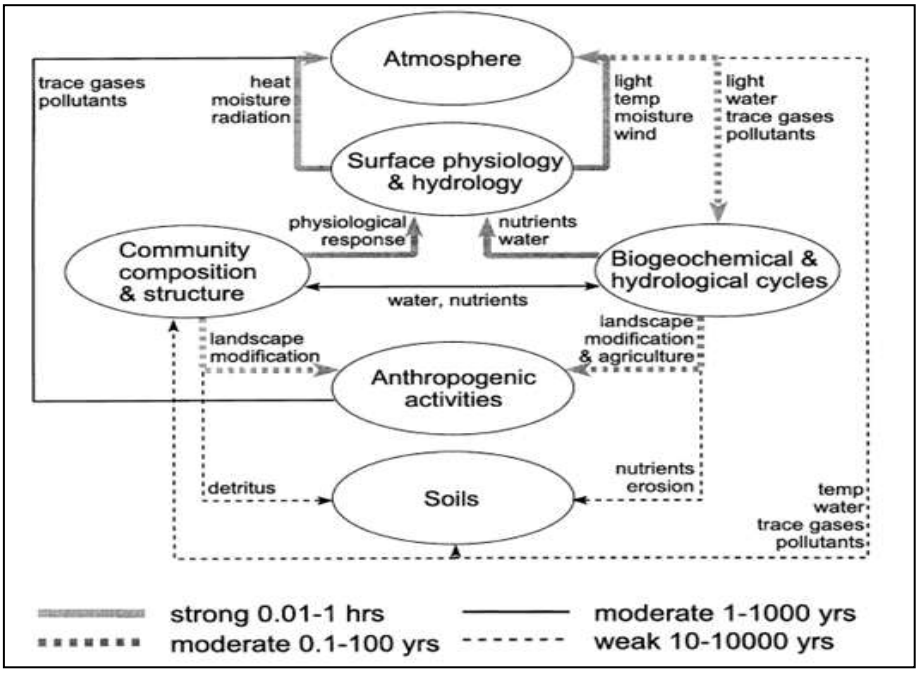


Figure 1.1: General overview of hydrobiogeochemical interactions and pathways, at various temporal scales. (adapted from Hutjes et al., 1998).

1.2.1 Climatic and geological setting

Climate refers to the long-term average of weather conditions (Tyson and Preston-Whyte 2000). The concerns relating to how human actions are influencing the natural functioning of the global climate is arguably the most widely researched and fervently debated topic of the last few decades (IPCC 2013). With respect to how climatic processes impact upon WQ, they function as causative mechanisms which influence both the distribution of acidity in precipitation and consequently, in catchment surface waters and soils (Krug 1991).

Rainfall is chemically altered as it falls through the atmosphere when it dissolves and incorporates aerosols from various sources. Sulphates (SO_4^{2-}), nitrogenous compounds (NO_X, NH_3, NH_4) and organic acids (HCOOH, CH_3COOH, $H_2C_2O_4$ and C_2H_5COOH) dissolve in tropospheric rainwater

during precipitation forming weak acids. These ions are derived from both natural sources such as volcanoes, wetlands and natural fires; as well as from human sources; such as agriculture, power plants, smelters and automobile emissions (Zhu and Schwartz 2011). Atmospheric carbon dioxide (CO_2) also equilibrates with rainwater forming weak carbonic acid (H_2CO_3), effectively giving unpolluted rainwater a widespread average pH of between 5 and 6 (Charlson and Rohde 1982, Sigha-Nkamdjou et al., 2003; Mphepya et al., 2004). In addition to wet deposition, dry deposition occurs when rainwater reacts with dry particulate aerosols which accumulate at the ground surface between precipitation events (Zhu and Schwartz 2011).

Catchment WQ is also largely influenced by the lithological composition (mineralogy) of rock in the Earth's crust. Weathering of rock material occurs either mechanically, chemically or biologically where the rate is largely determined by climatic conditions (*i.e.* temperature and precipitation) (Oliva et al., 2003). Physical and chemical weathering processes occur concurrently and in varying degrees to one another, respectively yielding different products. Physical weathering produces smaller fragments of pre-existing rocks and minerals, which are clastic in nature; whilst chemical weathering results in chemical transformation of the original lithological mineralogy, hence producing material in a chemically altered state to that of the parent rock (Chapin et al., 2002).

Chemical weathering of rock results in an alteration of water chemistry through the release of various nutrient ions and cations into catchment soils and water bodies (Ellery et al., 2007). Certain geological formations are more resistant to weathering than others owing to a variety of factors. However and with respect to this research, a basic understanding that geological factors exert a significant influence on geochemical cycling and catchment WQ is the important point to bear in mind. In the South African context and more specifically the Waterberg (still currently in a moderately natural state), it is expected that catchment WQ will be largely influenced by the underlying geological structure and composition (Hohls et al., 2002).

1.2.2 Macronutrient cycles

Nitrogen

N exists in nature in various forms and valences, and cycles between both organic and inorganic states. An in depth account of the global N cycle can be found in Söderlund and Svensson (1976) whilst Galloway et al., (2004) provide a contextual explanation of how the scientific understanding of the N cycle has changed and evolved over time. Included in the paper by the 2004 authors are several mass balance models and approaches which serve to explain fluxes and cycles in the global N budget.

What's important to elucidate in the context of this research is that the predominantly stable form of N (atmospheric dinitrogen/N_2) is naturally convertible to reactive forms mostly via biological N fixation and to a much smaller degree by lightning (Chapin et al., 2002). Pathways and processes involve either the reduction or oxidation of the N atom by biological or chemical means (Söderlund and Svensson 1976).

Reactive forms of N include inorganic, reduced forms such as ammonia (NH_3) and the ammonium ion (NH_4^+). Inorganic, oxidised forms (NO_X) include nitrate (NO_3^-), nitrite (NO_2^-), nitric acid (HNO_3) and nitrous oxide (N_2O). The more complex organic compounds include nucleic acids, proteins, amines, chlorophyll, hormones and urea (Galloway et al., 2004). N is an essential nutrient for plant growth (Söderlund and Svensson 1976) and constitutes most of the building blocks of life (Galloway et al., 2004). N has been identified as the major growth-limiting nutrient in coastal-marine systems (Howarth 1988; Nixon et al., 1996) whereas P is more often believed to be the growth limiting nutrient in freshwater lentic and lotic systems (Schindler 1974, Hecky and Kilham 1988, Francoeur et al., 1999, Biggs 2000). However, research has also shown that freshwater systems can switch between both N and P limitation at varying temporal and spatial scales (D´Elia et al., 1986, Elser et al., 1990, Malone et al., 1996, Downs et al., 2008, Mcdowell et al., 2009).

The N cycle is a complex one ranked against other major elements. Having seven oxidation states, numerous pathways for interspecies transformation and a range of environmental sinks and sources, tracking anthropogenic N through ecosystems is a major challenge (Galloway et al., 2004). Yet the impacts of human-induced loading of multiple forms of N into freshwater systems have numerous potential harmful effects on aquatic and non-aquatic life, and ultimately detrimental knock-on-effects on WQ and human health (DWA 2011).

Phosphorus

Similarly to N, P (as phosphate) is an essential macronutrient which organisms accumulate and use in the building of nucleic acids and in the storage and use of energy in cell respiration (metabolism) (Chapin et al., 2002). Whilst elemental P does not occur naturally in the environment and there are no biotic pathways which bring new P into ecosystems (Chapin et al., 2002), phosphate minerals within the apatite group are the most abundant types found predominantly in igneous rocks (Oelkers and Valsami-Jones 2008). Various inorganic and organic forms also occur in water and soil as dissolved and particulate species. Of these, pentavalent orthophosphate species such as H_2PO_4 and HPO_4^{2-} are the only soluble forms of inorganic P that aquatic biota (microbes and phytoplankton) are able to utilise (Rigler 1956).

The natural derivation of P in water bodies is from the weathering of rocks containing non-volatile compounds of P (USEPA 1986) as well as from the decomposition of organic matter (Wetzel 2001, DWAF 2011). Surface waters tend to receive most P from surface flows when phosphates bind to soils and sediment via sorption (Filippelli 2008). Exceptions occur when catchment streams are superimposed upon crystalline, igneous (volcanic) geologies and/or in upland sections of a catchment, with gradual increases in P occurring in lowland catchment reaches that are often largely comprised of sedimentary deposits (Filippelli 2008, DWAF 1996).

Sulphur

Depending on the surrounding environment, S occurs in various oxidation states ranging from +6 in aerobic conditions (as sulphate) to -2 in anaerobic conditions (as sulphide) (Zhu and Schwartz 2011). S (as sulphate) is an essential component of proteins and various other organic molecules and once assimilated and metabolised by microorganisms, undergoes transformation into either a reduced or oxidised state (Granat et al., 1976, Zhu and Schwartz 2011). The biogeochemical cycling of S is comprised of many steps and processes but as a starting point, the source of S originates from igneous rocks such as pyrite (Fe_2S) found in the Earth's geology (Chapin et al., 2002, Coetzee et al., 2010). S is a major by-product of coal combustion (DWAF 1996, Lampert and Sommer 1997) with human activities having doubled the global fluxes of S through mining and via industrial emissions (Chapin et al., 2002).

If the S cycle is considered from an input-output model perspective, the predominant natural inputs of mobile S into environmental media are weathering, volcanic activity, sea-spray and atmospheric (wet and dry) deposition. Natural outputs of S include river runoff and releases of reduced, volatile forms from waterlogged soils and wetlands (Granat et al., 1976).

1.2.3 Rivers: characteristics and components

Rivers are principally defined as exhibiting unidirectional water flow, fluctuating hydrological regimes, a high degree of spatial and temporal habitat heterogeneity and biota that are highly specialised within heterogenous (micro) habitats (Bernot and Dodds 2005). A brief description of the basic disciplines relating to rivers is provided in this section to integrate them within a conceptual framework which explains the roles of these disciplines in understanding the functioning of freshwater systems.

Fluvial geomorphology

Fluvial geomorphology is primarily concerned with sedimentation and transport processes that operate in river systems and the resultant landforms which are created (Rowntree and Wadeson 2005; Mutz et al., 2013). In the context of river health in South Africa, geomorphology is one component alongside several (*i.e.* water quality and hydrology) which is included in an overall assessment of ecosystem health and integrity (Kleynhans et al., 2005).

Stream hydrology

Stream hydrology is primarily concerned with the quantification of surface water flows and solute transport by focussing on the aspects of volume, timing, duration and magnitude of flows occurring at the catchment scale (Richter et al., 1996) and can also be conceived to interface at the land-atmosphere boundary where the distribution and movement of water, sediment and energy is strongly influenced by climate, biota and human interventions (O´Driscoll et al., 2010). In the context of this research, the effects of seasonality (*i.e*. timing and magnitude of flows) on water quality were investigated.

Water quality

The concept of *water quality* is a normative term used in management to describe the properties of water that determine its fitness for a range of uses and for the health of aquatic ecosystems (DWAF 1996). The required *quality* of the water will depend on the purpose for which it is intended and in South Africa, it is linked to a series of national water quality guidelines (DWA 2011). The properties of a given body of water are influenced by many different variables or constituents which are essentially used as descriptors to describe the *quality* of that water body. However, a basic description or statement about *water quality* does not offer sufficient contextual detail as to the desirability or acceptability of that given body of water exhibiting the stated properties (DWAF 1996).

As described in the 1996 South African Water Quality Guidelines, the concentrations of specific WQ variables are assessed against specific WQ *criteria*. These *criteria* are based on scientific and technical information for each of the WQ variables, with the rationale being that if the most sensitive species in major aquatic trophic groups are used as reference species for the setting of protection criteria, then all other species within the trophic group will fall under the same umbrella of protection. In a hypothetical scenario where the most sensitive aquatic species are present and flourishing, catchment WQ can be deemed to be in a good state, ecologically and in terms of being safe for human use and consumption (DWAF 1996).

1.3 Environmental impacts of declining water quality through anthropogenic activities

Dating back to the early nineteenth century, the industrial revolution has resulted in the progressive alteration and transformation of landscapes into what exists today as a globalised mosaic of predominantly industrial-based economies. The major land-use activities of agriculture, agro-forestry, mining, urbanisation and general industry have primarily been and continue to be driven by fossil fuel combustion (Smith et al., 1999, Hoff 2011). As a consequence, nutrient pollution of freshwater and nearshore marine ecosystems has become one of several prominent global concerns; the recent and short-term effects of which have and continue to be observed, but of which the long-term and ultimate impacts are yet to be fully known (Smith et al., 1999, Steffen et al., 2005, Diaz and Rosenberg 2008). Aquatic ecosystem pollution is largely attributable to anthropogenic activities operating simultaneously and often combining cumulatively, having adverse effects on both aquatic and terrestrial biotic communities, as well as human health (Hill et al., 2011).

1.3.1 Acidification

Freshwater acidification, one form of aquatic pollution, has been researched extensively over the last several decades, particularly in North America and Europe where the extent of acid-sensitive areas are much more widespread than was previously believed (Schindler 1988, Burns et al., 2011). The natural susceptibility of freshwater systems to acidification is largely dependent on the geology (parent rock) and soils of the catchment and additionally to natural inputs from precipitation and biological activity (Vitousek 1997, Bobbink et al., 2010).

The ability of catchments to buffer acidity is often defined in terms of their 'acid neutralisation capacity' (ANC) (Krug, 1991) which is the sum of the overall difference between their base cation and acid anion concentrations (Stumm and Morgan, 1981). Anthropogenic acid inputs into catchment systems in combination with natural inputs have the potential to influence the ANC of freshwater ecosystems beyond that which is naturally variable (Burns et al., 2011). The comparative magnitude of these influences have yielded some observable results in a number of large-scale catchment studies and research experiments (e.g. Lawrence et al., 1999, Likens et al., 2002, Likens 2004, Burns et al., 2011).

1.3.2 Eutrophication

Eutrophication, another form of pollution resulting from increased nutrient supply to aquatic primary producers, has further implications for the natural functioning of aquatic systems in that it disrupts and

potentially alters aquatic species compositions, community structures and food webs (Schindler 1988) and which is a similarly challenging problem to society in that it reduces the desired state of water quality necessary for safe human consumption (DWA 2011). Nutrient-induced eutrophication often leads to harmful proliferations (blooms) of algae and cyanobacteria, the latter often producing various toxins that contaminate water bodies and negatively impact upon human health, livestock, fish populations and wildlife (DWAF 1996, Johnson et al., 2010). In South Africa, problems associated with eutrophication were identified several decades ago (Toerien 1975, Toerien 1977) and have continued to exacerbate (e.g. Oberholster and Ashton 2008).

Hypoxia, a condition frequently associated with eutrophication, continues to deplete dissolved oxygen levels in many of the planet's estuaries, seas and coastal zones and in so doing, is potentially altering these ecosystems beyond the possibility of full recovery of their natural functioning. A number of 'dead zones' have already developed in the Baltic, Black Sea, Gulf of Mexico and the East China Sea with the phenomenon expected to persist and exacerbate if urbanisation, burning of fossil fuels and fertiliser application continues to load nutrient pollutants into river run-off and consequently, estuarine and marine environments (Diaz and Rosenberg 2008). Adapted from DWAF (2002) is a basic illustration of the factors and processes that act simultaneously, cumulatively and often synergistically to determine the overall trophic status of a given body of water (Figure 1.2).

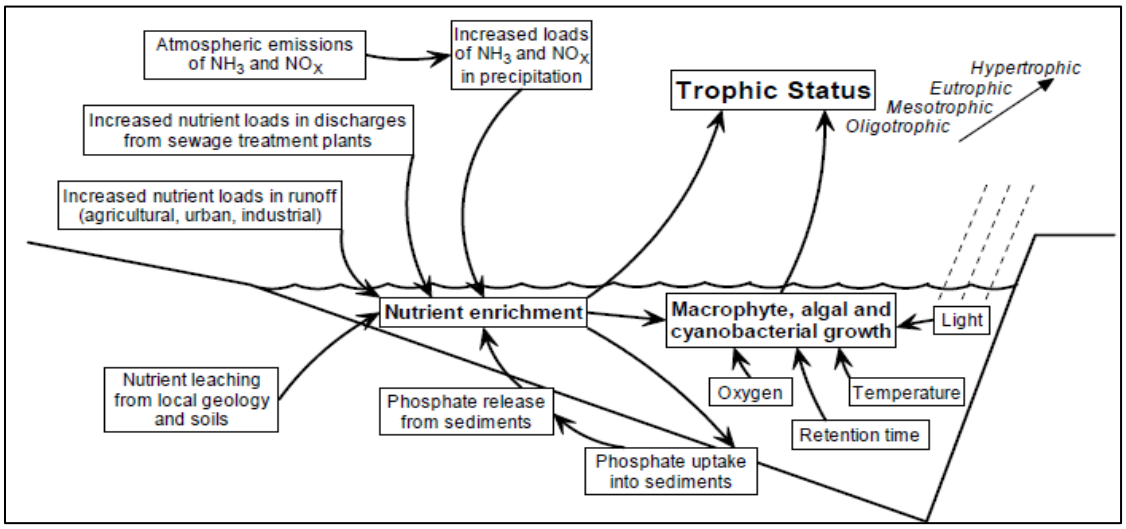


Figure 1.2: Natural and anthropogenic factors and processes which collectively determine the trophic status of waterbodies (Source: DWAF 2002).

1.4 Background to study site

From a geographical and jurisdictional perspective, all study sites are located within the Waterberg district municipality which falls within the Limpopo province of South Africa. From a drainage basin/catchment perspective, the quaternary catchment study sites lie within the Limpopo Water Management Area (WMA). The locations of the quaternary catchments are shown (Figure 1.3) with those being selected for inclusion in the study based on (i) the primary source location of SO_X and NO_X emissions and (ii) the historical hydrological data record. The data record needed to be sufficiently detailed if the respective trend analyses and tests were to yield robust and meaningful results.

In geological terms, the broader Limpopo region is comprised largely of slate and quartzite (having been metamorphosed from mudstone and sandstone) belonging to the Transvaal Supergroup; a broad basin-shaped formation which inwardly dips towards a central basin. The interior of this basin contains the Bushveld Igneous Complex which is largely characterised by various layers of different igneous rock types which have weathered at differing rates, resulting in the current landscape of today (McCarthy and Rubidge, 2005). Gabbro (previously predominating the lower layers) gradually weathered to produce the black turf soils of today whilst the upper layers contained more resistant granites and rhyolites. These latter rock types are visible today as elevated terrain in parts of the Waterberg west of Mokopane. The younger sedimentary rocks deposited during the Proterozoic eon include resistant quartzites of the Waterberg and Soutpansberg Groups which currently form the high ground of the Waterberg and Soutpansberg (Ellery et al., 2007).

The climate of the Limpopo is predominantly dry and typically receives only summer rainfall between the months of September and March (Vermeulen et al., 2011). Whilst local rainfall figures range between 200 and 2000 mm per annum, the provincial mean annual rainfall is 530 mm. The mean annual evapo-transpiration is 1970 mm per annum with spatial variations occurring between 800 and 2400 mm (ARC 2013). The mean annual temperature for the Waterberg ranges between 19 and 22°C (DST 2010).

The entire study area is situated within the Savanna Biome which is comprised of four Low and Rebelo vegetation types. These include the Waterberg Moist Mountain Bushveld which occurs on sandstone and quartzite of the Waterberg Mountains, the Clay Thorn Bushveld which is widely distributed on flat plains of black or red vertic clay soils derived from basalts, the Sweet Bushveld which occurs largely in areas of low rainfall and high grazing pressures and the Mixed Bushveld comprising numerous plant communities ranging from dense short bushveld to open tree savanna (Low and Rebelo 1996). Several

maps have been included showing in more detail the locations of the study site catchments and sample sites.

The locations of the six quaternary catchment study sites, at the WMA and national scale, are shown (Figure 1.3). Maps showing the quaternary catchment boundaries were created in Google Earth version 7.0 (https://www.google.com/earth/) (Figures 1.4, 1.5 and 1.6). These maps show higher resolution spatial detail and provide an enhanced perception of the various catchment scales. The catchment boundary data was sourced from the Municipal Demarcation Board (http://196.3.166.156/index.php/downloads/boundary-data/limpopo-4/dc36/google-earth-kmz-file-32/10982-dc36-all-kmz).

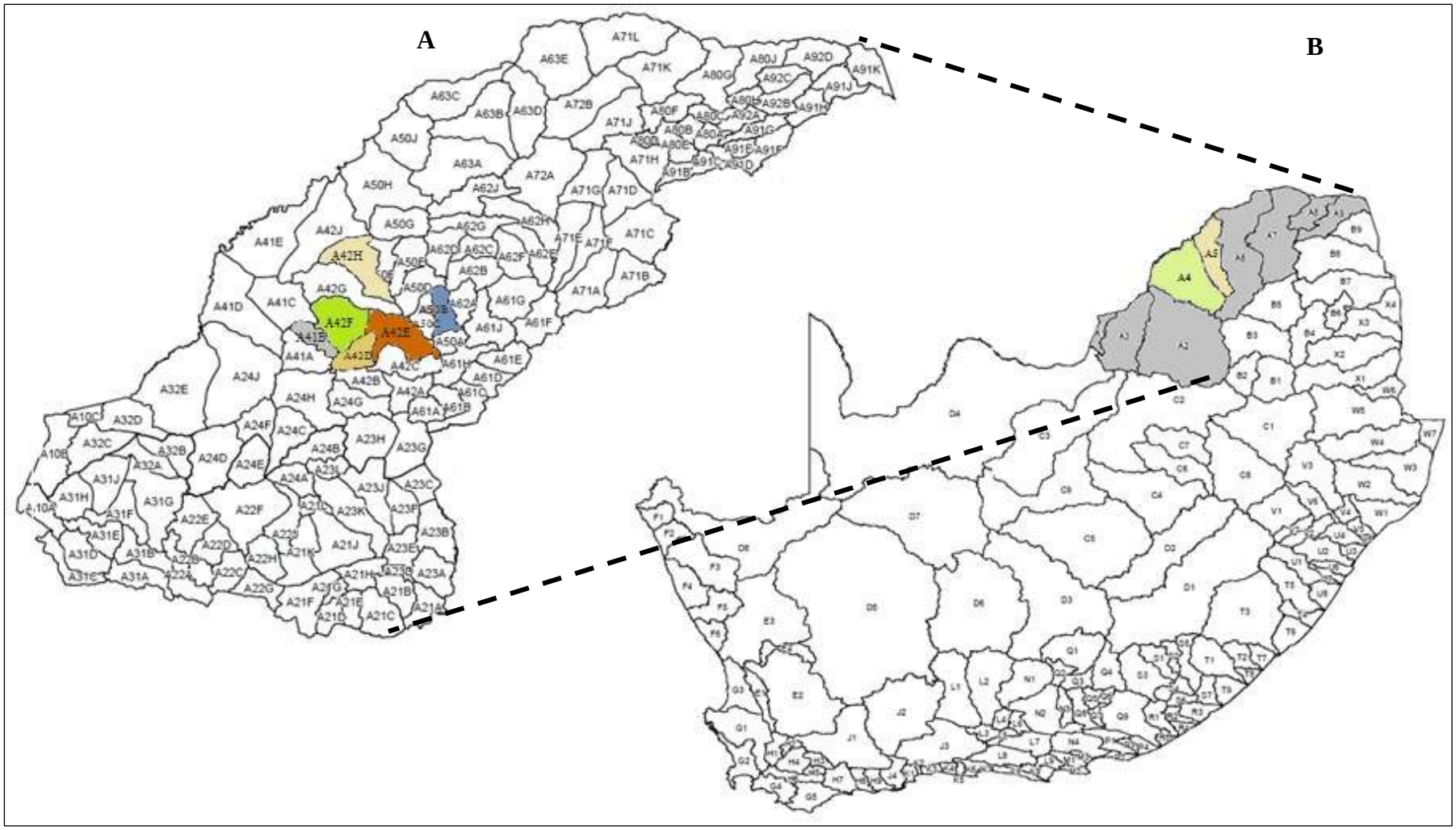


Figure 1.3: Location of the Limpopo WMA shaded in grey on the national map (B). Secondary catchments (A4 and A5) relevant to the study are shown in green and light brown. The 6 shaded quaternary catchments on the left map (A) are those located in the Waterberg region which had sufficient data records and which were specifically included in this study. (Maps adapted from Macfarlane et al., 2006).

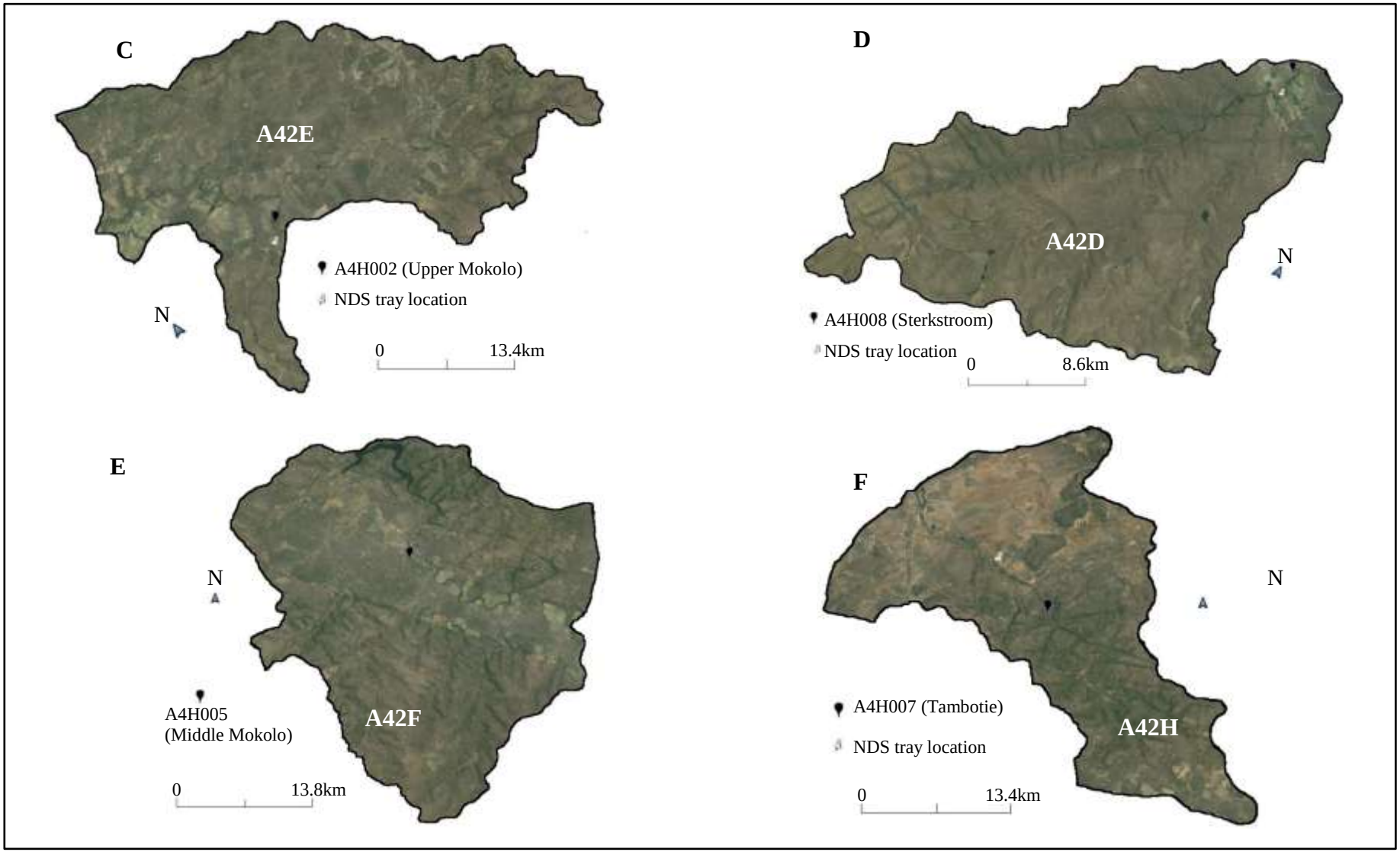


Figure 1.4: Quaternary catchments located within tertiary catchment A42, showing location of sampling sites.

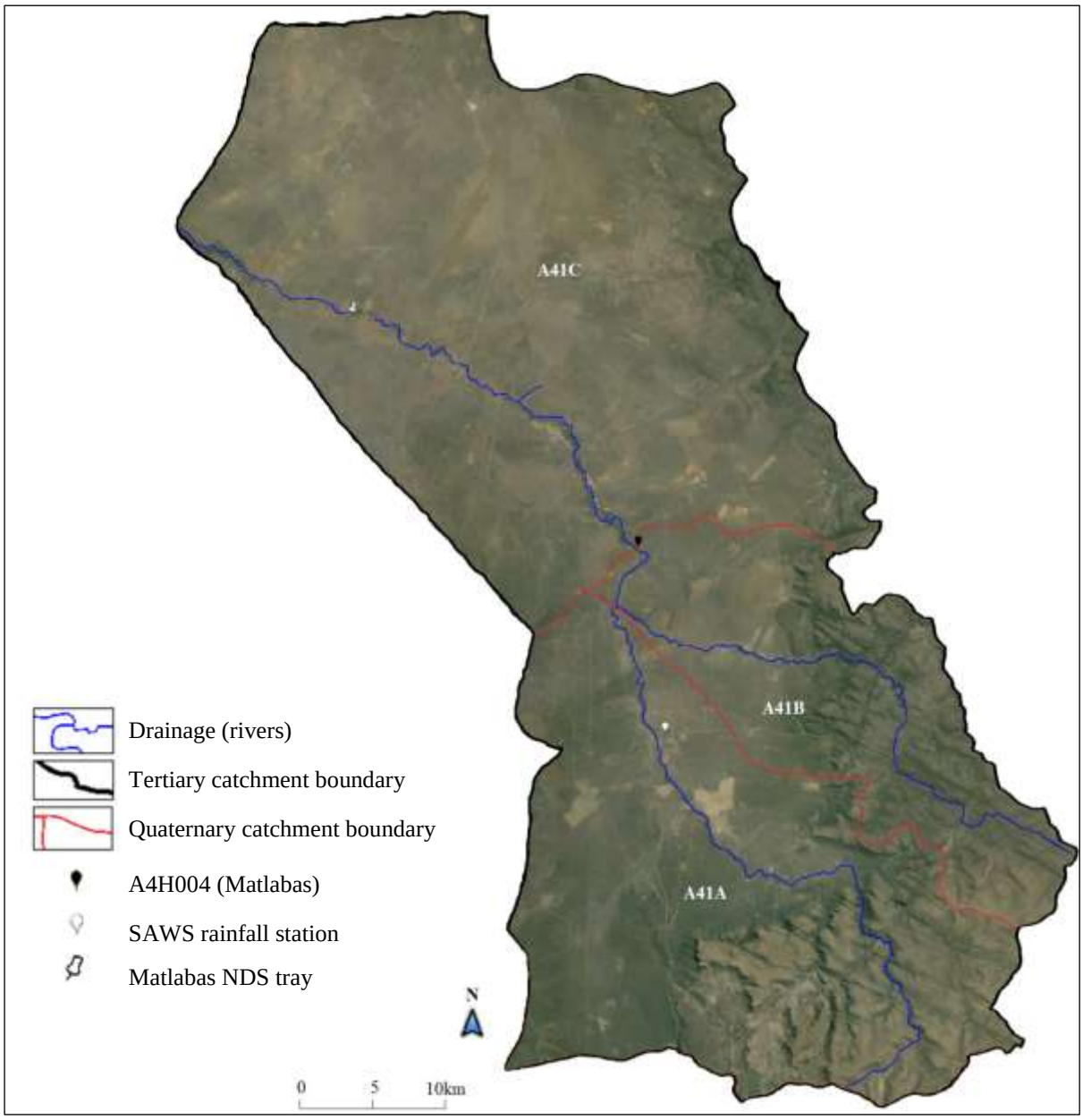


Figure 1.5: Tertiary catchment A41 showing location of sampling sites.

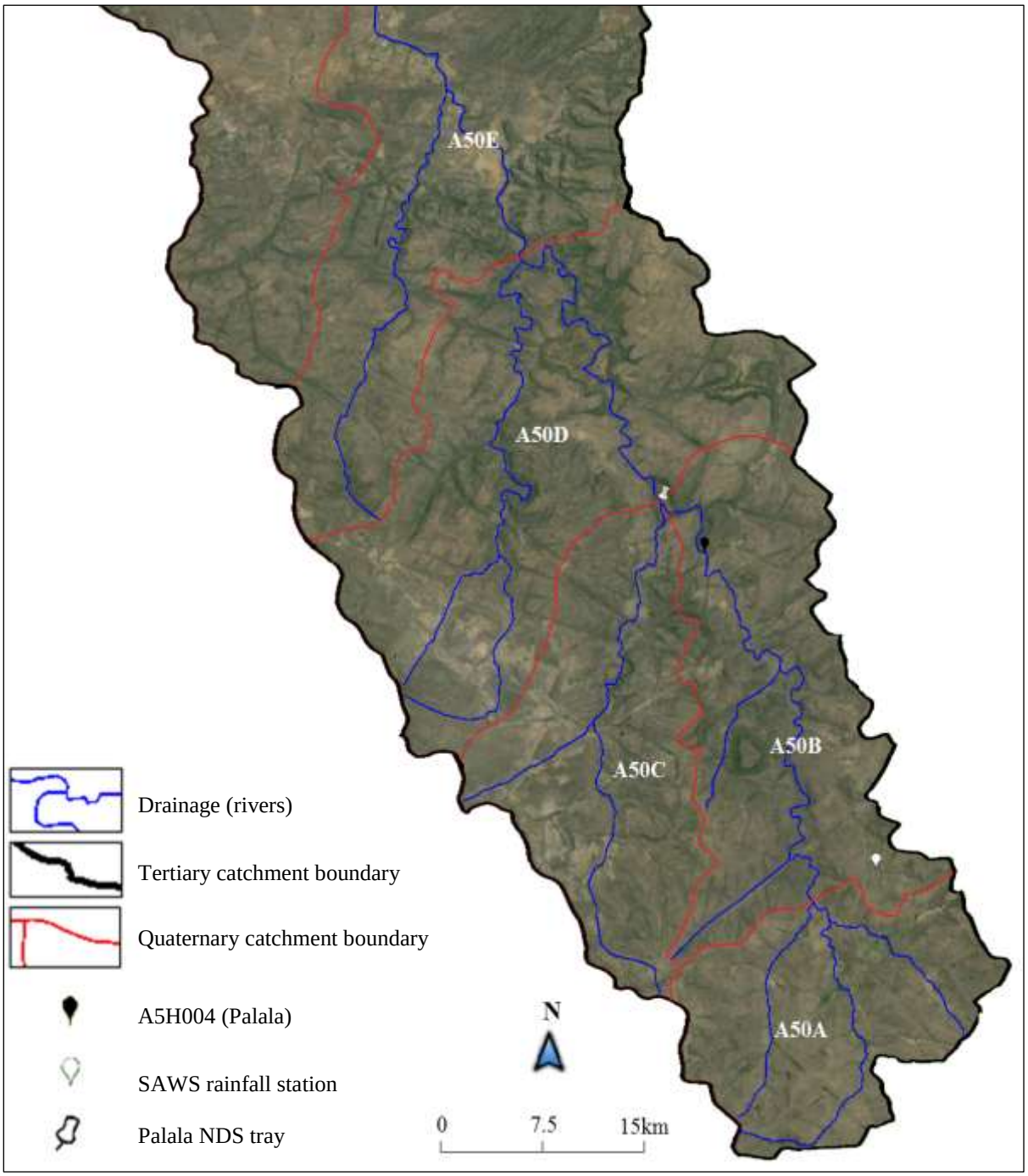


Figure 1.6: Tertiary catchment A50 showing drainage and location of sampling sites.

1.5 Ancillary data

To assist with understanding the mechanisms of any potential trends in WQ post-analysis, some relevant ancillary data have been included in this section. The tables and figures provide regional, spatial background information where basic descriptions have been provided but for which detailed analyses would go beyond the scope of the project.

1.5.1 Land use

Human populations

In terms of regional human populations in the Waterberg district, historical changes from censuses conducted in 1980, 1991, 1996, 2001 and 2011 are provided (Figure 1.7 and Table 1.1). Although the figures suggest the regional population to have more than tripled since 1980, the figures from 1980 and 1991 are somewhat ambiguous to the extent that those censuses excluded demographic groups based on previous governance policies. The initial two censuses did not include the former native homelands of Lebowa and Bophuthatswana. Post-1994 and after the first all-inclusive census of 1996, there were several jurisdictional changes and name changes which were effected nationally, including the then Transvaal province.

According to national census data the total population of South Africa was approximately 25 million in 1980, almost doubling to 50 million people 30 years later, as reported during the 2011 national census (http://interactive.statssa.gov.za:8282/webview/). If by inductive reasoning the 30-year national increase is inferred to the regional level, the overall population in the Waterberg district has probably more or less doubled rather than tripled between 1980 and 2011. Whilst an estimated 680 000 people were residing in the Waterberg District in 2011, exact population densities upstream in each quaternary catchment were not specifically quantified. However, densities are estimated to be relatively low given the respective land uses and spatial information (map G of Figure 1.8).

To focus more closely on the influence of human settlements in the study site catchments, it is useful to look at both maps G and H (Figure 1.8). The only town with a human settlement cluster of any significance upstream of the Mokolo gauging stations is Vaalwater. This cluster is considerably smaller than the closest surrounding towns of Lephalale, Thabazimbi and Bela Bela, the first of which is situated downstream and the latter two being located within other tertiary drainage regions (map H of Figure 1.8). It can therefore be deduced that the impacts of human settlements on stream WQ could be a factor in the Mokolo (catchments A42E and A42F) with respect to the town of Vaalwater. All

other study-site catchments have relatively low numbers of people inhabiting them, where the current land uses are chiefly a combination of ecotourism and/or game farming with very little agriculture (Waterberg EMF 2010).

Table 1.1: Historical population figures for the Waterberg District, Limpopo Province, South Africa.

Magisterial District (Former Transvaal province)	Warmbaths	Thabazimbi	Waterberg	Potgietersrus
1980 census	11797	101136	80298	25750
Total	**218981**			

District (Former Transvaal province)	Warmbaths	Thabazimbi	Ellisras	Nylstroom (*Waterberg*)	Potgietersrus
1991 census	41692	48844	24530	48991	69571
Total	**233628**				

Local Municipality (Waterberg District, Limpopo province)	Bela-Bela (*Warmbaths*)	Thabazimbi	Lephalale (*Ellisras*)	Mookgopong (*Naboomspruit*)	Modimolle (*Nylstroom*)	Mogalakwena
1996 census	47619	58528	86952	14549	50057	256521
2001 census	52124	65533	85272	34541	69027	298439
2011 census	66500	85234	115767	35640	68513	307682
Population density in 2011 (persons per km²)	8	8	50	6	15	20
No. of additional people between 1996 and 2011	26706	28815	51161	21091	18456	18881
% increase over 15 years	46	33	20	145	37	40
2011 Total	**679336**					

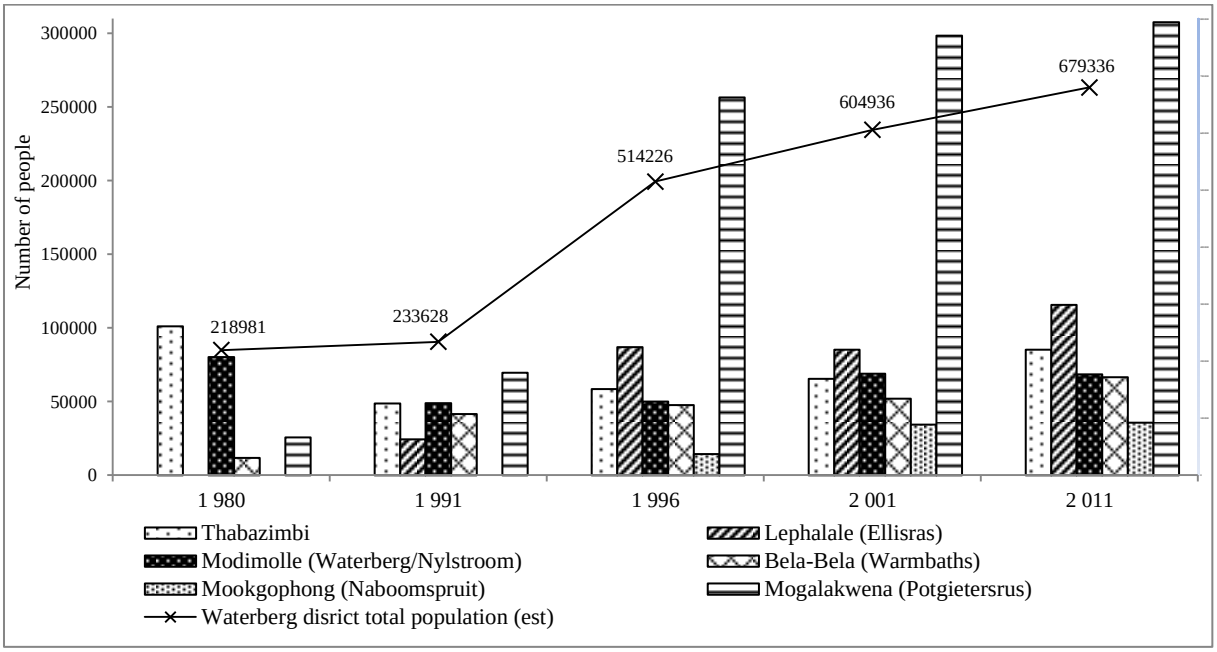


Figure 1.7: Historical human population estimates for what is today the Waterberg District Municipality, comprising 6 local municipalities. The former municipal names are included in parentheses. The 1980 and 1991 census estimates do not include the former homelands of Lebowa and Bophuthatswana. (Source: Stats SA http://www.statssa.gov.za/?page_id=964).

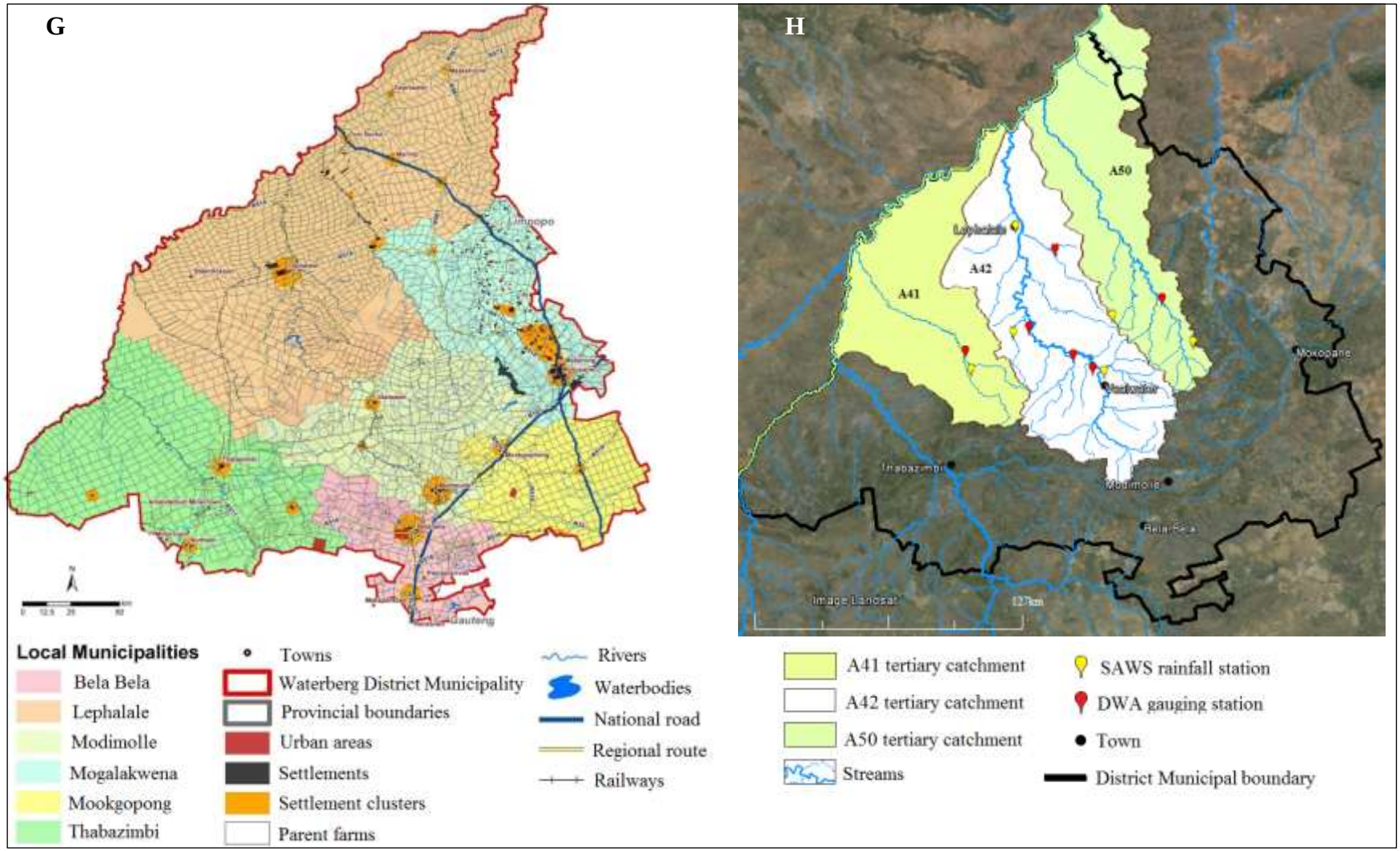


Figure 1.8: Waterberg district municipality (G) showing human population settlement clusters (Source: Waterberg EMF 2010) and study sites showing tertiary catchment boundaries (H).

Agriculture

A study which formed part of the National Land Cover (NLC) project indicates that cultivated agricultural areas in the Limpopo fell from a total land cover of 14.4% in 1994 to 10.6% in 2005 (Schoeman et al., 2010). The change in forestry and plantation areas was minimal where only 0.8% of the available land in 2005 was utilised for these activities. Although the NLC study focused more generally at a provincial level, research conducted by the Department of Water Affairs in 2001 integrated the same 1995 spatial dataset (www.dwaf.gov.za/iwqs/gis_apps/drain2/lc/sec_lc.html) to create land cover maps for each secondary drainage region. These maps had more specific relevance at a catchment level and are presented in this study.

Secondary catchment A4 (Figure 1.9) shows the various land uses in terms of area and corresponding percentage cover, based on 1995 land cover data. Five of the six study site quaternary catchments fall within the boundaries of this catchment where the hydrological gauging stations are labelled as M, UM, MM, S and T on the map. The headwaters of the Matlabas originate mostly in the thickets and bushlands of Marakele National Park upstream of M in quaternary catchments A41A and A41B. The national park is free of human settlement and agriculture, as are most of the areas outside the park (Figure 1.8 and Figure 1.9). Upstream of T in A42H, the source of the Tambotie flows through some cultivated land only in the far upper reaches, after which it flows through predominantly undisturbed savanna-type woodland and grassland. The Tambotie is a lower order tributary stream of the Mokolo river system and confluences with it just south of the town of Lephalale.

Two of the six hydrological stations applicable in this study are located in the Mokolo River, one in the middle reaches upstream of the Mokolo Dam (MM in A42F) and one in the upper reaches a short distance downstream from Vaalwater (UM in A42E). A significant proportion of the south western section of A4 is cultivated agricultural land where climate and soil conditions are favourable for such purposes. Visual assessment of historical Landsat imagery (http://glovis.usgs.gov/) indicates that agricultural activities have been underway in that region since 1972 and probably even earlier. The influence of agriculture coupled with the gradual expansion of the human population in Vaalwater was considered in the WQ trend results pertaining to those two stations (A4H002 and A4H005).

In terms of the Sterkstroom gauging station (S in A42D), the upstream headwaters originate in relatively natural and undisturbed savanna bushveld characterised by both forest woodland and thicket vegetation, interspersed with pockets of grassland. In terms of land use, the source tributaries arise in both the Marakele National Park and Welgevonden Nature Reserve, the latter sharing a border with the north-eastern edge of Marakele. As a result, virtually no commercial agriculture is found in the upper reaches

with only some occurring in the lower reaches towards the Sterkstroom and Mokolo confluence (map C and D of Figure 1.4 and Figure 1.9).

Secondary catchment A5 (Figure 1.10) also shows respective land use information based on 1995 land cover data. The Palala gauging station (denoted as Pa on the map) is located in quaternary catchment A50B (also see Figure 1.6). In terms of the NLC study and observations from recent and historical satellite imagery (http://glovis.usgs.gov/, http://landsat.usgs.gov/ and http://landsatlook.usgs.gov/) agriculture in the upper (*i.e.* southern) reaches of A5 is and has been largely limited to tracts of land in the south western part. It is also important to note that the DWS hydrological station used in this study (A5H004) is located upstream of those tributaries draining the tracts of agricultural land (Figure 1.10). The first tributary flowing into the Palala is Melk Rivier, which occurs approximately 5km downstream from A5H004. With the relatively minor exception of a small exotic forest plantation a few square kilometres in extent, water flowing via this gauging station is largely unaffected by human activities. Much of the surrounding land use is conservation and ecotourism (Waterberg EMF 2010).

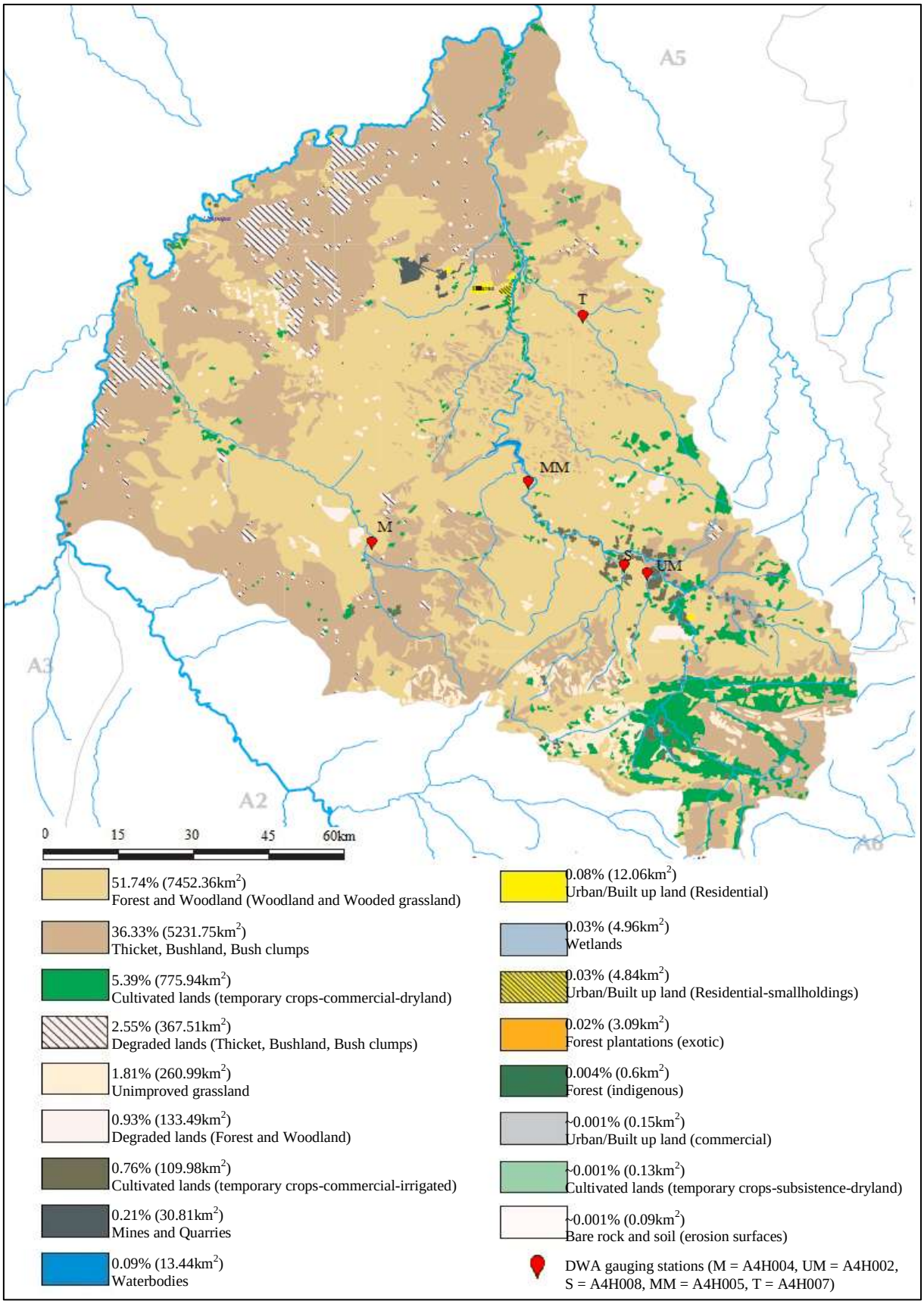


Figure 1.9: 1995 land cover map for secondary catchment A4.
(Source: https://www.dwaf.gov.za/iwqs/gis_apps/drain2/lc/lc_A4.PDF).

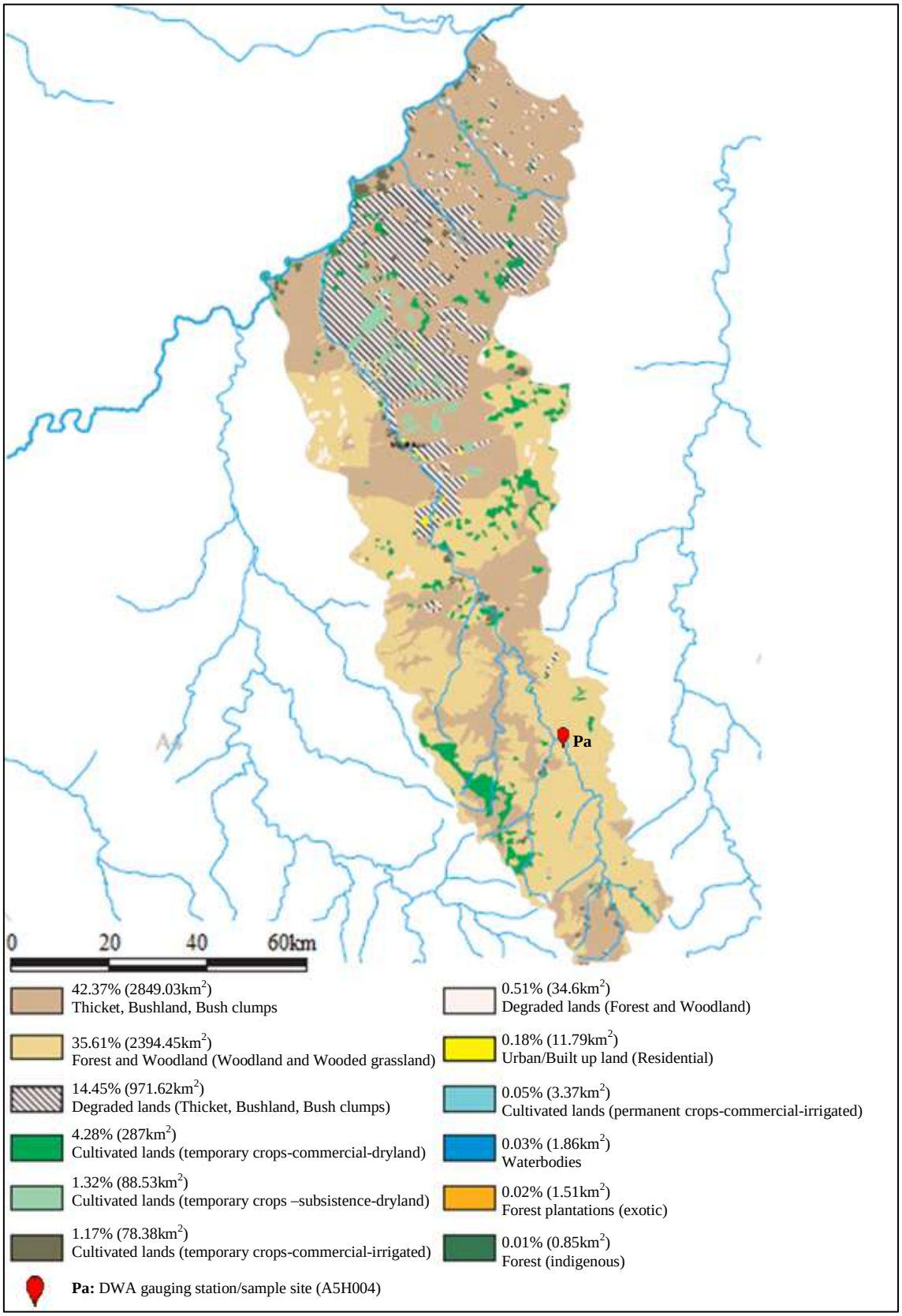


Figure 1.10: 1995 land cover map for secondary catchment A5.
(Source: https://www.dwaf.gov.za/iwqs/gis_apps/drain2/lc/lc_A5.PDF).

1.5.2 Physical

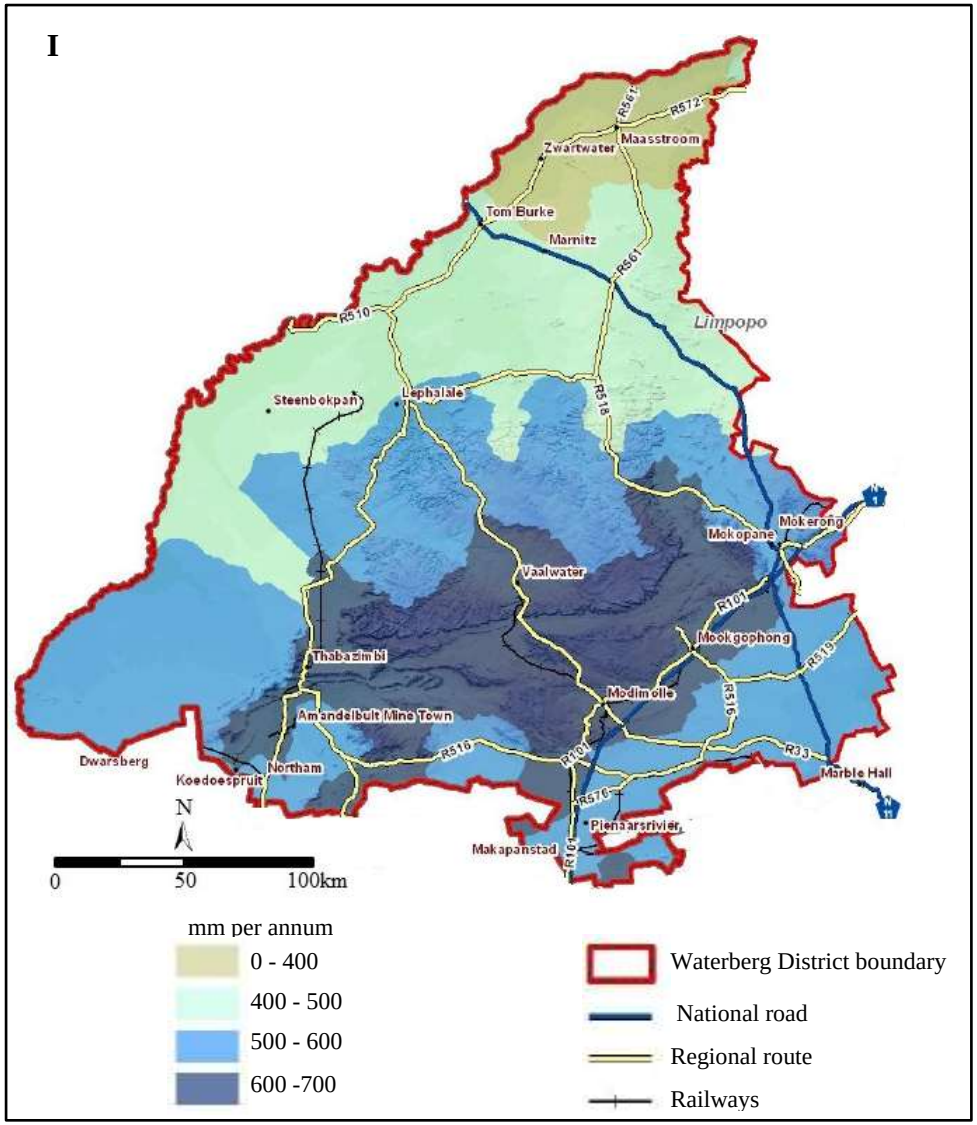
Climate

Average rainfall and mean temperatures for the Waterberg district (Figure 1.11) and average wind speeds and directions (Figure 1.12) are provided (Waterberg EMF 2010). The southern and eastern regions receive more rainfall than the northern and western region, the latter having a hotter and more arid climate. Maximum and minimum summer temperatures range between 16°C and 33°C with winter figures decreasing to between 5°C and 25°C. The regional wind field and wind directions are influenced largely by the local area topography and macroscale air circulation patterns which are predominantly anticyclonic in southern Africa (Tyson and Preston-Whyte 2000).

In the region of Lephalale where atmospheric NO_X and SO_X emissions have arguably been impacting on air quality for several decades, the wind blows primarily from the north-east (WDM 2009). The wind rose for the Marakele towers monitoring station, which lies more centrally in the study area, shows a similar pattern where the wind blows from an easterly direction most of the time (Figure 1.12). These regional wind patterns had an influence in the setting of the study hypotheses, where it was proposed that WQ in streams downwind from (*i.e.* the Matlabas) and closer to (*i.e.* the Tambotie and Middle Mokolo) emission sources would decline from ~1994 onwards. Impacts on WQ due to emissions were proposed to be negligible for the other study site streams located upwind or further away from the main emissions source.

Geology and soils

The simplified geology and 26 dominant rock types in the region are presented (Figure 1.13). All the study site streams flow through the Waterberg Group comprised of shale, quartzite and rhyolitic rocks. The shallow soils in the upland catchments through which the headwater streams flow are derived largely from sandstone and quartzite, are weakly developed and mostly not suitable for farming due to their sandy and acidic properties (Waterberg EMF 2010). Recent studies further suggest that a large proportion of minerals in the parent rock may be prone to acid generation with sandy and infertile soils being potentially susceptible to both S and N saturation and acidification (Vermeulen et al., 2011). The soil pH map (Figure 1.14) shows that the study area is dominated by soils slightly acidic in nature, with almost none of the region having neutral or alkaline soils (ISRIC 2013).



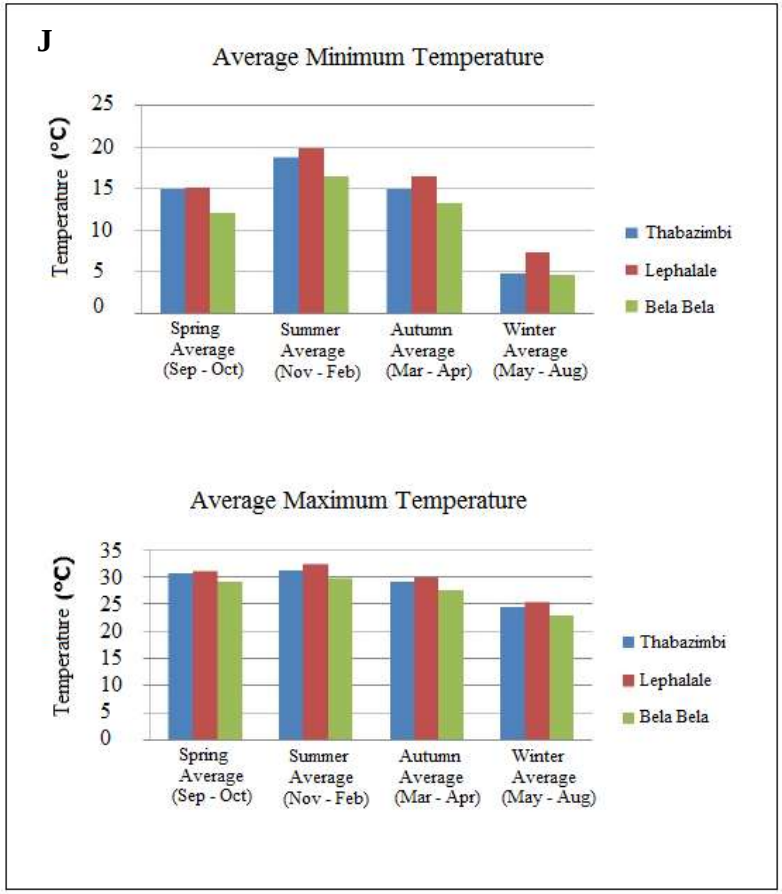


Figure 1.11: Mean annual precipitation per quaternary catchment (I) and average maximum and minimum temperatures (J) (Source: Waterberg EMF 2010).

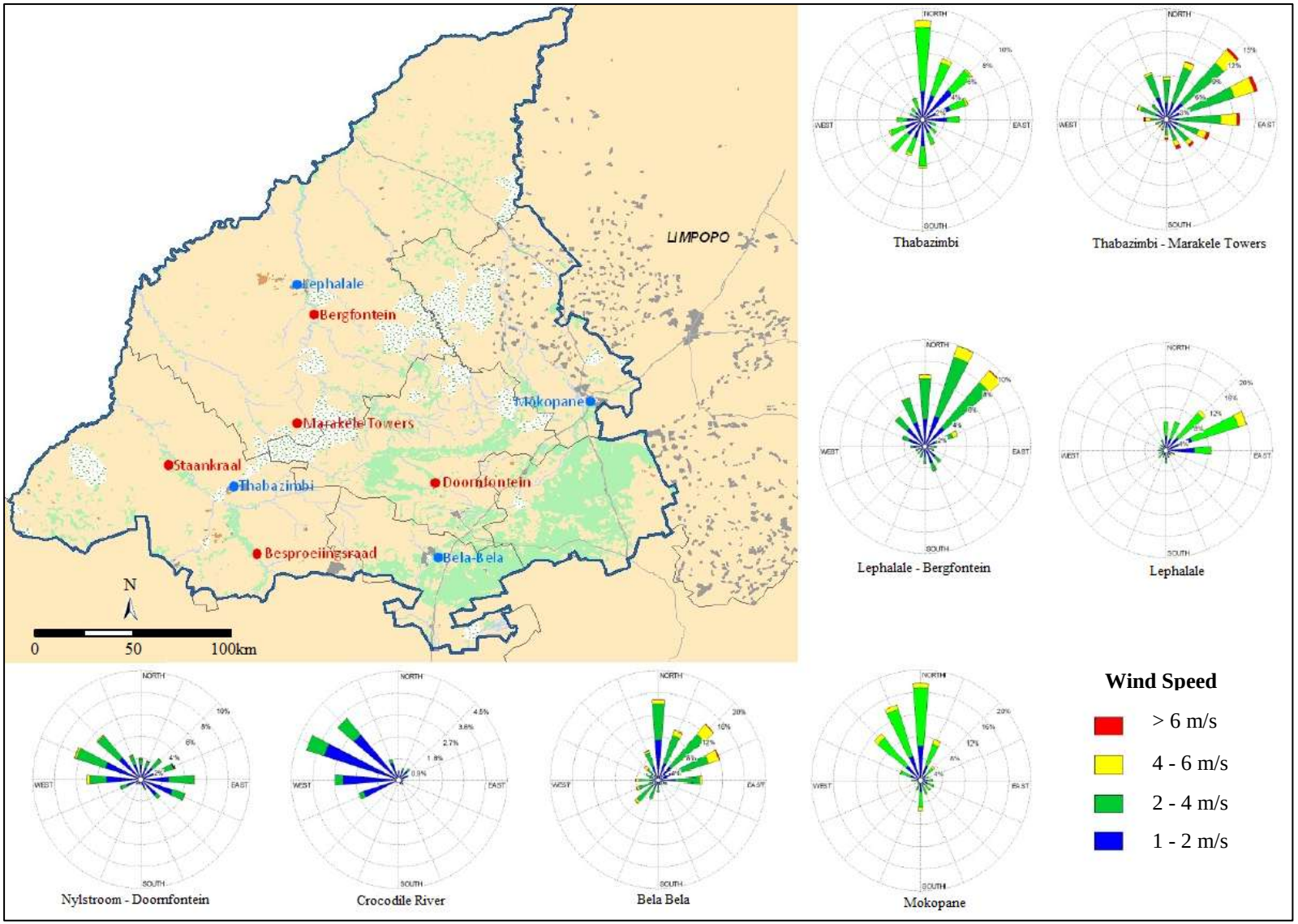


Figure 1.12: Average wind speed and direction for the Waterberg region (Source: Waterberg EMF 2010).

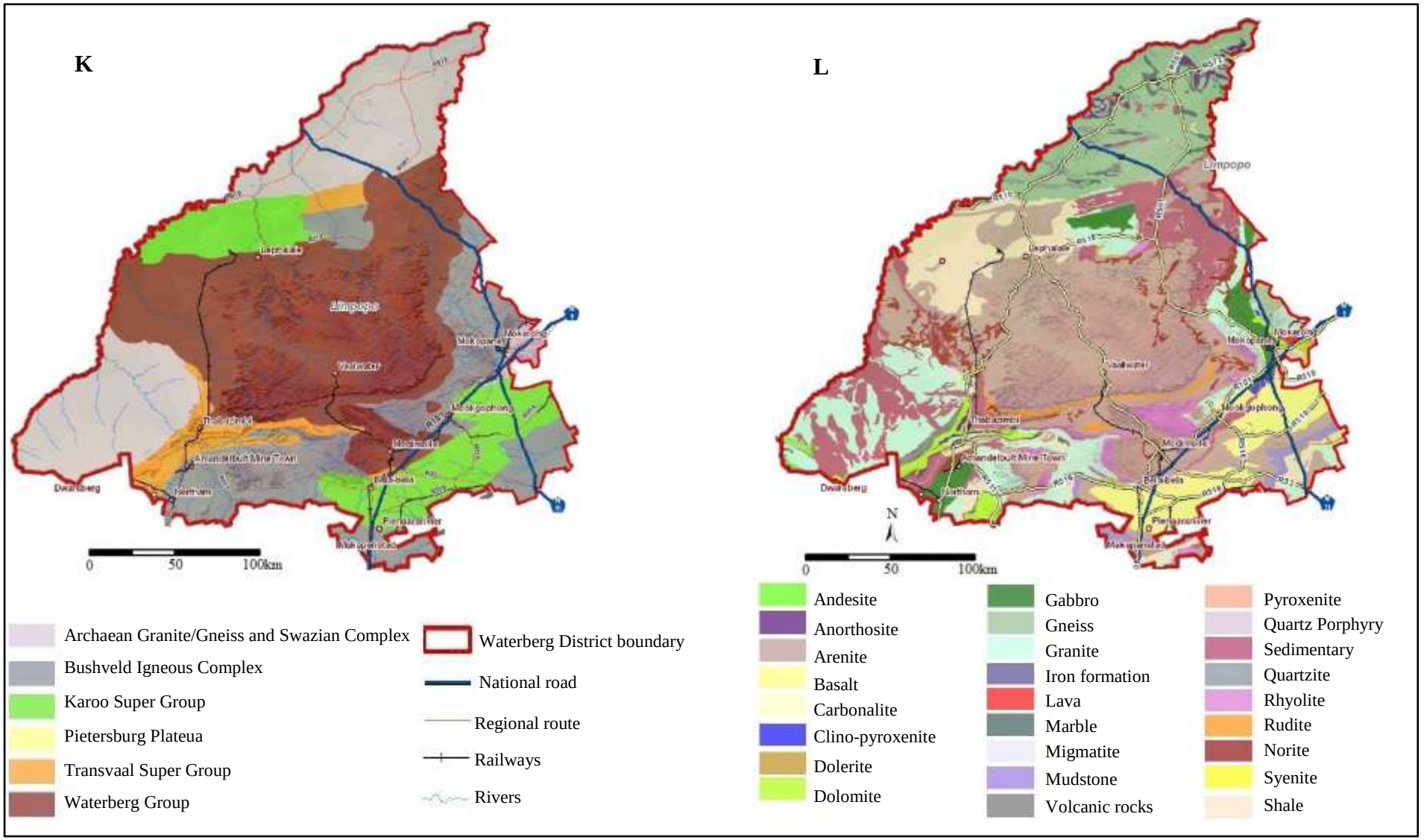


Figure 1.13: Simplified geological map (K) and dominant rock types (L) (Source: Waterberg EMF 2010).

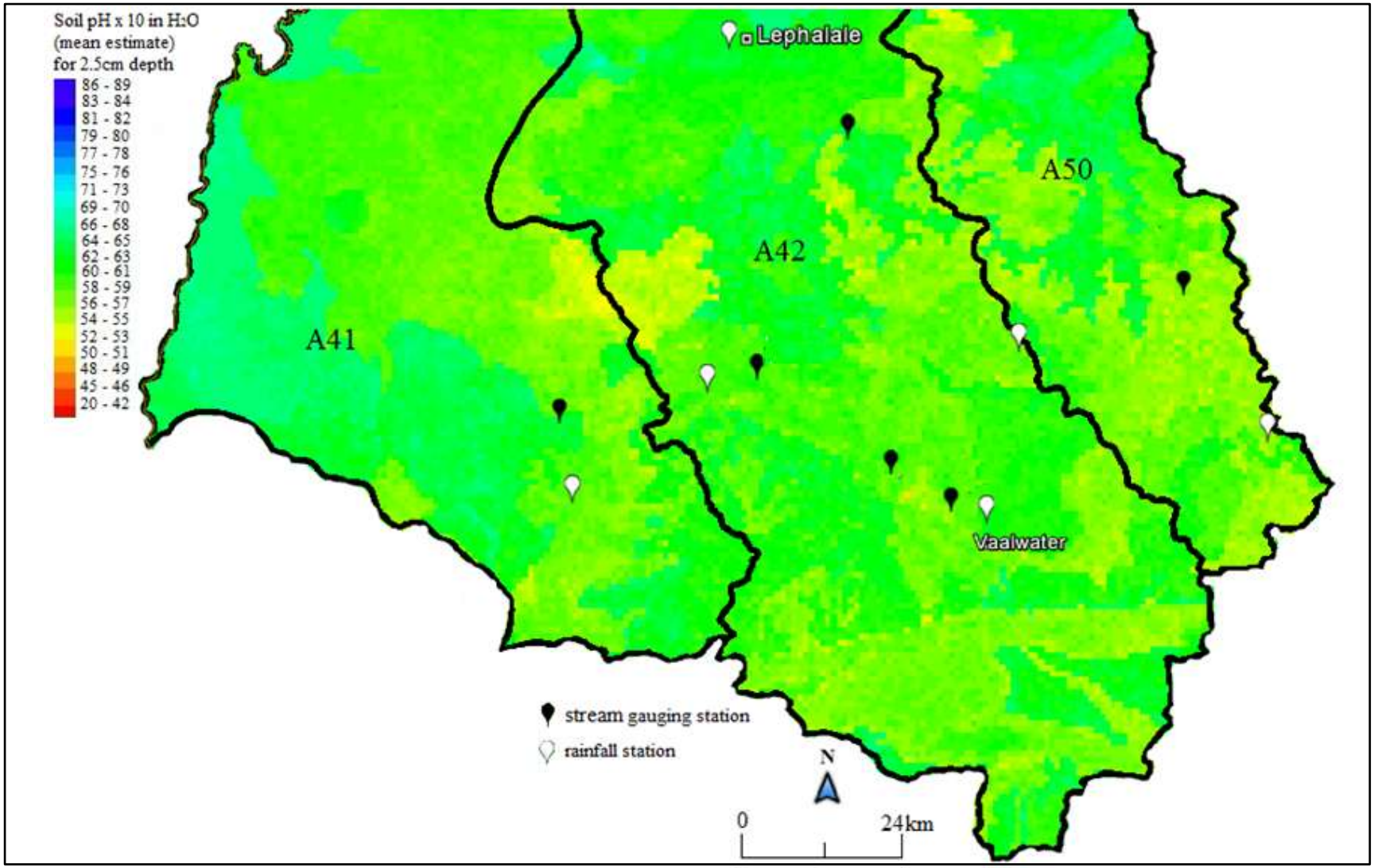


Figure 1.14: Soil pH map for tertiary catchments A41, A42 and A50 (ISRIC 2013).

2 Water quality and quantity trend analyses for several Waterberg streams

2.1 Introduction

The exploratory methods and tests used to characterise the raw data and adapt them prior to subsequent trend testing have been described (Appendix A). Tests for normality, correlation, first-order autocorrelation (AR[1]) and heteroscedasticity were carried out. I included the Exploratory Data Analysis (EDA) approach as an entire section in Appendix A as it provides the reader with context and reasoning for the two trend tests selected for this study and described in this chapter. These were the Pettitt's homogeneity test and the Mann Kendall (M-K) trend test, the latter which included methods for dealing with seasonality and autocorrelation.

The variables tested included rainfall, discharge (Q), pH, electrical conductivity (EC), dissolved calcium (Ca^{2+}), dissolved sulphate (SO_4^{2-}), dissolved nitrate + nitrite nitrogen (NO_3^-+NO_{2-}^--N), dissolved ammonium nitrogen (NH_4^+-N) and orthophosphate as phosphorus (PO_4^{3-}-P) for the respective six rivers. The EDA approach and all associated trend tests followed the methods of Helsel and Hirsch (2002) and were performed in Microsoft Excel© using the add-in software program XLSTAT-Pro and XLSTAT-Time, version 2014.5.02 (http://www.xlstat.com/en). Wherever there were missing data in the record, they were excluded from the respective analyses as an option allowed by the software. Data gaps equated to either missing discharge values, missing concentration values or both corresponding values missing.

2.1.1 Data acquisition

The majority of raw chemical concentration data for all catchments in the country are maintained on the national Water Management System (WMS) database (www.dwaf.gov.za/iwqs/wms). Data that were required for this study were obtained by request from the Resource Quality Services (RQS) offices located at Roodeplaat Dam north of Pretoria. RQS is part of the Water Resource Information Management Chief Directorate which falls under the Policy and Regulation Branch of the Department of Water and Sanitation (DWA 2012b). Verified discharge data for all drainage regions are available and were obtained from the DWS hydrological database website (https://www.dwaf.gov.za/hydrology). Historical rainfall records from local meteorological stations in the study area were obtained by request from the South African Weather Services (SAWS) central office in Midrand, Gauteng. The locations of the rainfall and stream gauging stations in

each respective catchment are shown (Figures 1.5, 1.6 and 1.8[H]). The various time series records (Q, chemical concentration and rainfall) comprised data from January 1982 to July 2013.

Study site streams were selected based on availability of sufficient data. Samples from the original DWS data record constitute a screened remainder of all water samples recorded at the respective stream gauging stations. For quality control reasons, a proportion of the original samples are not officially recorded on the central WMS after screening (Hohls pers comm. 2013). Sample screening is also conducted to improve the reliability of the data and eliminate human or technical measurement errors. Wherever a parameter had an observed result below the minimum detection limit (MDL), the value reported was exactly half of that respective MDL (Hohls pers comm. 2015).

2.1.2 Summary statistics

Descriptive statistics (Tables 2.1 to 2.4), including the gaps in the time series (Table 2.5) are summarised for the raw hydrological datasets. This is for reference purposes and precedes section 2.2, which explain in more detail the specific trend tests performed on the transformed and averaged data. I have also included boxplots as additional visualisation techniques for plotting and summarising the data (Appendix B).

Table 2.1: Total number of samples for chemical concentration data (January 1982 – July 2013).

Station	**River**	**Quaternary catchment**	**Water quality parameter**						
			pH	EC	SO_4^{2-}	PO_4^{3-}-P	NO_3^-+NO_2^--N	NH_4^+-N	Ca^{2+}
A4H005	M. Mokolo	A42F	216	247	216	216	216	216	216
A4H002	U. Mokolo	A42E	386	429	373	372	382	383	382
A4H008	Sterkstroom	A42D	465	514	455	455	463	463	463
A4H004	Matlabas	A41B	139	152	139	139	139	138	139
A4H007	Tambotie	A42H	64	79	63	64	64	64	64
A5H004	Palala	A50B	131	156	130	130	128	129	128

Table 2.2: Number of samples used in the T_1 (January 1982 – December 1987) trend analysis, prior to major regional industrial emissions.

Station	River	Quaternary catchment	Water quality parameter						
			pH	EC	SO_4^{2-}	PO_4^{3-}-P	NO_3^-+NO_2^--N	NH_4^+-N	Ca^{2+}
A4H005	M. Mokolo	A42F	23	55	23	23	23	23	23
A4H002	U. Mokolo	A42E	34	78	32	32	32	34	32
A4H008	Sterkstroom	A42D	39	90	39	39	39	39	39
A4H004	Matlabas	A41B	10	24	10	10	10	10	10
A4H007	Tambotie	A42H	6	22	6	6	6	6	6
A5H004	Palala	A50B	10	37	9	9	9	10	9

Table 2.3: Number of samples used in the T_2 (Jan 1994 – July 2013) trend analysis, after progressive (additive and cumulative) industrial emissions.

Station	River	Quaternary catchment	Water quality parameter						
			pH	EC	SO_4^{2-}	PO_4^{3-}	NO_3^-+NO_2^-	NH_4^+-N	Ca^{2+}
A4H005	M. Mokolo	A42F	151	150	151	151	151	151	151
A4H002	U. Mokolo	A42E	308	307	297	296	306	305	306
A4H008	Sterkstroom	A42D	353	351	343	343	351	351	351
A4H004	Matlabas	A41B	104	103	104	104	104	103	104
A4H007	Tambotie	A42H	46	44	45	46	46	46	46
A5H004	Palala	A50B	115	121	119	121	121	119	119

Table 2.4: Descriptive statistics for discharge and chemical concentration variables used in the study (based on raw data between January 1982 and July 2013). The discharge statistics are based only on Q values observed at more or less same point in time (+/- several hours either way) as the other WQ variables.

River (gauging station)	**Stream order**	**Parameter**	**Q**	**pH**	**EC**	**Ca^{2+}**	**SO_4^{2-}**	**PO_4^{3-}-P**	**NO_3^-+NO_2^--N**	**NH_4^+-N**
		Unit	m^3s^{-1}		$ms.m^{-1}$	mg/l	mg/l	mg/l	mg/l	mg/l
Upper Mokolo (A4H002)	3rd	Min, Max	0, 8.9	4.60, 8.78	4.0, 18.2	0.5, 10.2	0.375, 30	0.003, 4.82	0.005, 0.65	0.02, 0.19
		Median	0.72	7.53	8.68	5.44	5.300	0.014	0.074	0.020
		Mean	1.52	7.62	8.50	5.27	5.731	0.030	0.109	0.030
		Variance (n)	3.61	0.175	3.46	1.60	13.284	0.060	0.010	0.000
		SD	1.9	0.418	1.86	1.26	3.645	0.246	0.100	0.021
Middle Mokolo (A4H005)	3rd	Min, Max	0.046, 80.3	5.1, 8.28	3.9, 30	1.4, 20.3	1.5, 17.8	0.003, 0.24	0.02, 0.38	0.02, 0.13
		Median	1.28	7.490	6.600	3.700	5.200	0.011	0.020	0.020
		Mean	4.56	7.333	6.95	3.868	5.511	0.015	0.049	0.032
		Variance (n)	94.2	0.274	5.13	2.597	11.235	0.000	0.003	0.001
		SD	9.70	0.523	2.26	1.612	3.352	0.019	0.053	0.023
Sterkstroom (A4H008)	2nd	Min, Max	0.002, 5.22	4.18, 8.46	1.0, 27.3	0.5, 14.56	0.38, 16.4	0.003, 1.46	0.005, 7.42	0.015, 0.98
		Median	0.47	7.162	3.6	2.16	2	0.012	0.02	0.02
		Mean	1.065	7.065	4.19	2.53	3.65	0.018	0.067	0.034
		Variance (n)	2.08	0.366	4.82	2.61	6.31	0.005	0.148	0.004
		SD	1.442	0.605	2.20	1.62	2.51	0.069	0.384	0.07
Tambotie (A4H007)	2nd	Min, Max	0, 2.234	5.14, 8.2	1.4, 11.9	0.5, 5.35	1.5, 10.4	0.003, 0.14	0.02, 1.877	0.02, 0.234
		Median	0.06	6.90	6.56	2.20	4.15	0.014	0.02	0.02
		Mean	0.21	6.87	6.58	2.42	4.22	0.019	0.086	0.04
		Variance (n)	0.168	0.22	2.93	0.85	6.78	0.000	0.083	0.002
		SD	0.41	0.47	1.71	0.92	2.61	0.019	0.287	0.04
Matlabas (A4H004)	2nd	Min, Max	0.003, 13.6	5.26, 8.48	2.3, 58.6	1.3, 10.8	1.5, 13.02	0.003, 0.18	0.02, 1.402	0.02, 0.15
		Median	0.15	7.4	5.9	3.5	2	0.011	0.02	0.02
		Mean	0.947	7.3	8.29	3.89	3.96	0.017	0.041	0.035
		Variance (n)	4.73	0.32	54.6	3.36	7.25	0.001	0.014	0.001
		SD	2.17	0.565	7.39	1.83	2.69	0.028	0.118	0.026
Palala (A5H004)	3rd	Min, Max	0.005, 49.8	4.84, 7.85	1.0, 7.56	0.5, 6.81	0.38, 9.5	0.003, 0.1	0.005, 0.51	0.015, 0.22
		Median	0.44	7.05	3.89	1.61	2	0.01	0.02	0.02
		Mean	1.47	6.98	3.73	1.75	3.05	0.015	0.036	0.027
		Variance (n)	19.72	0.174	1.61	0.90	4.20	0.000	0.003	0.000
		SD	4.44	0.417	1.27	0.95	2.05	0.017	0.051	0.021

Table 2.5: Missing data (months and seasons) expressed as a percentage (%) in relation to the respective time periods used in the trend analyses. Where more than 50% of the data were missing, the figures are denoted in blue font.
'*Before'* time period relates to Jan 1982 to Dec 1987 (72 months/12 seasons).
'*During'* time period relates to Jan 1988 to Dec 1993 (72 months/12 seasons). *These data were included in the Pettitt's homogeneity tests but omitted from the Mann-Kendall trend tests.
'*After'* time period relates to Jan 1994 to Aug 2013 (236 months/40 seasons).

Parameter		**pH**		**EC**		**Ca^{2+}**		**SO_4^{2-}**		**PO_4^{3-}-P**		**NO_3^-+NO_2^--N**		**NH_4^+-N**	
River (Gauging station)	**Time period**	monthly	season	monthly	season	monthly	season	monthly	season	monthly	season	monthly	season	monthly	season
Upper Mokolo (A4H002)	*Before*	58	8	43	0	58	17	58	17	58	17	58	17	58	17
	During	46	17	46	17	46	17	46	17	46	17	46	17	46	17
	After	38	5	38	5	38	5	38	5	38	5	38	5	38	5
Middle Mokolo (A4H005)	*Before*	71	33	68	33	71	33	71	33	71	33	71	33	71	33
	During	47	33	42	33	47	33	47	33	47	33	47	33	47	33
	After	73	52	73	52	73	52	73	52	73	52	73	52	73	52
Sterkstroom (A4H008)	*Before*	50	0	25	0	50	0	50	0	50	0	50	0	50	0
	During	18	0	18	0	18	0	18	0	18	0	18	0	18	0
	After	29	0	29	0	29	0	29	0	29	0	29	0	29	0
Tambotie (A4H007)	*Before*	92	50	83	50	92	50	92	50	92	50	92	50	92	50
	During	83	67	83	58	83	67	83	67	83	67	83	67	83	67
	After	82	57	82	57	82	57	82	57	82	57	82	57	82	57
Matlabas (A4H004)	*Before*	86	42	79	42	86	42	86	42	86	42	86	42	86	42
	During	68	25	68	25	68	25	68	25	68	25	68	25	68	25
	After	59	20	59	20	59	20	59	20	59	20	59	20	59	20
Palala (A5H004)	*Before*	86	75	82	75	87	75	87	75	87	75	86	75	87	75
	During	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	After	56	22	63	22	56	22	56	22	57	22	57	22	57	22

2.2 Trend analysis

In the context of this research, trend analysis is synonymous with time series analysis. The intention in trend analysis is to determine whether a series of observations of random variables have changed over time. Statistically, this is whether the probability distributions from which the values have been derived have changed over time (Helsel and Hirsch 2002). In the event that any trends are identified, they can either be increases or decreases to the central value of the distribution, the median in the case of this study. No trend would equate to zero change (0) over time. Although rainfall is a primary driver of streamflow and will always influence stream WQ, the weak correlation and other EDA results (Appendix A) provided more support to use discharge as the explanatory variable in the trend tests.

Helsel and Hirsch (2002) categorise five types of trend test based on (i) the statistical nature of the data and (ii) whether it is possible to remove some or most of the variation caused by other exogenous variables, denoted by X (Table 2.6). An explanatory X variable is that which is used to explain changes in a response Y variable and in terms of the variables analysed in this study, all variables were continuous. Both non-parametric tests illustrated in Table 2.6 were carried out. In addition, a non-parametric homogeneity test was also conducted as an objective analysis to compare with results of the trend tests and check whether both sets of test results corroborated with each other. The Pettitt's homogeneity test differed in that it did not minimise the effects of seasonality as does the seasonal M-K test and it also checked for possible time trends in Q itself in addition to the WQ (Y) variables. The X-adjusted M-K test (denoted as [b] in Table 2.6) removes the effects of Q and then checks for trends only in the WQ residuals.

If any major disparities were found between the respective test results in terms of the timing and magnitude of trends over time, it would arguably be more justifiable to use the M-K test results, where both Q and seasonality effects were minimised.

Table 2.6: Classification of five types of test for trend (after Helsel and Hirsch, 2002).

	[a] Not adjusted for X	[b] Adjusted for X
Non-parametric	Mann-Kendal trend test on Y	Mann-Kendall trend test on residuals (R) from LOWESS of Y on X
Mixed	-	Mann-Kendall trend test on residuals (R) from regression of Y on X
Parametric	Regression of Y on T	Regression of Y on X and T

2.2.1 Methods

Homogeneity tests

Homogeneity tests determine whether a series may be considered as homogeneous over time or if there is a point in time at which a change occurs. There are a number of types of tests which can be used depending on the numerous possible alternative hypotheses, which can include either changes in the average or distribution or whether a trend is present or not. Pettitt's test is a nonparametric homogeneity approach that requires no assumption about the distribution of data and is an adaptation of the tank-based Mann-Whitney test which allows identifying the point in time at which the shift occurs.

As described in Pettitt (1979), the null hypothesis states that the variables follow the same distribution (*i.e.* are homogenous) whilst the alternative hypothesis states there is a time *t* at which there is a change of distribution. The test was conducted on median monthly pH, median monthly WQ variables, median monthly Q (log transformed) and monthly rainfall. All tests were carried out at a 5% significance level with 10 000 Monte Carlo simulations with test interpretations:

H_0*: Data are homogeneous*

H_A*: There is a date at which there is a change in the data*

Mann-Kendall trend tests

The Mann-Kendall (M-K) trend tests are based on the calculation of Kendall's tau (a measure of association between 2 samples) which is itself based on the ranks with the samples. During the test computations, the first series is an increasing time indicator generated automatically for which ranks are obvious, which simplifies the calculations. This test is the result of the development of the nonparametric trend test first proposed by Mann (1945), studied further by Kendall (1975) and improved by Hirsch et al., (1982) and Hirsh and Slack (1984). The most latter of which allowed for the inclusion of seasonality effects into the test. XLSTAT also allows for the removal of the effects of autocorrelation after the method in Yue and Wang (2004).

The existence of trends (as Sen's slopes) was further determined by the Sen's method which was calculated as part of the M-K tests. As defined by Theil (1950), the Theil-Sen estimator of a set of two-dimensional points is the median of the slopes determined by all pairs of the sample points. Sen (1968) extended this definition to handle the case in which 2 data points have the same x-coordinate (in this case the modelled WQ response variables versus time as the explanatory variable). In Sen's method, the median of the slopes is taken only from pairs of points having distinct x-coordinates.

Sen's method is insensitive to missing data, is not affected by gross data errors or outliers and makes no assumption about the distributions of the data. It goes a step further by estimating the value and confidence intervals for slope. Only the Sen's slope values are presented (Appendix E and F). The confidence intervals were also test outputs but have not been included. Any significant trends and the respective directions (up or down) are confirmed by the Sen's slope values.

[a] *Mann-Kendall trend tests on medians with no flow-adjustment*

This is a simple trend test where no adjustment for X (Q) as the explanatory variable was carried out. It was conducted on monthly medians, with the exception of the Tambotie where the test was applied to seasonal median values due to numerous missing data years. To compute the p-values for this test, XLSTAT calculates, as in the case of the Kendall tau test, exact p-values if there are no ties in the series and if the sample size is less than 50. If exact calculations were not possible, normal approximations were used for which corrections for continuity were applied. The autocorrelations were taken into account and removed in the M-K trend tests using the method in Yue and Wang (2004). Insufficient data existed for the Tambotie for the T_1 time period hence no results were generated for all variables in this case. Due to the same reason of large data gaps regarding this river, seasonal medians were used instead of monthly medians for the T_2 analysis.

In terms of the statistical theoretical principles, the null hypothesis (H_0) is always the same for the Mann-Kendall (M-K) trend test, and this applied in all cases and for all tests used in this research:

H_0*: There is no trend in the series*

There are 3 alternative hypotheses (H_A) that are applicable in the test computations, namely:

H_{A1}*: There is a non-null trend in the series (two-tailed)*

H_{A2}*: There is positive trend in the series (upper-tailed)*

H_{A3}*: There is negative trend in the series (lower-tailed)*

All 3 alternative hypothesis tests above were included and iteratively computed (where tau≠0, tau>0 and tau<0), where variations in outcomes were possible depending on river and WQ parameter.

Given the theoretical outcomes for the 3 alternative M-K test hypotheses and the research hypotheses (section 1.1.2), the test interpretations were as follows:

1) For streams upwind/far from SO_X and NO_X emission sources (Palala, Upper Mokolo and Sterkstroom) and for both time periods (T_1 and T_2):

H_0*: There is no trend in all the series*

H_{A1}*: There is a non-null trend in all the series*

2a) For streams downwind/close to SO_X and NO_X emission sources (Matlabas, Tambotie, Middle Mokolo) and for the T_1 analysis (1982-1987):

H_0*: There is no trend in all the series*

H_{A1}*: There is a non-null trend in all the series*

2b) For streams downwind/close to SO_X and NO_X emission sources (Matlabas, Tambotie, Middle Mokolo) and for the T_2 analysis (1994-2013):

H_0*: There is no trend in the series*

H_{A1}*: There is a non-null trend in the series*

H_{A2}*: There is a positive trend in the series*

H_{A3}*: There is negative trend in the series*

[b] *Mann-Kendall trend tests on residuals after adjusting for discharge*

A second approach for performing the M-K test involves testing the residuals from a LOWESS of Y (the response variables) on X (the explanatory variable) (Table 2.6). LOWESS (Locally Weighted Scatterplot Smoothing) is the non-parametric technique analogous to linear regression. The reason to run the tests separately on both the median values and flow-adjusted values (LOWESS residuals) was to check if similar trend patterns would arise in both sets of results. This would give some indication as to how strong an influence discharge had on WQ in the different rivers. One of several functions of LOWESS is to remove the effect of an explanatory variable without assuming the form of the relation by smoothing the two variables (X and Y) prior to using the residuals in subsequent trend analyses (Helsel and Hirsch 2002).

As with the simple M-K tests performed on the median values in [a] (Table 2.6) the study-specific hypothesis test interpretations for the LOWESS residuals [b] were exactly the same and are hence not repeated. The only difference was in the wording of the hypothesis tests where the word '*series*' in the simple M-K test hypotheses should be replaced with the word '*residuals*' for the M-K tests after LOWESS. The autocorrelations were also taken into account and removed using the method after Yue and Wang (2004).

Seasonal Mann-Kendall tests

Stream chemical concentration data often shows strong seasonal patterns, with discharge almost always varying greatly from season to season owing to variations in rainfall. Some of the observed variation in WQ can therefore be explained by accounting for seasonal variations in discharge, which is essentially a by-product of rainfall. Given that seasonality can be a major source of variation in the Y variable(s), seasonal variation must be compensated for or removed (deseasonalised) in order to reduce the variation (as background noise) and better discern possible time trends in Y.

As discharge exhibits seasonality, detrending (*i.e.* minimising) the effects of discharge using the LOWESS method would have eliminated a portion of the seasonality in the respective WQ time series.

However there are also cases when some other seasonal effects remain, even after the effects of Q have been removed (Hirsch et al., 1982). Some other possible factors contributing to seasonal patterns include biological activity, agricultural activity and seasonal rises and falls in groundwater levels (Helsel and Hirsh 2002).

The seasonal M-K test takes into account and checks for seasonality in the time series by testing whether there are any trends not due to seasonality. The test checks for seasonal trends between one season of summer with another and so on, and between one season of winter with another and so on. Kendall's tau is first calculated for each season followed by an overall average Kendall's tau. In XLSTAT the variance of tau is calculated assuming that the series are either independent or dependent, which requires the calculation of a covariance. XLSTAT allows both (*i.e.* serial dependence or not).

I defined there to be two seasons per annum, so the period assigned in the seasonal M-K tests was '2'. The summer/wet season was October to March inclusive whilst the winter/dry season was April to September inclusive. Calculation of the p-values involves XLSTAT using a normal approximation to the distribution of the average Kendall's tau, for which a continuity correction was used. As was carried out on the 'monthly medians' to compute 'monthly residuals' in the M-K tests, the same procedure (LOWESS) was repeated on the 'seasonal medians' to create 'seasonal residuals' prior to seasonal M-K testing.

The hypothesis test interpretations for all seasonal M-K tests were:
H_0*: There is no trend (due to seasonality) in the series/residuals*
H_A*: There is a trend (due to seasonality) in the series/residuals*
Due to some of the sample sizes being too small, some sequences were constant for the Seasonal M-K test yielding no output of results, denoted as n/a (Appendix E and F).

2.2.2 Results

Change detection in water quality variables for the entire time period

The Pettitt's homogeneity tests identified changes in the time series independent of the expectations predicted by the research hypotheses and without any compositing of the data. The aim of the tests were to objectively analyse the entire raw time series data prior to grouping them into time periods, where the effects of exogenous variables, in this case discharge, were not removed.

Explanatory catchment variables

The Pettitt's test results indicate discharge and rainfall do not correspond in parallel, contrary to what would generally be expected. Except for the Tambotie River where no trends were detected, statistically significant increasing discharge trends over time were identified in all other rivers (Table 2.7). For the tertiary catchment (A42), upward step trends in both Mokolo River stations (Upper and Middle) and the Sterkstroom were identified at the end of 1995 in the months of November and October respectively. An upward step trend in the Palala River (tertiary catchment A50) also occurred in November 1995 whilst it was over a decade later (November 2007) for an upward trend to occur in the Matlabas. When comparing the time series plots from the Pettitt's tests (Figures' 2.1 to 2.3), where monthly median values were used, with the EDA plots (A1 and A2, Appendix A), where monthly flow volumes were used, similar trend directions exist in both sets of figures. At the quaternary catchment level, downward step trends in rainfall were only identified in A41B (April 2000) and A50B (March 1998).

Table 2.7: Pettitt's test results for explanatory variables in the quaternary catchments. Where the trends confirm the alternate hypothesis of non-homogeneity in the data, the p-values are shown in bold.

4°ry catchment		A41B	A42F	A42E	A42D	A42H	A50B
River		Matlabas	M. Mokolo	U. Mokolo	Sterkstroom	Tambotie	Palala
Explanatory variable (X)	Unit						
ln Q (monthly mean)	cumecs	**0.001** (n=265)	**<0.0001** (n=281)	**<0.0001** (n=259)	**<0.0001** (n=374)	0.530 (n=188)	**<0.0001** (n=359)
Monthly rainfall (total)	Mm	**0.002** (n=265)	0.392 (n=378)	0.143 (n=374)	0.143 (n=374)	0.081 (n=188)	**0.002** (n=359)

Response catchment variables

Mixed trend results were obtained for the WQ response variables (Table 2.8). Owing to the vast number of individual variables (7) and rivers (6), all cases where a result of 'no change' was identified are not described in the text. There were 33 significant trends identified in 42 time series (Figures' 2.4 to 2.9). Also included are the dates when significant changes in the average occurred (as step trends) and whether such trends (mu2) were increases or decreases from the original average (mu1) (Table 2.9).

Table 2.8: Pettitt's test results for response variables in the quaternary catchments. Where the trends confirm the alternate hypothesis of non-homogeneity in the data, the p-values are shown in bold.

4°ry catchment		A41B	A42F	A42E	A42D	A42H	A50B
River		Matlabas	M. Mokolo	U. Mokolo	Sterkstroom	Tambotie	Palala
n (sample size)		123	110	196	243	27	91
Response variable (Y)	Unit						
pH		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.002**	0.036
EC	$ms.m^{-1}$	0.076	**0.001**	**0.006**	**0.01**	0.497	**0.001**
Ca^{2+}	mg/l	0.135	**<0.0001**	**<0.0001**	**0.002**	0.750	0.313
$NO_3^-+NO_2^-$	mg/l	**<0.0001**	0.153	**<0.0001**	**0.000**	0.154	**0.001**
NH_4^+	mg/l	**0.001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.03**	**<0.0001**
PO_4^{2-}	mg/l	**0.001**	**<0.0001**	**<0.0001**	**0.001**	0.075	**0.031**
SO_4^{2-}	mg/l	**<0.0001**	**<0.0001**	**0.006**	**<0.0001**	**0.04**	**<0.0001**

Table 2.9: Transition years where increases or decreases in the median values were deemed significant in the Pettitt's homogeneity tests. Significant trends are shown in bold with upward arrows indicating a step-up trend and downward arrows denoting a step-down trend. The onset of large scale coal combustion activity commenced between 1988-1992.

		Matlabas	Middle Mokolo	Upper Mokolo	Sterkstroom	Tambotie	Palala
Explanatory variable	Monthly Q	**Nov 07'↑**	**Nov 95'↑**	**Nov 95' ↑**	**Oct 95'↑**	No change	**Nov 95'↑**
	Monthly rainfall	**Apr 00' ↓**	No change	No change	No change	No change	**Mar 98'↓**
Response variable	pH	**Jul 91'↑**	**Apr 89' ↑**	**Apr 89'↑**	**Jun 91'↑**	**Jul 91'↑**	**Nov 82'↑**
	EC	No change	**Jul 95'↑**	**Jun 88'↑**	**Dec 95'↓**	**Dec 96'↓**	**Aug 02'↑**
	Ca^{2+}	No change	**Jul 95'↑**	**Aug 91' ↑**	**Aug 91'↑**	No change	No change
	$NO_3^-+NO_2^-$	**Mar 03'↑**	No change	**Feb 96' ↑**	**Dec 99'↑**	No change	**Sep 03'↑**
	NH_4^+	**Mar 93'↓**	**Apr 91'↓**	**May 93' ↓**	**May 92'↓**	**Jun 91'↓**	**Aug 06'↑**
	PO_4^{2-}	**Apr 06' ↓**	**May 91'↑**	**Apr 98'↑**	**May 91'↓**	No change	**Jun 09'↓**
	SO_4^{2-}	**Aug 09'↓**	**May 90' ↑**	**May 90'↑**	**May 94' ↓**	**May 94'↓**	**Sep 07'↓**

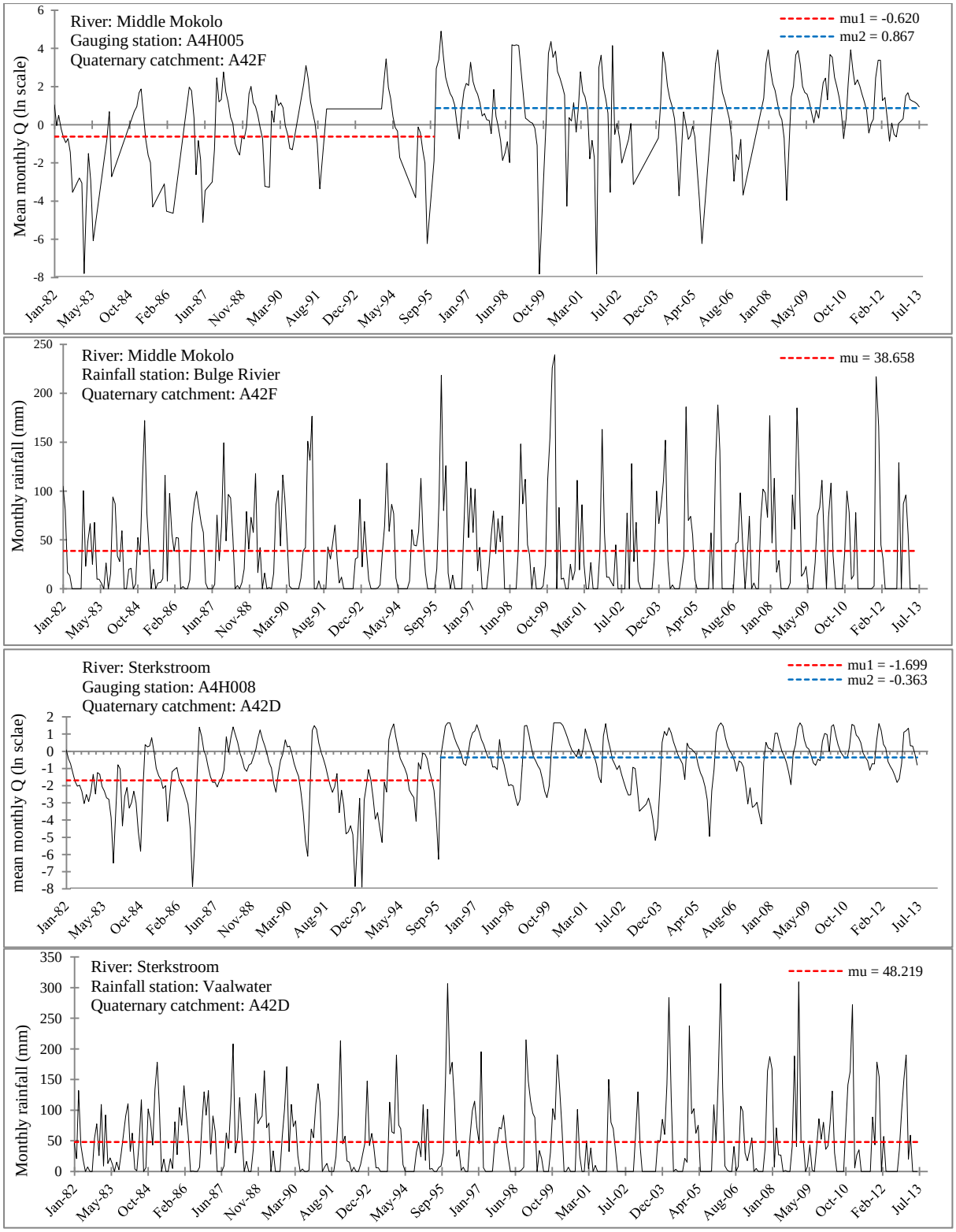


Figure 2.1: Monthly discharge and rainfall trends in the Middle Mokolo and Sterkstroom, based on Pettitt's homogeneity tests.

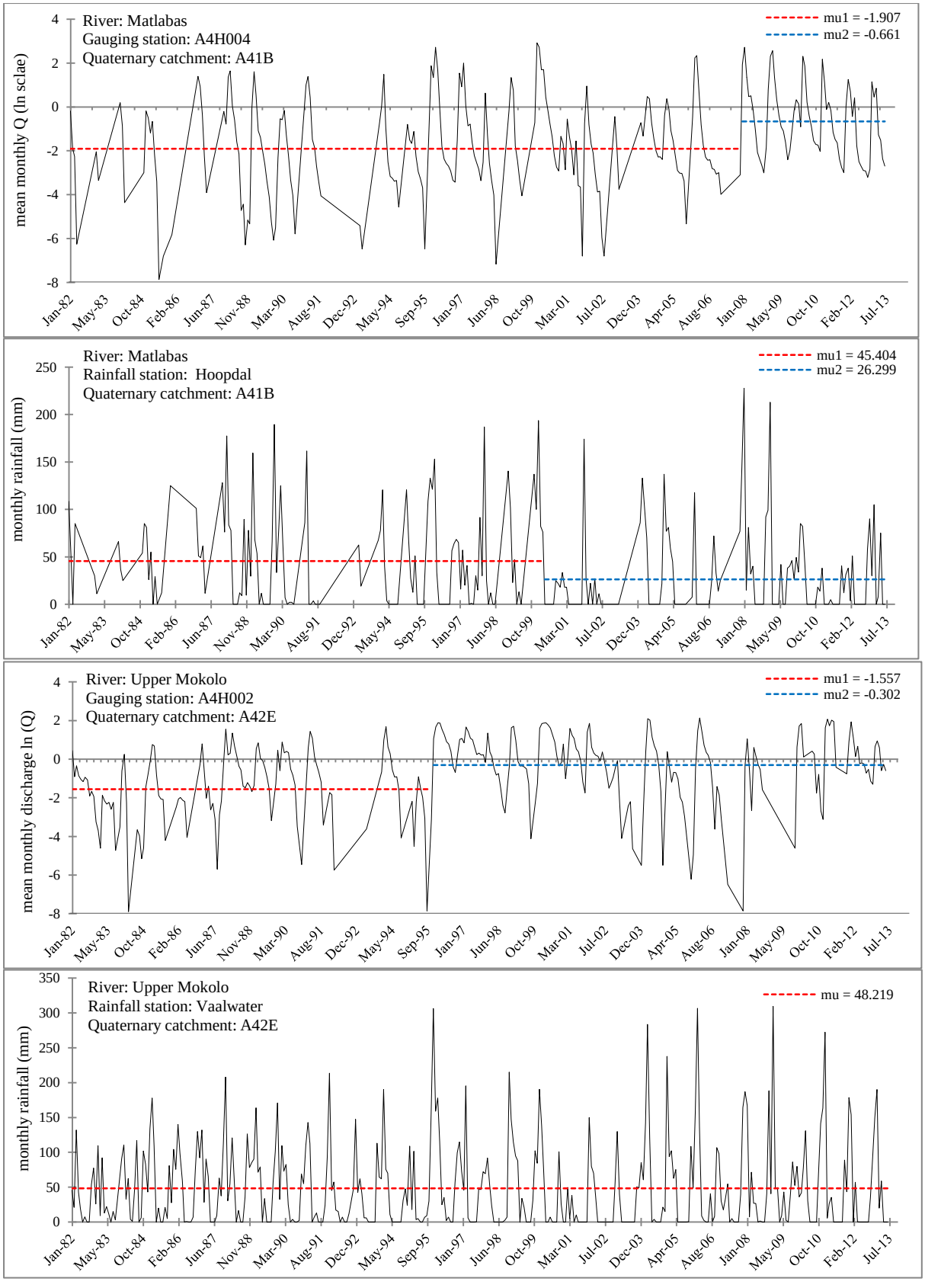


Figure 2.2: Monthly discharge and rainfall trends in the Matlabas and Upper Mokolo, based on Pettitt's homogeneity tests.

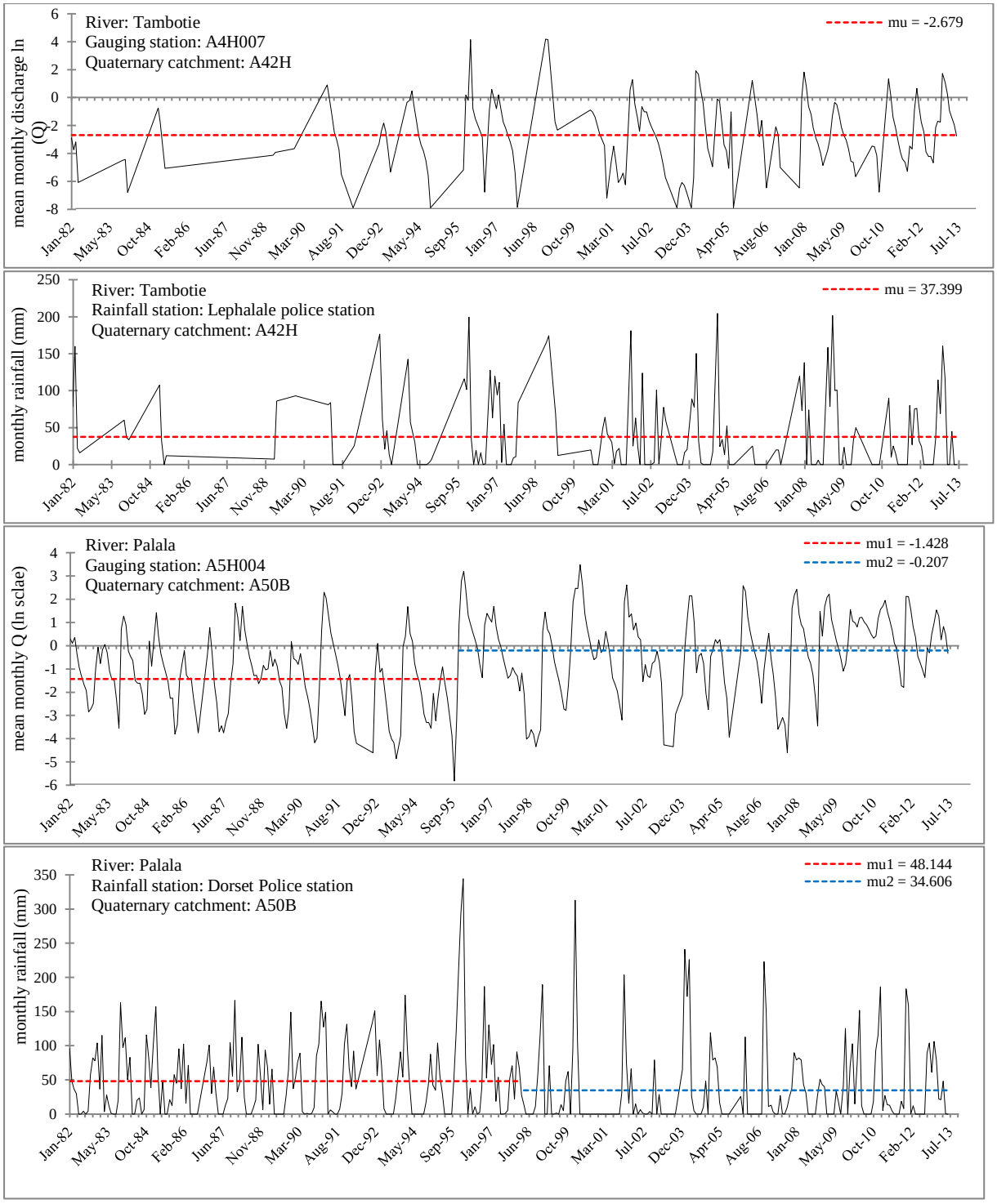


Figure 2.3: Monthly discharge and rainfall trends in the Tambotie and Palala, based on Pettitt's homogeneity tests.

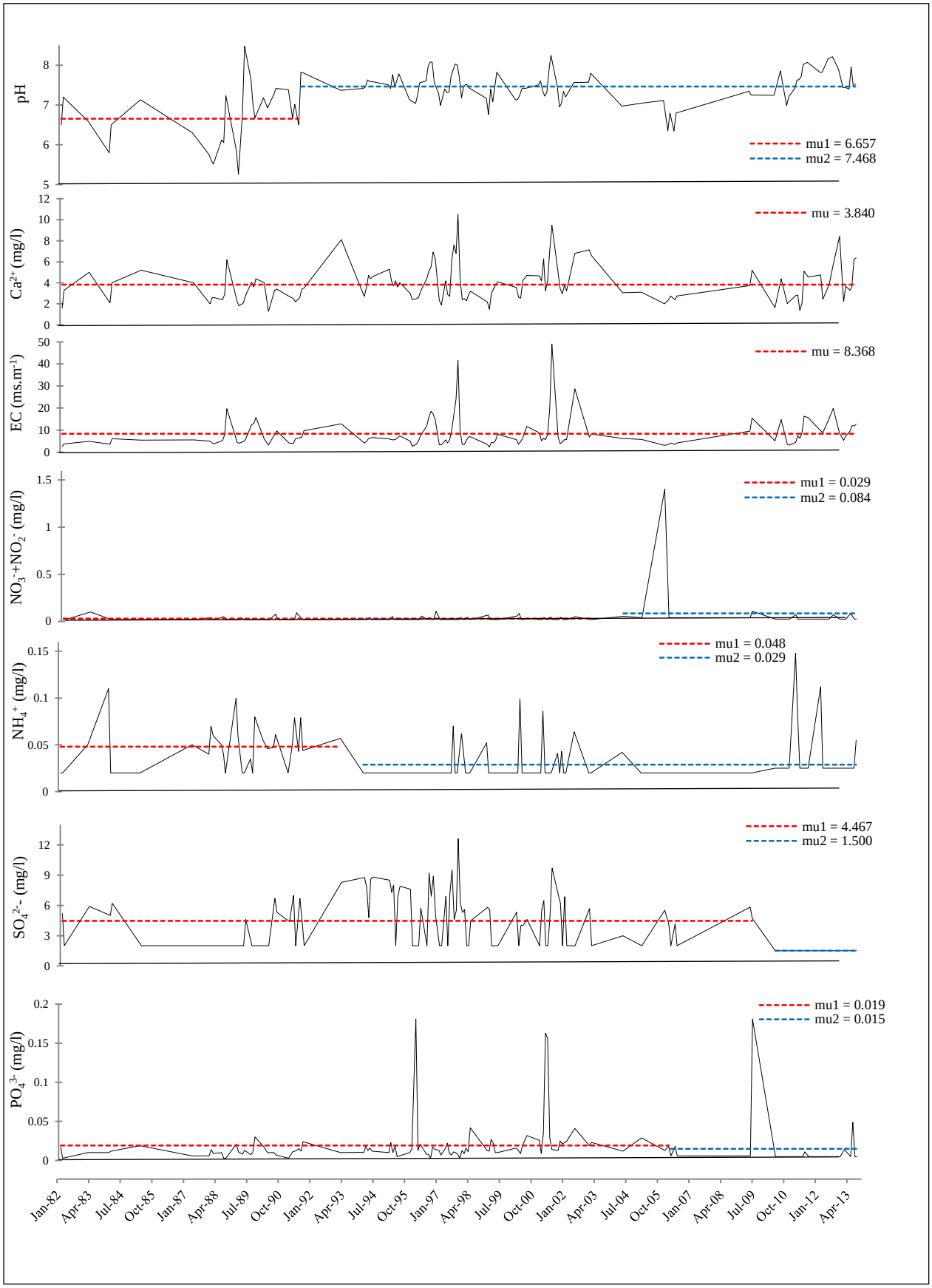


Figure 2.4: Water quality trends in the Matlabas (A4H004), based on Pettitt's homogeneity tests on monthly medians.

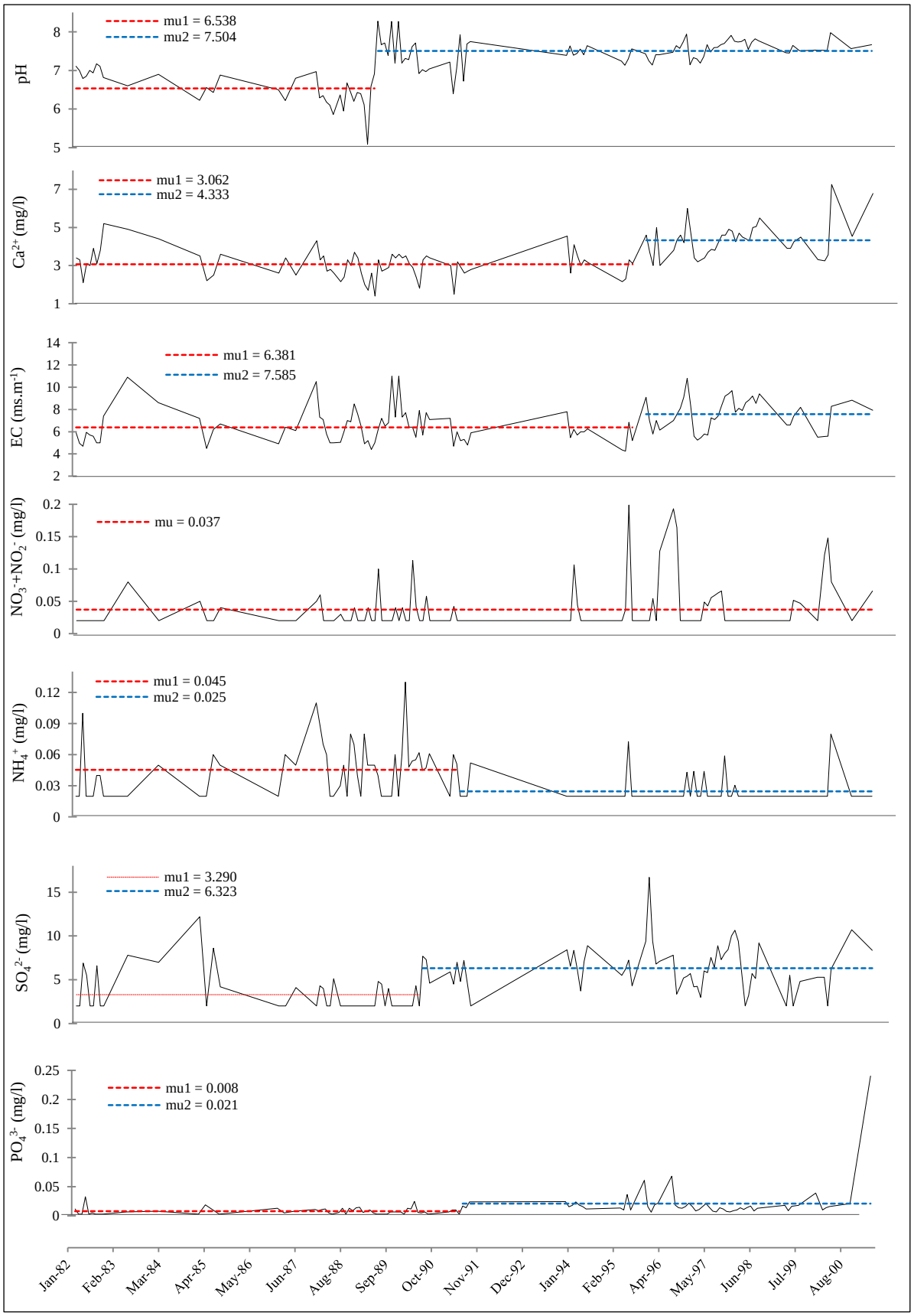


Figure 2.5: Water quality trends in the Middle Mokolo (A4H005), based on Pettitt's homogeneity tests on monthly medians (Jan 1982 – May 2001).

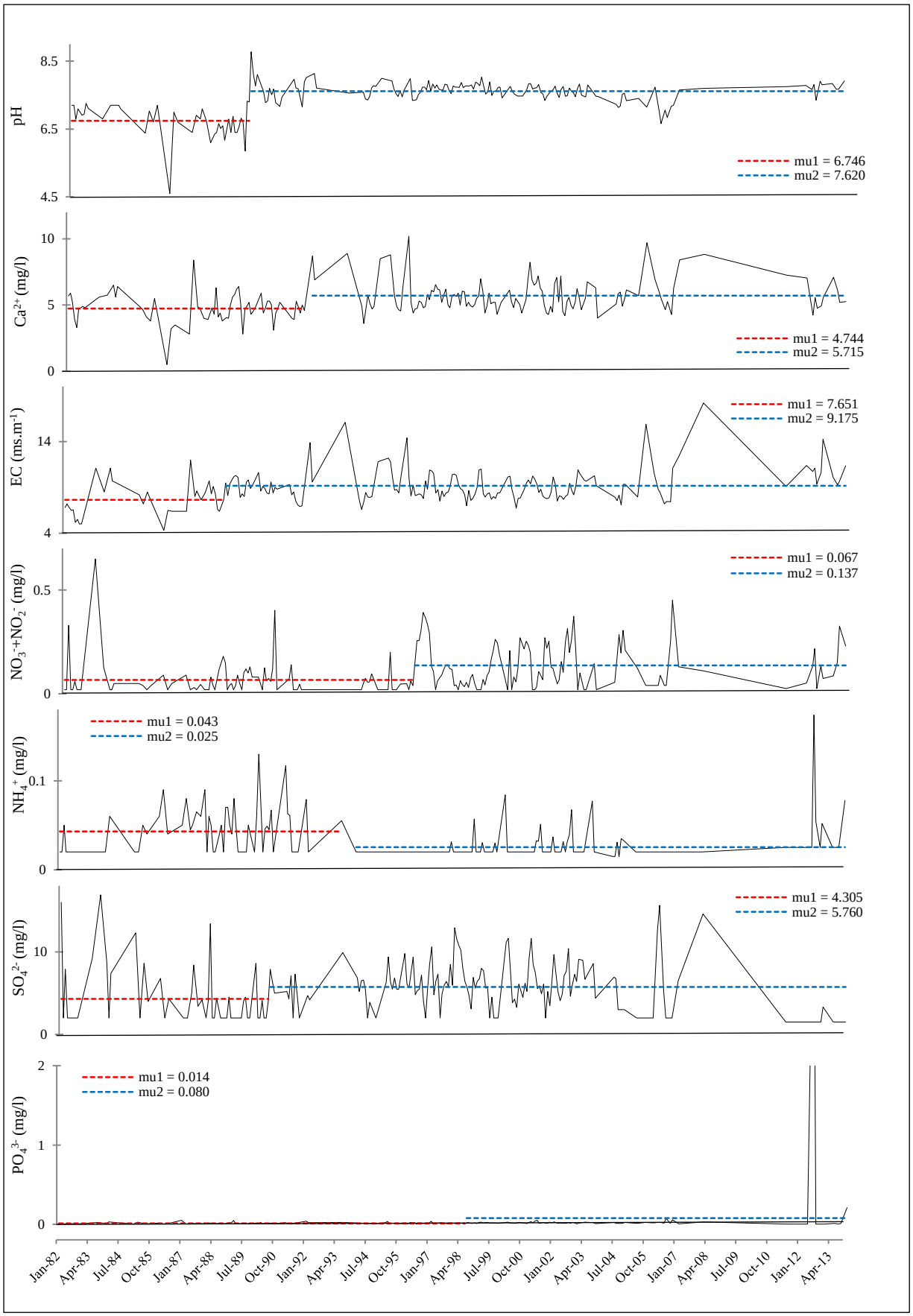


Figure 2.6: Water quality trends in the Upper Mokolo (A4H002), based on Pettitt's homogeneity tests on monthly medians.

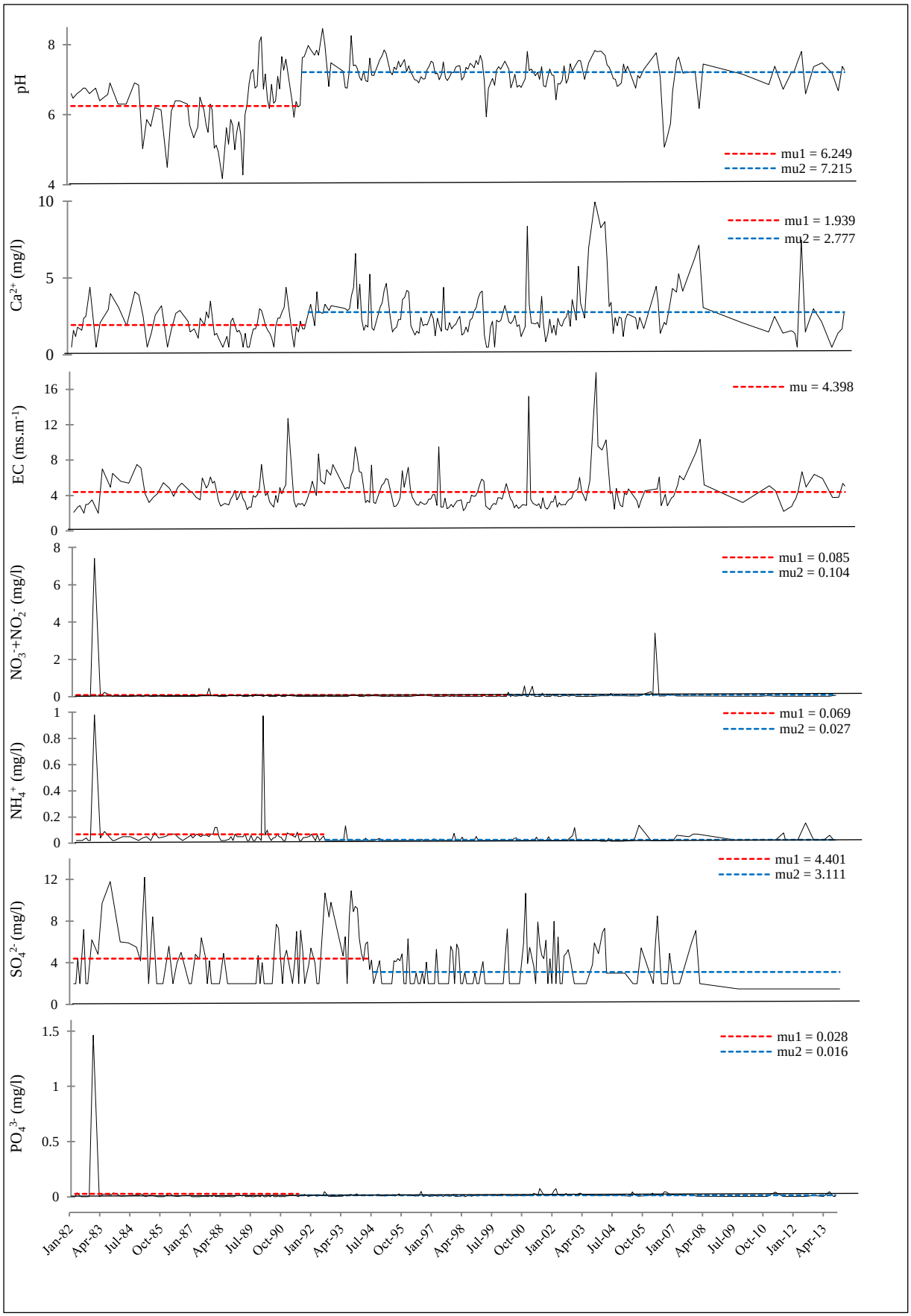


Figure 2.7: Water quality trends in the Sterkstroom (A4H008), based on Pettitt's homogeneity tests on monthly medians.

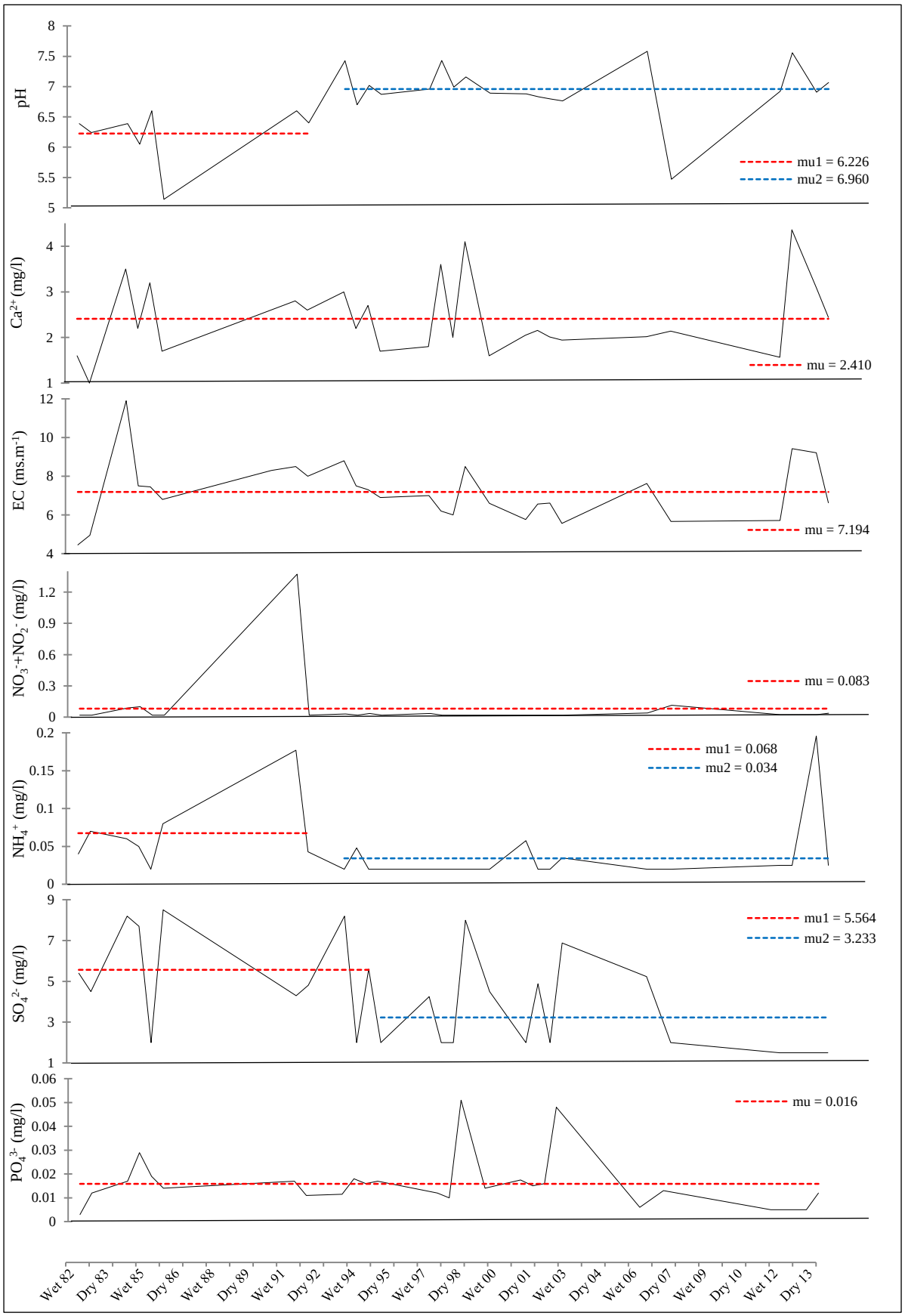


Figure 2.8: Water quality trends in the Tambotie (A4H007), based on Pettitt's homogeneity tests on seasonal medians.

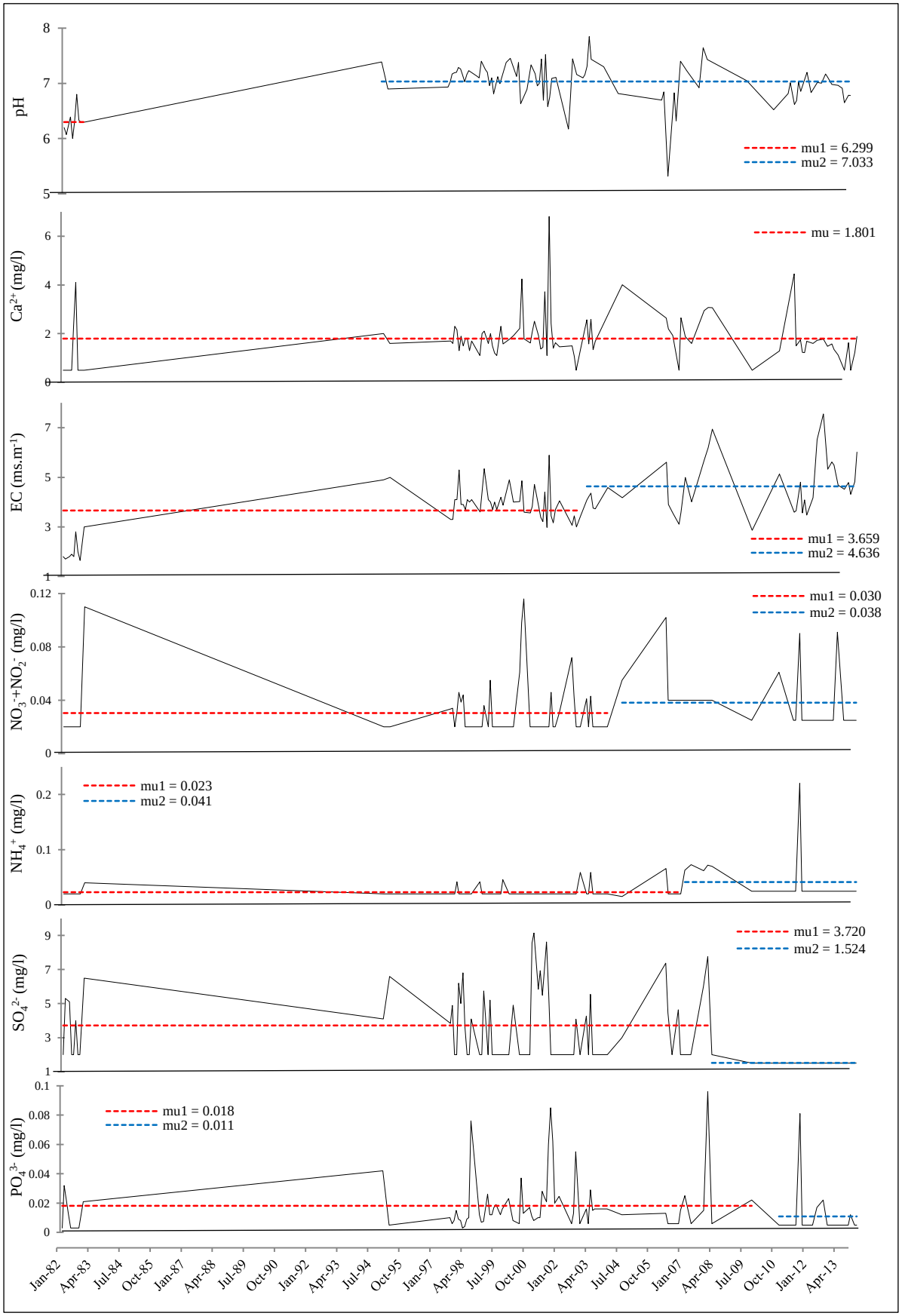


Figure 2.9: Water quality trends in the Palala (A5H004), based on Pettitt's homogeneity tests on monthly medians.

Intervention comparison pre- and post-commencement of large scale coal combustion

M-K trend tests on monthly medians with no flow-adjustment

The trend directions are summarised (Table 2.10) however these are derived only from the simple M-K test (Table 2.6[a]) which did not take into account and detrend the effects of discharge, nor the effects of seasonality. These results are therefore included in this manuscript for reporting and comparative purposes only, but were not used in the final interpretations and discussions on WQ (see section 2.3). The detailed summary statistics and test results are provided for further reference (Appendix E).

In terms of the explanatory (X) variables:

- Discharge trends were identified for both time periods in the Tambotie and Palala and for T_2 in the Matlabas. A decreasing trend (*i.e.* diminishing flows) occurred between 1982 and 1987 in the Tambotie and Palala followed by increasing flows in the Tambotie, Palala and Matlabas between 1994 and 2013.
- No rainfall trends were identified in any of the rivers for both time periods. This observed phenomenon contrasts with the oscillating discharge trends just described. Increasing or decreasing flows would appear not to have been caused by or strongly influenced by more or less rainfall.

Table 2.10: Summary of trend directions for the simple Mann-Kendall trend tests without flow-adjustment.

River		Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Time period		**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**
Parameter	ln Q	0	0	0	0	0	0	0	↑	↓	↑	↓	↑
	Rainfall	0	0	0	0	0	0	0	0	0	0	0	0
	pH	↓	↑	↓	0	↓	0	0	0	0	0	0	≠ 0
	EC	0	↑	0	0	↑	≠ 0	0	0	0	0	0	↑
	Ca^{2+}	0	↑	0	0	0	0	0	0	0	0	0	0
	SO_4^{2-}	0	↓	0	↓	0	↓	0	↓	0	0	0	↓
	PO_4^{3-}	0	↓	0	0	0	0	0	↓	0	0	0	↓
	NO_3^-+NO_2^-N	0	≠ 0	0	0	≠ 0	0	0	↑	0	0	0	0
	NH_4^+-N	≠ 0	≠ 0	↑	↑	↑	↑	0	↑	0	↑	0	↑

M-K trend tests on monthly LOWESS residuals after flow adjustment

The M-K trend directions on the LOWESS residuals are summarised (Table 2.11), where zero symbols indicate no trend. The detailed summary statistics and trend directions are also provided (Appendix E). Although this set of M-K trend tests went one step further than those computed on the medians which were not flow-adjusted (Table 2.10), the trend directions reflected (Table 2.11) may also be somewhat unreliable in that the tests did not account for the seasonal effects of the

winter (dry) and summer (wet) seasons. These results have been included for purposes of comparing the results with the Seasonal M-K tests performed on the seasonal medians after the LOWESS method.

Table 2.11: Summary of trend directions for the Mann-Kendall trend tests after flow-adjustment.

River		Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Time period		T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
Parameter	pH	≠ 0	↑	↓	↓	↓	0	0	0	n/a	0	0	0
	EC	0	0	0	≠ 0	↑	↑	0	≠ 0	n/a	0	0	↑
	Ca^{2+}	0	↑	0	0	0	≠ 0	0	0	n/a	0	0	0
	SO_4^{2-}	0	0	0	↓	0	≠ 0	0	↓	n/a	0	0	↓
	PO_4^{3-}	0	0	0	0	0	0	0	0	n/a	0	0	≠ 0
	$NO_3^-+NO_2^-$ N	0	0	0	≠ 0	≠ 0	0	0	↑	n/a	0	0	0
	NH_4^+-N	↑	0	↑	≠ 0	↑	≠ 0	↑	≠ 0	n/a	0	0	↑

Seasonal M-K trend tests on seasonal LOWESS residuals after flow adjustment

The Seasonal M-K tests constituted the final stage of the trend analysis where the results are tabulated in conjunction with the M-K trend test results (see Appendix F). The key difference is that the M-K trend test results are for residuals based on monthly medians whereas the seasonal M-K tests results are for residuals based on seasonal medians.

The overall trend directions are summarised (Table 2.12) where zero symbols indicates no trend. The time-series plots (Figures' 2.10 to 2.15) visualise the before (T_1) and after (T_2) residual time sequences. Red arrows show statistically significant upward trends whilst blue arrows indicate the contrary, also depicted in the two final catchment maps (Figure 2.16).

*These are the final results (*i.e.* Table 2.12 and Figure 2.16) which have been used for the final interpretations and discussion on trend directions.

pH: 4 negative trends; each for both time periods in the Upper Mokolo and the Sterkstroom.

Electrical Conductivity: 4 positive trends (H_{A2}) identified; 2 for both time periods (Sterkstroom) and one each for the Palala and Matlabas for T_2 only. 2 non-null trends (H_{A1}) identified for T_2 (Upper Mokolo and Matlabas).

Calcium (Dissolved): positive trends identified only for T_2 in the Middle Mokolo and Sterkstroom.

Nitrate + Nitrite nitrogen (Dissolved): only 1 positive trend identified for T_2 in the Matlabas.

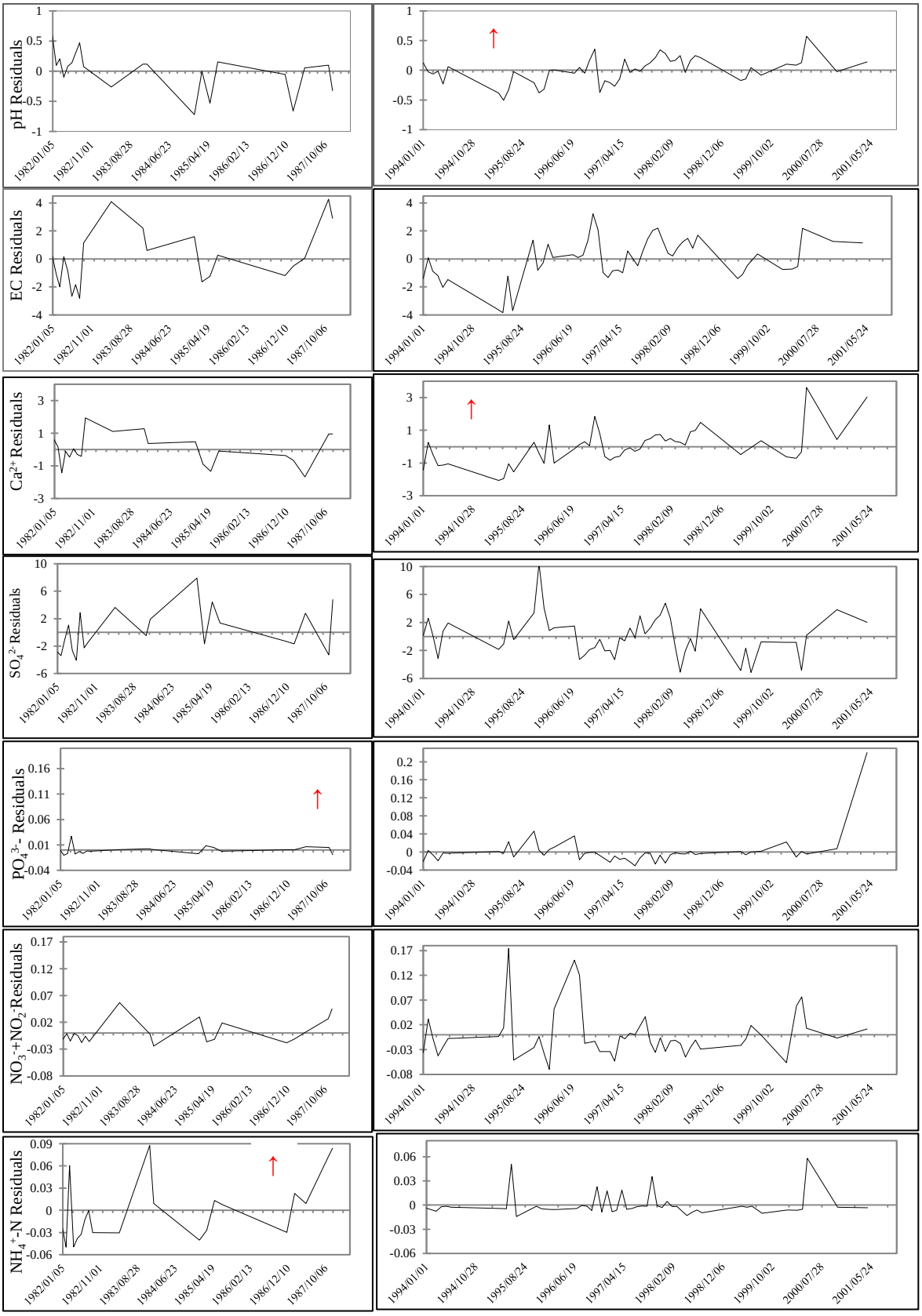
Ammonium nitrogen (Dissolved): 8 positive trends in total; 4 in T_1 in the Middle and Upper Mokolo, Sterkstroom and Matlabas and 4 in T_1 in the Upper Mokolo, Sterkstroom, Matlabas and Palala.

Phosphate phosphorus (Dissolved): 1 positive trend in T_1 in the Middle Mokolo, and 1 negative trend in the Palala for T_2.

Sulphate (Dissolved): 4 negative trends in T_2 in the Upper Mokolo, Sterkstroom, Matlabas and Palala.

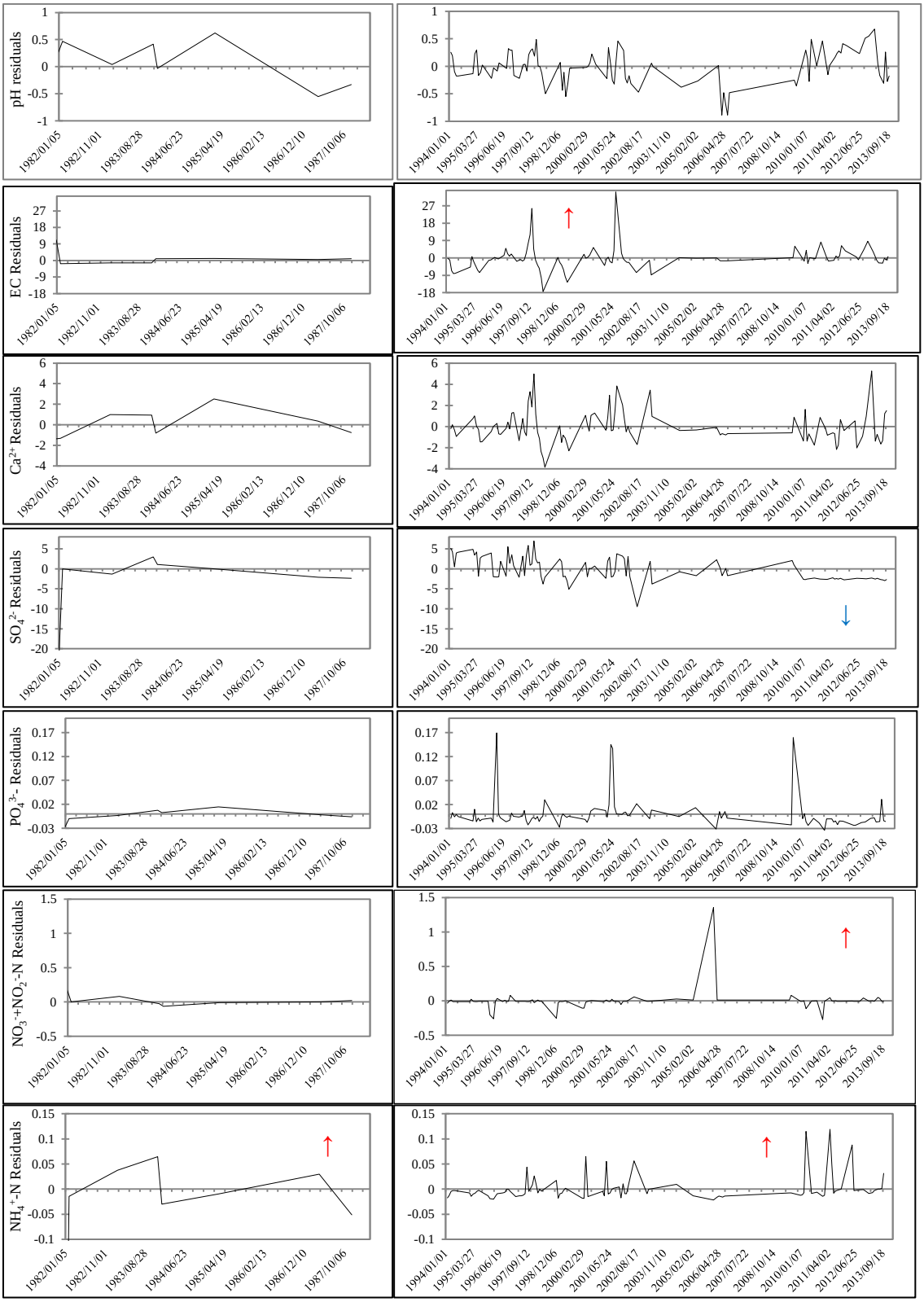
Table 2.12: Summary of trend directions after both flow-adjustment and deseasonalisation

River		Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Time period		**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**
Parameter	pH	0	0	↓	↓	↓	↓	0	0	n/a	0	0	0
	EC	0	0	0	0	↑	↑	0	↑	n/a	0	0	↑
	Ca^{2+}	0	↑	0	0	0	↑	0	0	n/a	0	0	0
	SO_4^{2-}	0	0	0	↓	0	↓	0	↓	n/a	0	0	↓
	PO_4^{3-}	↑	0	0	0	0	0	0	0	n/a	0	0	↓
	$NO_3^-+NO_2^-$ N	0	0	0	0	0	0	0	↑	n/a	0	n/a	0
	NH_4^+-N	↑	0	↑	↑	↑	↑	↑	↑	n/a	0	n/a	↑



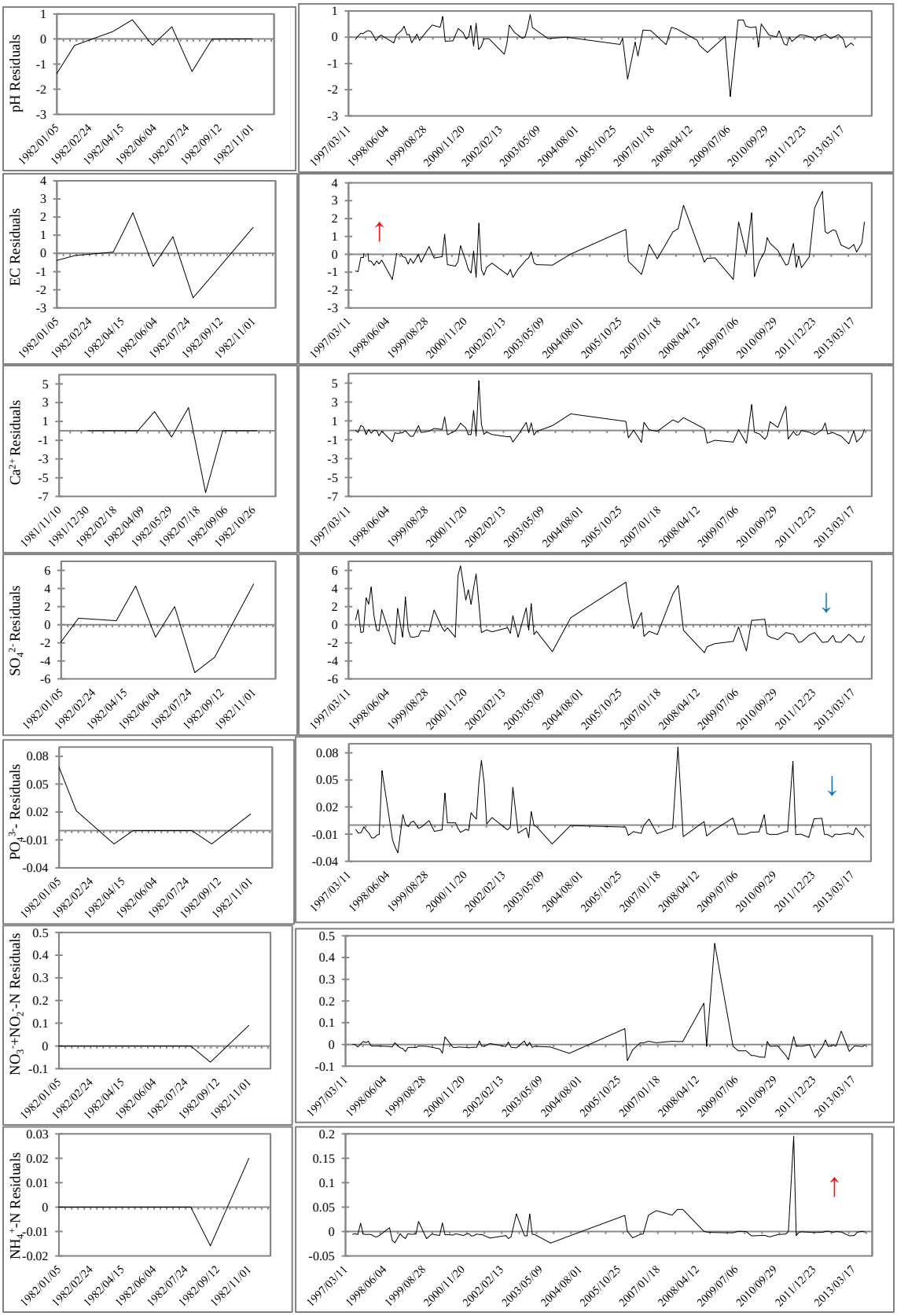
Before (T_1) *After* (T_2)

Figure 2.10: Post-LOWESS WQ residuals versus time for the Middle Mokolo river (A4H005).*Due to missing data for more than a decade after 2001, T_2 in this case was between January 1994 and May 2001.



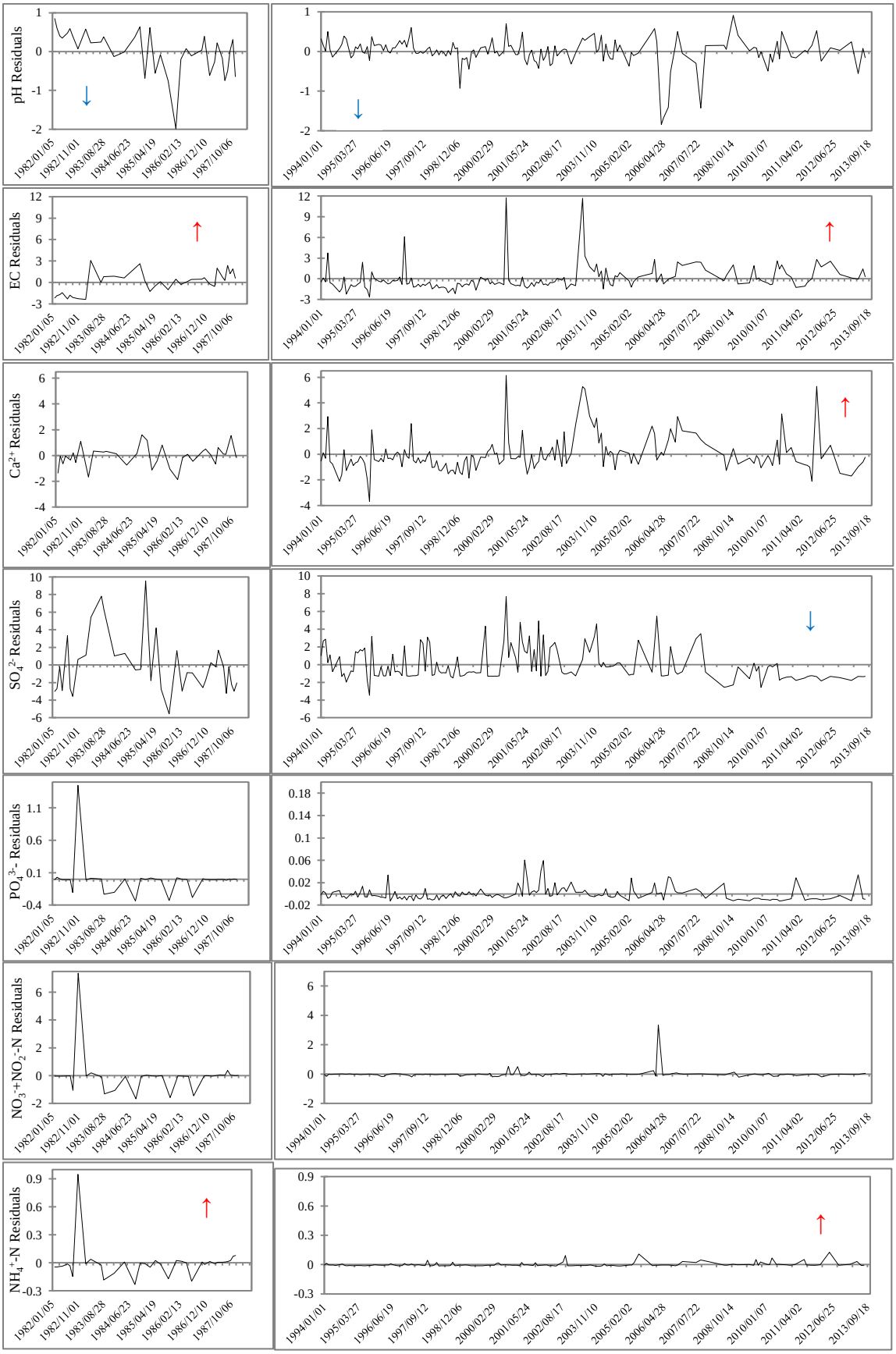
Before (T_1) *After* (T_2)

Figure 2.11: Post-LOWESS WQ residuals versus time for the Matlabas river (A4H004).



Before (T_1) *After* (T_2)

Figure 2.12: Post-LOWESS WQ residuals versus time for the Palala river (A5H004).* Due to missing WQ data from 1983 to mid-1997, T_1 represents the year 1982 only whilst T_2 included data from between June 1997 and June 2013.



Before (T_1) *After* (T_2)

Figure 2.13: Post-LOWESS WQ residuals versus time for the Sterkstroom river (A4H008).

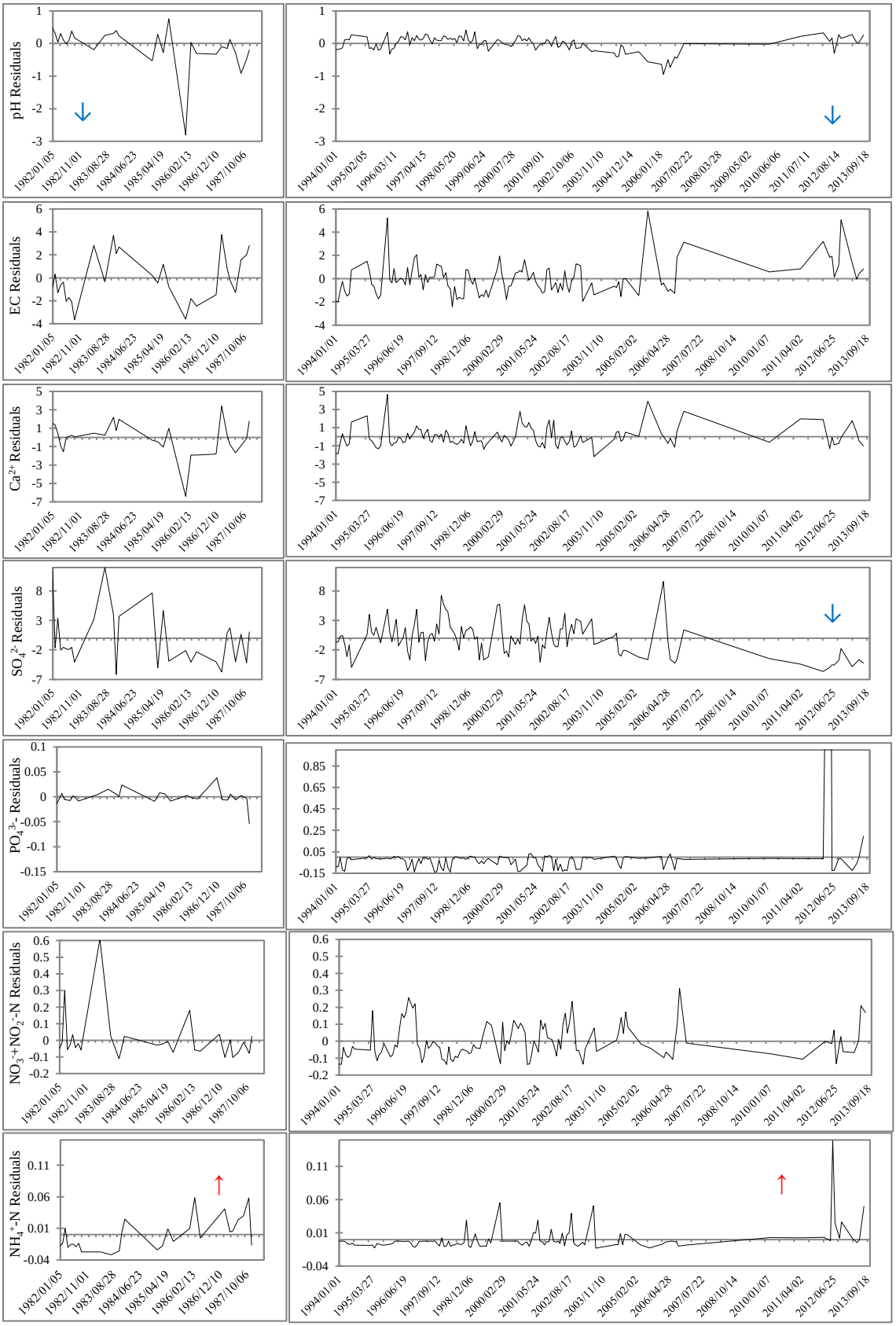


Figure 2.14: Post-LOWESS WQ residuals versus time for the Upper Mokolo river (A4H002).

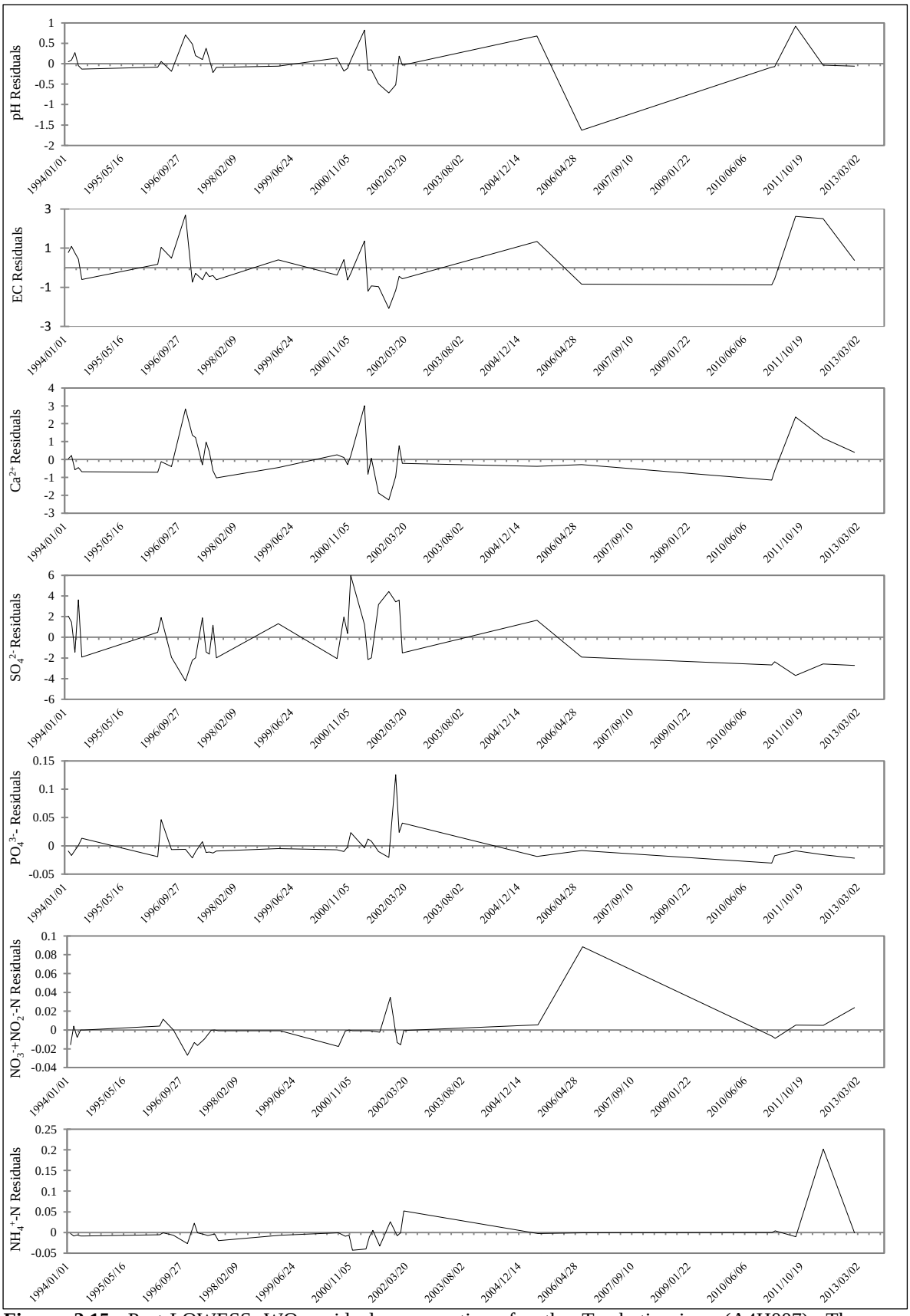


Figure 2.15: Post-LOWESS WQ residuals versus time for the Tambotie river (A4H007). There was insufficient data for a pre-LOWESS regression and trend tests for T_1 (only 6 observations between 1982 and 1987).

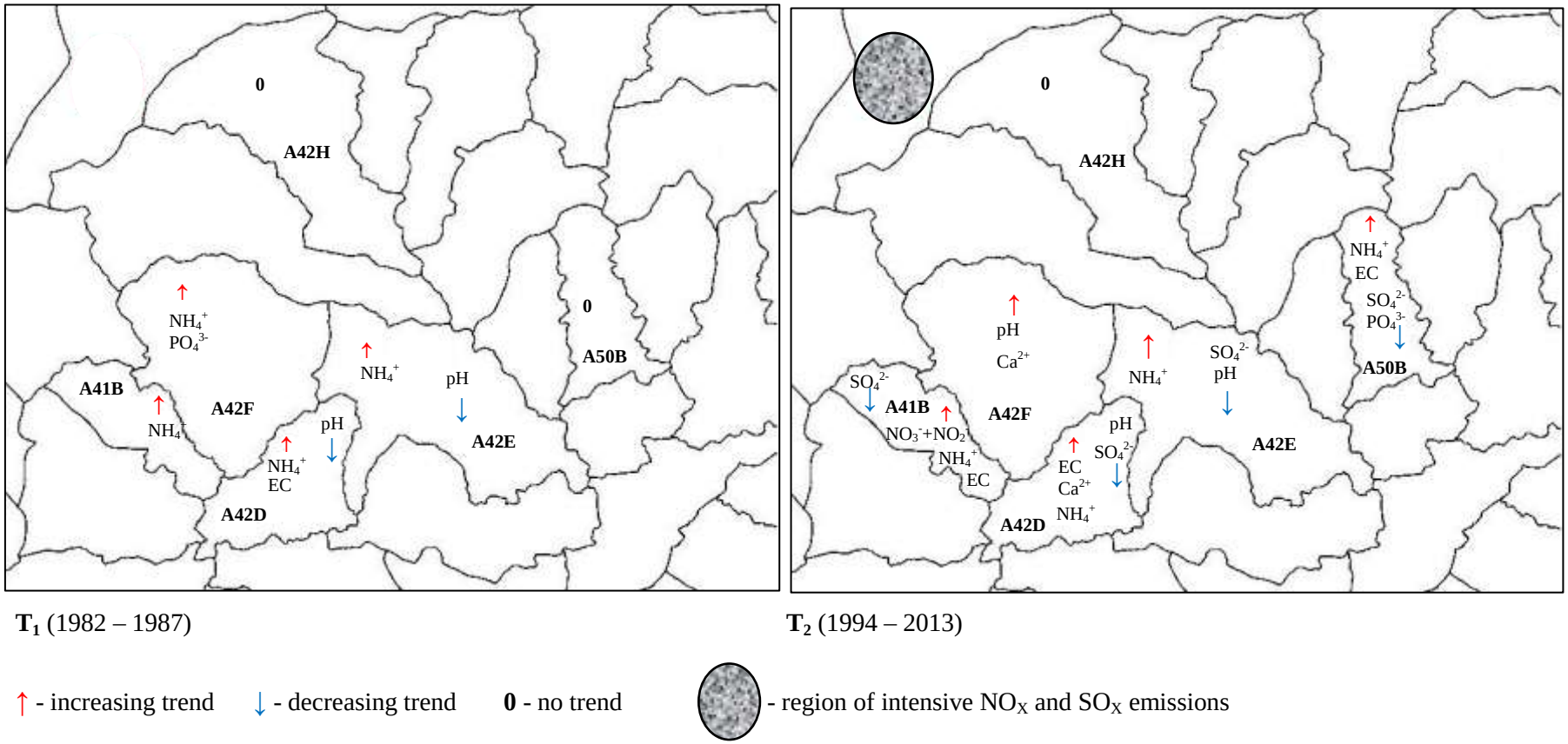


Figure 2.16: Before and after trend directions in the quaternary catchments of the Waterberg (on LOWESS residuals). Only variables with increasing or decreasing trends are shown. Detrending the effects of Q was achieved using the LOWESS method. Deseasonalisation was accomplished by the Seasonal M-K test, which represented the final stage of the overall trend analysis that was performed in addition to the M-K trend test.

In terms of the Seasonal M-K test hypotheses, intrinsic to the test and stated in section 2.3.1, the test outcomes are shown separately in terms of those streams close to/downwind from emissions and those far from/upstream from emission sources:

Streams close to/downwind from NO_X and SO_X emissions

T_1 (1982-1987)

Tambotie: no trends were identified in any variables, hence acceptance of H_0 in all cases.

Middle Mokolo: H_{A2} accepted for NH_4^+ and PO_4^{3-} (upward trends). H_0 accepted for all other variables.

Matlabas: H_{A2} accepted only for NH_4^+ (upward trend). H_0 accepted for all other variables.

T_2 (1994-2013)

Tambotie: no trends were identified in any variables, hence acceptance of H_0 in all cases.

Middle Mokolo: H_{A2} accepted for pH and Ca^{2+} (upward trends). H_0 accepted for all other variables.

Matlabas: H_{A2} accepted for EC, NH_4^+ and NO_3^-+NO_2^- (upward trends). H_{A3} accepted for SO_4^{2-} (downward trend). H_0 accepted for pH, Ca^{2+} and PO_4^{3-}.

Streams far from/upwind from NO_X and SO_X emissions

T_1 (1982-1987)

Upper Mokolo: H_A accepted only for NH_4^+ (upward trend) and pH (downward trend).

H_0 accepted for all other variables.

Sterkstroom: H_A accepted only for NH_4^+ and EC (upward trends) and pH (downward trend).

H_0 accepted for all other variables.

Palala: no trends were identified in any parameter, hence acceptance of H_0 in all cases.

T_2 (1994-2013)

Upper Mokolo: H_{A2} accepted only for NH_4^+ (upward trend) and H_{A3} accepted for pH and SO_4^2 (downward trends). H_0 accepted for all other variables.

Sterkstroom: H_{A2} accepted for Ca^{2+}, $NH_4^+$and EC (upward trends) and H_{A3} accepted for pH and SO_4^2 (downward trends). H_0 accepted for NO_3^-+NO_2^- and PO_4^{3-}.

Palala: H_{A2} accepted for $NH_4^+$and EC (upward trends) and H_{A3} accepted for PO_4^{3-} and SO_4^2 (downward trends).

2.3 Discussion

The step trends identified in the Pettitt's homogeneity tests were specific in providing dates of change as 'steps' up or down in the average for each WQ variable, in addition to discharge and rainfall. Although these step trends may not have accounted for other important and possibly confounding factors, the homogeneity tests were performed as a stand-alone to the M-K trend tests to compare and contrast both sets of results.

Time-based step trends over entire time period

As was the case, the step trend directions from the Pettitt's homogeneity test (Table 2.9) differed substantially with the final Seasonal M-K trend test results (Table 2.12). There are several cases where a parameter identified to trend upwards in the homogeneity test is identified to trend downwards in the M-K test. For example, pH increases (steps up) in all rivers between April 1989 and July 1991 in the homogeneity test results (except for the Palala where it steps up in 1982). Yet in contrast, the M-K tests identify no pH trend in four of the rivers and downward trends in the Upper Mokolo and Sterkstroom. There are other disparities and only a few similarities between other WQ variables for both tests. These differences present a dilemma in explaining which results are more robust and closer to reality.

Given the ability of the M-K trend test to deal more comprehensively with the effects of seasonality, autocorrelation and to filter out 'noise' caused by exogenous variables such as discharge (Hirsch et al., 1982; Helsel and Hirsch 2002) it is my opinion that it is the more reliable test of the two. Further justification for this is the fact that the homogeneity tests identified significant 'step up' trends in discharge at various times for each river, except the Tambotie (Table 2.8). The correlation tests also indicate both significant positive and negative correlation between discharge and the WQ variables, for about half of all test cases (Appendix A5). Hence the systematic removal of the influences of discharge from all the data using the LOWESS method and subsequent trend testing is more robust than leaving the latent effects of discharge to persist in the final results, which is effectively what the homogeneity test allowed.

Aside from discharge effects, the numerous data gaps characterising some of the hydrological records (particularly for the Palala, Tambotie and Matlabas) lowers the confidence in trends borne out in the homogeneity tests (Figures' 2.4 to 2.9). In summary there is a greater likelihood in concluding false trends based on the homogeneity test as compared to those trends borne out from the M-K test (Helsel and Hirsch 2002).

Water quality trends pre- and post-1994 after detrending the effects of flow and seasonality

When comparing the M-K trends (Table 2.11) with the Seasonal M-K trends (Table 2.12), it appears all were in mostly the same direction. Of the 84 possible outcomes pertaining to each parameter and time period, there were only 7 mismatches in the trend results. In other words, these few differences suggest WQ trends in these rivers were not greatly influenced by seasonal effects (*i.e.* seasonality appears not to have played a major role). When comparing the results from the simple M-K trend tests (Table 2.10) and the seasonal M-K tests (Table 2.12), there were 17 trend result mismatches from the 84 possible outcomes. This suggests that discharge as an exogenous variable had more of an influence on stream WQ than seasonality, whereby removal of its effects via the LOWESS method improved the reliability of the data prior to final testing. The effects of discharge and seasonality tend to be coupled in that discharge itself often exhibits a seasonal effect on WQ. Seasonal WQ patterns result from changes in rainfall and temperature, both of which affect streamflow and evapotranspiration rates. In other words, seasonal variation in discharge may result in seasonal variation in WQ. There are however seasonal effects that are not caused by discharge and which persist in the data even after discharge effects have been removed (*i.e.* detrended) (Helsel and Hirsh 2002).

Additional seasonal effects include nutrient run-off into streams from fertiliser application, which varies according to the agricultural growing season. Uptake of nutrients like N and P by natural vegetation also varies seasonally, tending to be greater in the summer (growing) season. Seasonal fluctuation in groundwater levels is another factor which can contribute to different water qualities in a given stream at different points in time. In a hypothetical catchment scenario, a wet season of high rainfall leading to widespread and high quantities of surface runoff from and through upper soil horizons would influence the observed stream WQ at a given gauging station. In the same catchment but in another season when a drought is experienced, stream discharge may primarily be derived from groundwater inputs into the stream via lower soil horizons or rock lithologies, which exhibit different sediment chemistries to the upper soil horizons (Helsel and Hirsch 2002).

Linkage with key question and study hypotheses

In terms of the WQ trends predicted by study site hypotheses I and II, there were agreements and conflicts to both. However all analyses in this study were largely exploratory in nature as opposed to predictive, so the hypothesis tests results do not provide exact causes for any of the observed WQ trends. Hypothesis I was based on the premise that minimal agricultural and industrial activities were underway in the Waterberg region prior to the late 1980s. Hypothesis II considered

that changes in certain WQ variables and in certain catchments would have occurred at a time after which coal combustion activity had commenced.

The cumulative and additive emissions of NO_X and SO_X (post-1994) were originally hypothesised to translate into upward trends in DIN and SO_4^{2-} and downward trends in pH in streams downwind and/or closer to the primary emission source. By hypothesising that emission-driven nutrient loading would increasingly occur, it was also predicted that EC would have trended upwards owing generally to more ions (in the form of nitrate and sulphate nutrients) being in solution post-1994. Trends in Ca^{2+} was not hypothesised owing to the potential catchment co-deposition resulting from coal combustion (Ca^{2+} ions are often a by-product) and the dual-process of Ca^{2+} ions leaching from catchments and entering streams due to emission-derived acid rain (Likens et al, 1998). There was some uncertainty regarding this variable as no historical data on the specific coal chemistry was available, it was rather assumed that Ca^{2+} ions may have been deposited as a by-product of coal combustion.

Acid rain is a natural phenomenon and can be exacerbated by activities such as coal combustion, however an in-depth account and differentiation between natural and anthropogenic acid rain inputs went beyond the scope and context of this study. The final trends illustrated (Figure 2.16) suggest however that any inputs of acid rain, either in the form of weak nitric and sulphuric acid derived from coal combustion or otherwise natural, had no measurable effect on stream pH in the downstream and/or nearby sites (where such impacts was projected to be more likely). A decline in pH occurred only in two catchments, both far from the primary emission source (Sterkstroom and Upper Mokolo). This decline is more likely due to upstream land-use activities rather than industrial emission-derived acid rain inputs.

Some studies in Europe and North America suggest exchangeable calcium in soils to have decreased over time as a result of leaching by acidic deposition due to NO_X and SO_X emissions (Rodhe et al., 1995; Cerny, 1995, Wesselink et al., 1995). Only two upward trends with respect to calcium were identified post-1994 in the Middle Mokolo and Sterkstroom (Figure 2.16). Incidentally, both sites where these trends were identified are located far from primary emission sources. The role of acidic deposition in soil calcium status and how it affects the ANC of streams remains a subject of research worldwide (Lawrence et al., 1999) and owing to the confounding effects of co-deposition of calcium, any trends in this ion were not included in hypothesis II. Given this study did not examine specific soil chemistry data to infer with the hypothesis tests results with

respect to the two calcium trends, it is my opinion that less certainty exists in terms of explaining which causal mechanisms led to or made greater contributions to these specific trends.

In terms of the Tambotie (A42H) where the gauging station is located close to emission sources (~ 20km), both hypotheses were nullified given that no significant WQ trends were identified. This catchment however does lie to the east of the source which is upwind of the (predominantly) prevailing wind direction, which by inference means that NO_X and SO_X deposition probably only rarely occurs in A42H. Minimal impacts from other upstream land-use activities are also likely to have accounted for the lack of WQ trends over the entire time period. Most of A42H lies within the Waterberg Biosphere where ecotourism and conservation are and have been the main land uses, where the vegetation exists in a largely untransformed and natural state.

Hypothesis I was true for the Palala (A50B) prior to the onset of emissions, however this stream was not explicitly included in hypothesis II owing to the gauging station being located far from (~ 85km) and upwind from primary emission sources. I considered A50B to be located well out of range of wind-deposited NO_X and SO_X emissions, both of which were not likely to be responsible for or to have contributed to the positive trends in NH_4^+ and EC and negative trends in SO_4^2 and PO_4^{3-}. Although difficult to quantify, a combination of natural biological activity, underlying geology and some human-related activity are more likely to be responsible for the trends in these parameters.

Similar to the Palala, the gauging station in the Sterkstroom (A42D) is located far from (~ 75km) and out of range of wind-transported NO_X and SO_X emissions. The five variables trends depicted in Figure 2.16 (for both time periods) contradict hypothesis I and II. Given that A42 is located far from primary industrial emission sources and that streamflow via the gauging station is derived from pristine, natural vegetation (Marakele and Welgevonden), neither industrial nor anthropogenic activity is likely to be responsible for the trends identified in this catchment.

With respect to the Mokolo system, hypothesis I was invalidated for A42E in terms of the upward trends in NH_4^+ and PO_4^{3-} and for A42F in terms of the upward trend in NH_4^+ and downward trend in pH. Only NH_4^+ increased in the Matlabas (A41B), also contrary to hypothesis I. For the rest of the variables, hypothesis I was found to be true. Hypothesis II implied directly to the Middle Mokolo (A42F) and Matlabas as sites downwind from emission sources, and was validated in only the following cases: pH and Ca^{2+} increased in A42F whilst DIN and EC increased in A41B. For the rest of the variables, other WQ trends predicted by this hypothesis (II) did not arise.

2.4 Conclusions

The trend analyses approach focused on the detection of statistically significant changes in WQ as a result of factors other than natural variations induced by discharge. M-K trend tests are well suited for analysing non-parametric data with many missing values and outliers and were therefore selected for application in this study. Although difficult to quantify, the observed WQ trends (Figure 2.16) could hypothetically be mostly due to catchment variability ascribed to other natural and physical exogenous variables such as soil type, underlying geology, biological activity, groundwater inputs and climate. The inception of large scale coal combustion in the early 1990s and consequential loading of S and N in the downstream/nearby catchments does not appear clearly traceable as related trend signals in historical stream WQ records. However this is not to say that NO_X and SO_X emissions have had zero impact on stream pH, sulphate and inorganic nitrate concentrations.

Other types of historical data (*i.e.* for other exogenous variables) which would have provided more certainty on the effects of industrial NO_X and SO_X emissions were either not available, or an attempt to include such data would have gone beyond the scope of the study. Chemical concentrations of the coal (pre-combustion) and historical air quality data records would have improved in explaining more definitively and accurately the WQ trends depicted (see Figure 2.16). As such and in the context of this study, any potential effects of these un-modelled variables remains unexplained. It is therefore suggested that data from this research be combined with additional data variables and modelled in further catchment studies to acquire a better holistic understanding of cause and effect relating to water quality trends.

The natural variability of biogeochemical cycles in relation to any of the observed WQ trends were also not explored to any degree, yet modelling and incorporating the respective roles of these cycles in catchment processes and how they influence stream WQ would vastly improve in trend analysis studies of this nature. Catchment-scale experiments such as the Hubbard Brook Experimental Forest in the USA represents an ‘outdoor laboratory’ where multiple ecological studies are carried out, including biogeochemical cycles and the relationship between vegetation (*i.e.* forest cover) and water quality (Lawrence and David, 1996).

In a study by Likens et al., (1996) an analysis of historical stream chemistry records inferred that the loss of soil cations limited the response of stream WQ to declining rates of acidic deposition. In other words, soil base depletion seemingly had a reversal effect on stream water acidification (*i.e.*

declining pH). Related studies have indicated a similar phenomenon (Kirchner and Lydersen 1995). Research conducted by Bird (2011) has also inferred that atmospheric co-deposition of base cations, such as calcium, may add to the buffering capacity of catchment soils on the South African Highveld. Further research on the biogeochemistry and soil buffering capacities in the Waterberg catchments may therefore be warranted. If the various sources of anthropogenic pollution are to be more conclusively and accurately distinguished, stable isotope analysis studies would also add considerable value in that elemental isotopic signatures are able to provide objective insight regarding the origin of various stream pollutants (Hill et al., 2011). Research carried out in a multidisciplinary and transboundary manner would provide a better overall understanding on what mechanisms are causally-linked to changes in stream water quality.

3 Inorganic nitrogen and phosphorus as limiting nutrients to growth of stream periphyton

3.1 Introduction

Nitrogen (N) and phosphorus (P) are fundamental macronutrients used by primary producers. Their concentrations in waterbodies are also important factors which contribute to any given water quality. The natural and cultural origins of these two nutrients and the associated impacts on water quality have already been described in the introductory chapter (Chapter one). Important to recongise is that the N and P trends (Chapter two) do not reflect the in-stream total for either nutrient. A large component remains bound in stream periphyton where even small changes in N:P ratios can strongly affect primary production (PP).

Studies by Redfield et al. (1963) indicated that a reasonably constant molar ratio of carbon, nitrogen and phosphorus of 100:16:1 exists during growth of marine phytoplankton. Since then this ratio, known universally as the Redfield ratio has been commonly used as an indicator of both terrestrial and aquatic PP (Schindler et al., 2008). In terms of whether N or P normally limits PP in freshwater systems, a number of studies have found that in most cases and with some exceptions, algal growth tends to be P-limited (Mcdowell et al., 2009). As carbon is almost always in abundant supply it is generally considered to be a non-limiting nutrient. If light and temperature conditions are optimal, these factors are also considered to be non-limiting.

The supply of both N and P and their bioavailability relative to each other therefore plays a considerable role in aquatic PP, more so when human-induced disturbances combine with natural nutrient cycles to influence or alter catchment water quality (DWAF 1996). Industrial NO_X emissions are one such disturbance which can add to and raise levels of inorganic N to above that of natural sources (Chapin et al., 2002). In addition to catchment run-off of N and P into streams, primarily derived from biological activities, human and animal waste products and agricultural fertilisers, wet and dry deposition of NO_X emissions have likely become a major additive source of N in Waterberg streams since the early 1990s. Prior to this date energy generation from coal combustion and associated NO_X emissions was not a contributory factor but since then, approximately 14.8 million tonnes has been burnt annually in the Waterberg (http://www.eskom.co.za/Whatweredoing/ElectricityGeneration/PowerStations/Pages/Matimba_Power_Station.aspx). In terms of various criteria used in categorising trophic status, the 1996 National Water Quality Guidelines use the average summer inorganic N concentration as a one classification

criterion. In basic terms, any waterbody with inorganic N levels below 0.5mg/l is classified to be oligotrophic (DWAF 1996).

Given the potential consequences of excessive nutrient loading in aquatic systems and the intensification of NO_X emissions in the Waterberg (Chapter one), it was investigated whether N or P limited PP in these rivers and if loading of industrially-derived N had influenced inorganic N:P ratios in any way. In this study, growth of periphyton was used as a proxy measure to PP. The degree to which limitation occurred and whether different nutrients became limiting at different time and space scales was also examined. According to Francoeur et al. (1999), one of the least ambiguous approaches to assessing nutrient limitation in streams is through nutrient bioassay experiments.

Key question 4 and hypothesis III had relevance in this chapter and are respectively reiterated below:

(4) Which nutrient, if any, is limiting stream periphyton growth and what are the potential (eutrophication) implications of cumulative NO_X emissions?

(III) Whilst human-induced land-use changes may have influenced inorganic N:P ratios, growth of stream periphyton will predominantly be limited by the availability of P.

3.2 Experimental design

In relation to my research, there are previous studies of a similar nature which have employed stream nutrient bioassays to assess the effect of land-use on nutrient enrichment in aquatic systems (e.g. Schanz and Juon 1983, Chessman et al., 1992, Corkum 1996, Mcdowell et al., 2009). Discrete bioassay nutrient experiments manipulating the availability of inorganic N and P were carried out in five Waterberg rivers during the summer season (February 2014). The measurement of mean chlorophyll-*a* (chl-*a*) per unit area was chosen in this study as a representative proxy to total periphyton biomass as it is the most abundant pigment in periphytic algae and cyanobacteria (Biggs and Kilroy 2000; Wetzel 2001). A more detailed description of the design of the bioassays has been included (Appendix F).

To complement the bioassay experiments and the results borne out from those, historical N:P ratios were calculated based on the original hydrological data used in the trend analyses (Chapter two). The dissolved inorganic nitrogen (DIN) to soluble reactive phosphate (SRP) ratio was used to group data into 3 nutrient limitation classes, which were by mass:

≤10:1 (N-limited),
between 10:1 and 20:1 (co-limitation),
and ≥20:1 (P-limited).

These ratios have been used in a number of previous studies (e.g. Schanz and Juon 1983 in Borchardt 1996; Ewart-Smith and King 2012). Thus wherever the term 'N:P ratio' is referred to in this chapter, it applies only to inorganic forms of N (*i.e.* NO_3^-+NO_2^- +NH_4^+) and P (*i.e.* PO_4^{3-}). It should be borne in mind however that there are several reasons why caution should be exercised when using experimental nutrient enrichment bioassays to infer nutrient limitation, some of which are discussed further on (see section 3.4).

3.2.1 Laboratory methods

As described in Biggs and Kilroy (2000), the nutrient diffusing substrate (NDS) method was followed for the nutrient bioassay field experiments (Appendix F). Five identical bioassay trays (5 replicates by 4 treatments) were prepared 24hours prior to deployment in the selected stream sites. Grade 542 Whatman filter papers (quantitative, ashless, 70mm diameter) functioned as contact surfaces with the nutrient-enriched agar substrate, through which the nutrients diffused and upon which colonisation of stream periphyton occurred. These were attached to the agar-nutrient treatment surfaces in each jar using rubber bands. An illustrative description of assay preparation and basic design of the trays is provided in Appendix G (adapted from Biggs and Kilroy 2000).

Each assay had 5 replicates of 4 treatments:
N treatment- 0.5 molar solution of sodium nitrate ($NaNO_3$) in granulated agar
P treatment - 0.05 molar solution of trisodium orthophosphate ($Na_3PO_4.12H_2O$) in granulated agar
NP treatment - both 0.5 molar solution of sodium nitrate and 0.05 molar solution of trisodium orthophosphate in granulated agar
C (control) treatment - no nutrients added to granulated agar

To measure chl-*a* as a proxy for periphytic algal growth, the filter papers from the NDS trays were prepared for analysis in the laboratory, post-retrieval, using ethanol extraction prior to UV detection via spectrophotometry. These procedures are detailed by Sartory and Grobbelaar (1984) and also described in Biggs and Kilroy (2000). For a more detailed description of the extraction and detection methods, refer to Appendices G and H.

To correct for the phaeopigment absorption peak interfering with that of chl-*a*, an acidification step to remove the magnesium ions from the chlorophyll molecules was carried out (Biggs and Kilroy 2000). Acidification serves to convert the whole samples to phaeopigment. Chl-*a* concentrations were calculated by multiplying the spectrophotometer readings by an absorption coefficient of 28.66 for chl-*a* in ethanol (after Sartory and Grobbelaar 1984). Following the protocol used by Sartory (1982), an acid ratio of 1.72 for chl-*a* in ethanol was used in the set of phaeocalculations (Figure 3.1).

Chl-*a* (mg per sample)	=	$[(absorbance_{665\ before} - absorbance_{665\ after})*28.66*$sample volume*extractant volume] / filtered sub-sample volume
Phaeopigments (mg per sample)	=	$[(1.72*absorbance_{665\ after}) - absorbance._{665\ before}]*28.66*$sample volume*extractant volume / (filtered sub-sample volume)
Chl-*a* (mg/m^2)	=	chl-*a* (mg/sample) / area of sample (m^2)

Figure 3.1: Formulae used to calculate the final chl-*a* concentrations per unit area.
(from Biggs and Kilroy 2000).

3.2.2 Field methods

A preliminary site survey was carried out in November 2013 to identify and select suitable locations to deploy the NDS bioassays within each of the 5 rivers. A summer sampling campaign involving deployment, retrieval and preservation of samples was subsequently carried out in February 2014. It was not possible to deploy the NDS trays at an earlier stage due to some of the rivers having insufficient or no flow prior to the onset of summer rainfall in late-January 2014. The locations of the five NDS trays are shown in Chapter one (Figures' 1.4, 1.5, & 1.6).

One NDS tray (comprising 5 replicates of 4 treatments) was deployed in each of the following streams:
Matlabas in sub-catchment A41C, +/- 50km SSW of primary emission sources
Mokolo in sub-catchment A42E, +/- 85km SSE of primary emission sources
Sterkstroom in sub-catchment A42D, +/- 75km SSE of primary emission sources
Tambotie in sub-catchment A42H, +/- 20km E of primary emission sources
Palala in sub-catchment A50B, +/- 85km ESE of primary emission sources

The stream deployment sites were assessed to ensure as homogenous as possible conditions for all trays across all sites (*i.e.* similar light exposure, similar flow velocity and similar depth to the stream bed). Once deployed, the bioassays were left for an incubation period of 9 days prior to

retrieval to allow for adequate algal growth on the substrate surfaces. Any longer than 14 days may have resulted in the development of thick algal mats and sloughing, distorting the final results. To reduce the potential effect of grazing by benthic invertebrates, the trays were placed in stream riffles with moderate flow velocities. *In situ* depth and velocity measurements were recorded during deployment and retrieval, using a tape measure and flow meter respectively (Table 3.1). Two landowners provided assistance in visual monitoring of the trays every second day after deployment and after a 9-day incubation period, when sufficient growth was observed, the trays were retrieved. Trays were deployed over 3 consecutive days on the 16th, 17th and 18th February 2014 and retrieved in the same order on the 25th, 26th and 27th February 2014.

During the retrieval phase, the substrate surfaces (filter papers) were removed from the NDS agar jars, folded inwards and in half, inserted into tinfoil (to prevent UV light degrading the chlorophyll molecules) and inserted into pre-labeled plastic bags for identification purposes at a later stage during laboratory analysis. Prior to transporting the samples, the plastic bags were placed directly in a dry ice cool-box for near-instant freezing and preservation. Freezing keeps periphyton samples intact for several months prior to chl-*a* analysis and is recommended by Biggs and Kilroy (2000). Transportation to the laboratory and subsequent chl-*a* analysis was carried out on the 29th and 30th February 2014.

3.2.3 Data analysis

After the chl-*a* extraction and calculation of mean chl-*a* concentrations (in mg per m^2), a series of one-way ANOVA tests and two different multiple comparison tests were run on the means. The ANOVA procedure was performed to check which nutrient had the greatest influence on the growth of periphyton and whether there were significant differences between the 4 NDS categories (N, P, NP and C). It tested for differences between the respective nutrient treatments within each river. Differences between treatments in all sites (*i.e.* river versus river comparisons) were not tested for. All tests were conducted using a 95% confidence interval ($\alpha = 0.05$) where the ANOVA hypothesis test interpretations were:

H_0: *No effect of nutrient type on algal growth.*

H_A: *Nutrient type has a significant effect on algal growth.*

Where differences were found to be significant in the ANOVA tests, the post-hoc Tukey HSD test was subsequently performed to determine which of the groups differed. The Tukey HSD test is a pairwise analysis procedure which checked for differences between the four NDS groups, where

each group was tested iteratively against the other three groups. The two-sided Dunnett's test, another post-hoc multiple comparison test, was also carried out where the control (C) treatment was assigned as the control group in the test and compared against each of the other three treatments (N, P and NP). The smaller confidence intervals with respect to the Dunnett's test give it greater statistical power than pairwise comparisons methods when comparing . All tests were performed in Microsoft Excel© using the add-in software program XLSTAT-Pro (Version 2014.5.02) (http://www.xlstat.com/).

3.3 Results

3.3.1 In-situ stream measurements

During both phases where measurements were taken, stream velocities did not exceed 0.2 $m.s^{-1}$ at the surface of the NDS trays (this was the maximum velocity measured in the Mokolo). The rate was more-or-less half the maximum in the Sterkstroom, a quarter in the Matlabas and $1/20^{th}$ in the Tambotie (Table 3.1). Discharge and the depth of water covering the bioassays increased in all rivers over the incubation period, and were between 1 and 14 cm. The smallest depth increases were for the Tambotie and Matlabas (1 and 3 cm respectively) with the other three rivers rising between 12 and 14 cm. This was attributed to the increased frequency and amount of rain which fell between the 16^{th} and 25^{th} February 2014. Although the in-stream average velocities may have significantly differed to those I recorded, the trays were deliberately placed in stream reaches where minimal changes to velocity would have occurred for the duration of the experiment.

Table 3.1: NDS tray locations and corresponding stream conditions during deployment and retrieval. The depth measurement was from the stream water surface to the NDS tray growth surface. Velocity readings were measured at the tray surfaces.

River	GPS reading of NDS tray	Depth at deployment (cm)	Depth at retrieval (cm)	Deployment velocity ($m.s^{-1}$)	Retrieval velocity ($m.s^{-1}$)
Matlabas	S24 61. 411 E027 17.161	35	38	0.05	0.05
Sterkstroom	S24.14.499 E027 58.252	40	54	0.1	0.1
Mokolo	S24 15.761 E028 05. 788	25	37	0.2	0.2
Palala	S24 61. 411 E028 22.333	37	50	0.15	0.2
Tambotie	S23 411.864 E027 49.192	32	33	0.01	0.01

3.3.2 Historical N:P ratios

Based on the inorganic N and P stoichiometry calculations, it can be inferred that N-limitation across all rivers and for both time periods was more prevalent and common than cases where P-limitation or co-limitation occurred (Table 3.2). The post-1994 ratios which were calculated from a greater number of observations over a longer time period may be more reliable than the pre-1994 ratios. This is particularly so for the Tambotie, Matlabas and Palala where more pre-1994 data may have increased certainty regarding the ratios reported for these rivers. Over a six year period (1982-1987) the number of observations in these rivers was only 6, 9 and 11 respectively.

Overall however, the ratios do not appear to have shifted greatly over time in each river. Two streams in closer proximity and/or downwind from the source of emissions, which were assumed to have received more industrial fallout of N over time, did conform to what was expected. The number of observations where N was predicted to be limiting increased post-1994: by 18% to 85% (Matlabas) and by 24% to 84% (Middle Mokolo). Minimal changes were seen in the Tambotie and only a minor shift occurred in the Palala, where co-limitation increased from nil (pre-emissions) to 7% (post-emissions). The decrease in N-limitation by 12% in the Upper Mokolo and increase by 16% in the Sterkstroom are more likely to have been due to upstream land-use changes and not from fallout of industrial N. Possible reasons why are further discussed (see section 3.4).

Table 3.2: Historical inorganic N:P ratios before (T_1) and after (T_2) large scale industrial NO_X emissions. The ratios are based on seasonal means where the nutrient limitation class ≤10:1 denotes N-limitation, between 10:1 and 20:1 denotes co-limitation and ≥20:1 denotes P-limitation. The respective number of observations in each limitation class expressed as a percentage of the number of observations are included in parentheses.

River		Matlabas	Upper Mokolo	Middle Mokolo	Tambotie	Sterkstroom	Palala
DWA sample station ID		A4H004	A4H002	A4H005	A4H007	A4H008	A5H004
T_1 (No. of observations summer 82'-winter 87')		9	27	20	6	33	11
No. of pre-emission observations with **N:P ratio** (by mass):	≤ 10:1 (N-limitation)	6 (67%)	21 (78%)	12 (60%)	5 (83%)	23 (70%)	11 (100%)
	between 10:1 and 20:1	2 (22%)	6 (22%)	8 (40%)	0 (0%)	5 (15%)	0 (0%)
	≥20:1 (P-limitation)	1 (11%)	0 (0%)	0 (0%)	1 (17%)	5 (15%)	0 (0%)
T_2 (No. of observations summer 94'-summer 13')		95	146	67	48	176	104
No. of pre-emission observations with **N:P ratio** (by mass):	≤ 10:1(N-limitation)	81 (85%)	93 (64%)	56 (84%)	38 (79%)	151 (86%)	96 (92%)
	between 10:1 and 20:1	11 (12%)	40 (27%)	2 (3%)	4 (8%)	22 (12%)	7 (7%)
	≥20:1 (P-limitation)	3 (3%)	13 (9%)	9 (13%)	6 (13%)	3 (2%)	1 (1%)

3.3.3 Summary statistics

The growth of periphyton (as mg/m^2 of chl-*a*) after the 9-nine day incubation period in each river signifies growth to have been greater in the N and NP treatments across all sites (Figure 3.2 and Appendix J). Based on these results alone, N is evidently the limiting nutrient in these streams as enrichment of each system with identically controlled microcosms (in streams with similar physical conditions) produced similar results throughout.

Periphyton growth in the Tambotie was considerably greater compared with the other rivers, with a maximum mean of 29 mg/m^2 chl-*a* for the N treatment (Figure 3.2). The NP and C treatments were not much lower, at 22 and 25 mg/m^2 chl-*a* respectively. These figures contrast with the other rivers where the means for all treatments were all notably lower (Figure 3.2). For visualisation of the colour differences in the cuvettes prior to UV detection with the spectrophotometer, one set of the Mokolo treatments are illustrated (Appendix K).

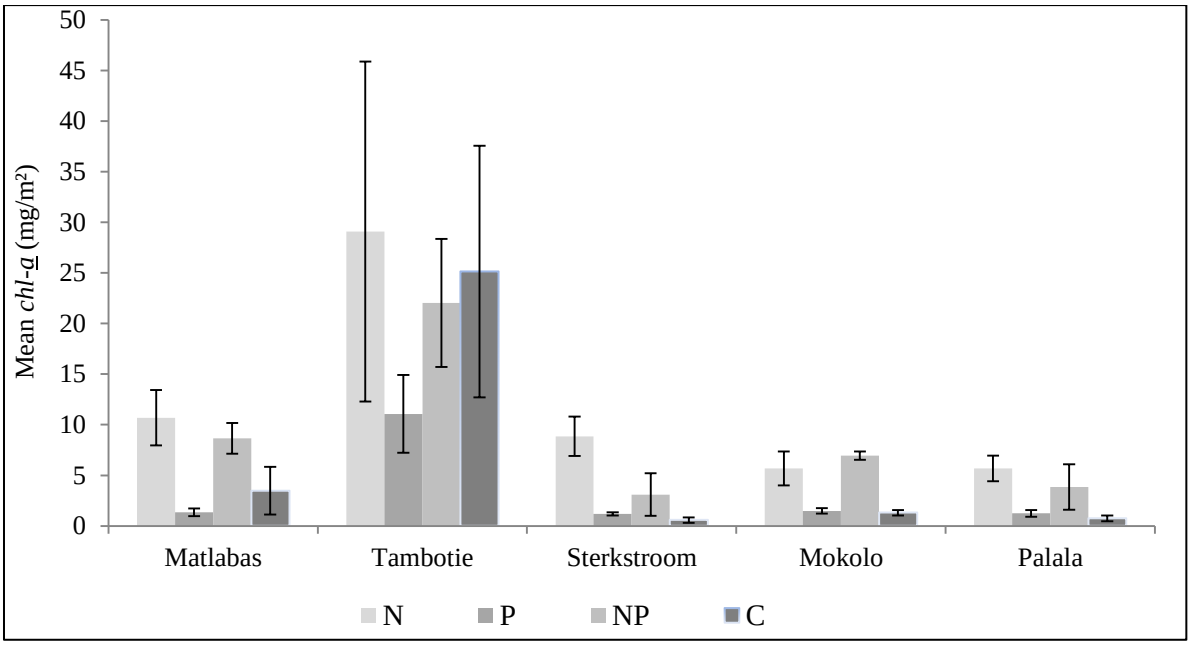


Figure 3.2: Periphyton growth, measured as mean chl-*a*, after 9 days incubation period. Standard deviation shown as Y-error bars.

3.3.4 ANOVA and multiple comparison tests

With the exception of the Tambotie, the Fisher's F values were high in all rivers with corresponding probabilities being less than 0.0001 (Table 3.3). In these cases there was less than a 0.01% risk in concluding the H_0 (*i.e.* no effect on growth) was wrong. Hence in all rivers except the

Tambotie, the nutrient treatments did have a significant effect on periphytic algal growth (*i.e.* there was no evidence of limitation by any of the nutrients in the Tambotie treatment). The Tambotie bioassay also differed from the rest with a low coefficient of determination (R^2) of 0.314. This effectively means that 31% of the variability in chl-*a* was explained by nutrient type (the explanatory variable). The remaining 69% variability remained hidden as random errors in other variables which were not modelled in the ANOVA. Less error existed for the other bioassays where R^2 values were all >0.75, where more of the variability was explained by nutrient type.

With the Tukey's HSD test (where $\alpha = 0.05$), no significant differences between the means in either paired treatment occurred in the Tambotie, as would have been the case anyway based on the ANOVA results (Table 3.4). For all rivers, there were also no significant differences between the means from the C vs P comparison. As would otherwise have been expected, the comparison between the means from the N versus NP treatments also yielded no significant differences in all rivers, except the Sterkstroom.

Most of the significant differences in the means were between both the N and NP treatments vs the C and P treatments, indicating N to have been the limiting nutrient that promoted algal proliferation when supplied in abundance. By assigning the C treatment as the control category, the Dunnett's test further confirmed and agreed with the Tukey's test results. Significant differences occurred when comparing the C means with those from the N and NP treatments, but not with those from the P treatments (Table 3.5).

Table 3.3: ANOVA with Fisher's F-test. p-values in bold indicate the rivers in which the treatments had a significant effect on algal growth.

River	Source	DF	Sum of squares	Mean square	R^2	Fisher's F	Pr > F
Matlabas	Model	3	284.777	94.926	0.821	24.441	< **0.0001**
	Residuals	16	62.143	3.884			
	Total	19	346.920				
Tambotie	Model	3	897.709	299.236	0.314	2.440	0.102
	Residuals	16	1961.960	122.623			
	Total	19	2859.669				
Mokolo	Model	3	106.129	35.376	0.837	27.424	< **0.0001**
	Residuals	16	20.640	1.290			
	Total	19	126.769				
Sterkstroom	Model	3	171.119	57.040	0.873	27.434	< **0.0001**
	Residuals	12	24.950	2.079			
	Total	15	196.069				
Palala	Model	3	80.081	26.694	0.751	15.667	< **0.0001**
	Residuals	16	27.261	1.704			
	Total	19	107.343				

Table 3.4: Tukey's HSD analysis on the differences between nutrient treatments with critical value d=4.046. p-values in bold indicate significant differences between the means.

River	Treatment category					
	N ~ P	N ~ C	N ~ NP	NP ~ P	NP ~ C	C ~ P
Matlabas	**< 0.0001**	**0.000**	0.387	**0.000**	**0.004**	0.357
Sterkstroom	**0.000**	**0.000**	**0.001**	0.114	0.288	0.929
Mokolo	**0.000**	**0.000**	0.829	**< 0.0001**	**0.000**	0.994
Palala	**0.000**	**0.000**	0.155	**0.009**	**0.029**	0.930
Tambotie	0.086	0.747	0.940	0.226	0.970	0.425

Table 3.5: Two-sided Dunnett's test results for multiple comparisons for the variable nutrient, where the C treatment was used as the control group. Values in bold indicate significant differences between the means Significant differences are indicated in bold, where the standardised differences are > critical d-value of 2.592.

River	Treatment category		
	N ~ C	NP ~ C	P ~ C
Matlabas	**5.790**	**4.153**	-1.698
Sterkstroom	**8.128**	2.480	0.604
Mokolo	**6.938**	**6.086**	0.258
Palala	**5.982**	**3.745**	0.601
Tambotie	0.567	-0.442	-2.007

3.4 Discussion

The results of the NDS experiments I conducted largely conformed to the predictions based on historical N:P ratios calculated from the DWS hydrological database, where the growth of stream periphyton in the Waterberg is limited by the availability of N as opposed to P. Hypothesis III is therefore contradicted in terms of the results borne out from the bioassay experiments. In contrast to the tried, tested and therefore general belief that PP in streams and lakes is typically limited by the availability of P (Schindler 1974, Hecky and Kilham 1988, Francoeur et al., 1999, Biggs 2000), it is arguably the case in these streams that the reverse is true. N-limitation has also been observed from a number of other studies (e.g. Elser et al., 1990, Malone et al., 1996, Downs et al., 2008, Mcdowell et al., 2009) which does not conform to the general (and former) acceptance that P-limitation was ubiquitous throughout freshwater systems (e.g. Schindler 1974).

The exception was the Tambotie River, where although periphyton growth was more abundant than the other rivers, no significant differences occurred between the treatments. Physical conditions rather than nutrients may have been the main factor resulting in the higher amount of algal growth.

Irrespective of the deliberate and controlled nutrient enrichment conditions intended by the bioassay, growth on the colonisation surfaces showed no clear distinction across the treatments, as was apparent in the other rivers. The stream velocity was also comparatively lower in the Tambotie and upon visual inspection during tray deployment and retrieval, biomass (*i.e.* both periphyton and macrophyte communities) was significantly more abundant compared to the other river sites. Owing to the low velocity readings (where flow appeared almost stationary to the naked eye) and potential micro-eddies caused by both rooted and suspended macrophytes, cross-contamination of the diffusing nutrients across all treatment surfaces may also have occurred in the Tambotie. Although homogenous conditions were strived for, accessibility to large sections of all rivers was not possible and sites were ultimately determined by this constraint.

With respect to the A41 gauging stations from which historical data were obtained, those for the Mokolo and Sterkstroom are located furthest away from the primary NO_X emission source. Figure 1.8 (Chapter one) illustrates where the main source of emissions is located (referred to as 'Mines and Quarries' in the map legend entry). Instead of industrial N emissions contributing to the shifts in N:P ratios over time, it is more likely to have been other catchment land-uses that have played more of a role, alongside natural seasonal variations induced by climate and biological activity.

Some cultivated agriculture does occur in A42E and A42F (Mokolo and Sterkstroom) with a few pattern shifts in N:P ratios occurring after 1994. N-limitation increases from 70% to 86% in the Sterkstroom and from 60% to 84% in the Middle Mokolo. These observed shifts (Table 3.2) may be more likely due to seasonal variations in agricultural fertiliser application and/or the possible influx of other point sources of pollution upstream. Although the human population in Vaalwater is relatively small, it has grown steadily in the last few decades where effluents from surrounding communities could be a factor. There is an informal settlement (upstream of the Mokolo gauging station) which has expanded on the town periphery and although not quantified in this study, input of nutrients into the Mokolo system from this settlement is speculated.

It should be borne in mind that although the NDS experiments greatly increased the concentrations of bioavailable inorganic N and P above that which was already in the streams, organic forms of both N and P were not accounted for. Organic forms of N and P derived from biological activities forms a large part of both nutrient pools and inorganic forms alone do not fully reflect the rates of their supply to the system (Dodds 2006). In terms of the SRP treatments (inorganic P), these would have included forms of phosphorus in addition to phosphate (e.g. Hudson et al., 2000) and SRP is not always a good indicator of total P as is it a chemically, rather than biologically, defined

measure (Dodds 2006). Uptake rates of SRP in aquatic systems can also be highly variable depending on trophic status and even when phosphate concentrations are low, uptake can be rapid when mineralisation rates are high (Dodds et al., 1989). However the design of the bioassay experiments was such that the N and P concentrations were high enough to override the influence of existing, natural concentrations.

In terms of any possible eutrophication impacts in these rivers, Appendix L represents the summer inorganic N concentrations over the 31 year data record, where the averages for all rivers, (as well as most of the seasonal figures) are less than 0.5mg/l. This gives an indication that water quality upstream of all gauging stations used in this study has not experienced any major deterioration due to nutrient enrichment and subsequent eutrophication. There are other classification criteria such as mean annual chl-*a* and mean annual total P, however data for these criteria were not obtained or generated during this study.

3.5 Conclusions

All study rivers included in the bioassay analyses, with the exception of the Tambotie, were N-limited. The method of using inorganic N:P ratios to predict nutrient limitation is not flawless in that it does not account for total P, which includes organic P which has a rapid turnover rate in freshwater. Further NDS bioassay experiments in the winter season to compare the seasonal effects on the ratios may also have improved the ability of the experiment to predict which nutrient limited algal growth. However, the historical N:P ratios calculated from the WMS database still indicate N-limitation has predominated these streams for the last several decades.

The theoretical implications of further and additional N loading into the Waterberg catchments and associated streams, from either agricultural activities or industrial-derived N emissions, may not become clearly apparent in this region of the greater catchment. However at a continental scale, future impacts may arise further downstream in estuarine and near-shore marine environments. Eutrophication and hypoxia in many estuarine and nearshore coastal areas has resulted in a number of dead zones having developed, such as those in the Baltic, Black Sea, Gulf of Mexico and the East China Sea (Diaz and Rosenberg 2008). This global phenomenon is projected to persist and exacerbate if urbanisation, burning of fossil fuels and fertiliser application continues to load nutrient pollutants into river run-off and consequently, estuarine and marine environments. The greater Limpopo catchment, which encompasses the Waterberg region and the nearshore zone of

the Indian Ocean, is included in this projection and should therefore be monitored continuously in a collaborative effort by the respective nations bordering the greater catchment area.

4. Research synthesis and final remarks

The detection of trends (directional changes) in WQ over time are important to measure, quantify and explain in terms of human impact as far as is practically possible such that the consequences of our actions can be measured and better understood within the context of what is naturally variable. The more accurately our actions can be measured and quantified, the more robust and informed our management interventions will be to control the detrimental effects of such actions on freshwater ecosystems (DWA 2011).

From a water resource management perspective, the official, jurisdictional body mandated to oversee the nation's water resources and their use, the Department of Water and Sanitation (DWS) assumes a central role in spearheading the coordination, implementation and regulation of water policy in the country (DWA 2012a). Within the national water management framework, commonly referred to as Integrated Water Resource Management (IWRM), part of the mandate as contemplated in the National Water Act (Act 36 of 1998) includes the monitoring of WQ at various levels and scales. Monitoring programmes are necessary for the interpretation of possible trends and activities which may negatively impact and potentially disrupt ecological functioning of aquatic ecosystems (DWA 2012b).

A number of national monitoring programmes have been established and are underway to monitor water quality from various foci of expertise and, based on systematic principles and observations, provide scientifically credible advice and feedback into the IWRM structure. The objective of monitoring and modelling is to aid and facilitate the decision making process which often needs to take into consideration multiple criteria, conflicting demands and varying degrees of certainty in arriving at final decisions, at times with unintended consequences. It became apparent from this research that large gaps in much of the data record exist and it is my opinion that efforts to reduce such gaps would serve to further improve the existing programmes.

In terms of the impacts of climate change on freshwater resources and as projected by global circulation models, changes in the structure of the atmosphere are predicted to take place in the future (Seidel et al., 2008, IPCC 2013) with regional temperature rises in Africa expected to exceed that of the rise in the global average (DST 2010). With respect to rainfall projections over Southern

Africa, the observed trends are not as well defined and spatially consistent as the observed temperature trends (e.g. Kruger 2006) and the extent to which models can successfully downscale precipitation remains unclear (DST 2010). What is generally more certain is that the summer rainfall region of South Africa, within which the Waterberg lies, is projected to experience more intense and increased summer rainfall amounts and become drier during spring and autumn as a result of the more frequent formation of mid-level high pressure systems (Engelbrecht et al., 2009).

Based on the trend test results and outcomes of the nutrient limitation experiments, examined in conjunction with the accessory information provided (Chapter one, section 1.5), an overall conceptual framework for the Waterberg streams can be inferred where (i) climate and geo-hydrological factors have and will continue to function as primary drivers of spatial and temporal variation in catchment water quality (past and present), (ii) nutrient pollution from agricultural activities and human populations upstream in each quaternary catchment has had relatively minor effects on WQ over the last ~20 years and (iii) input loads of NO_X and SO_X have not yet had a major influence on surface water quality in the study-site quaternary catchments.

However with industrial development in the region scheduled to progressively intensify in the future, technology improvements and pollution abatement measures will have an important role to play in mitigating the cumulative impacts of NO_X and SO_X emissions on catchment water quality. The contribution of emissions to regional climate change and the consequential impacts on human and ecosystem health therefore requires rigorous implementation of effective intervention measures. Minimum Source Emission and Ambient Air Quality Standards have been legislated and the implementation process is still underway.

The Standards advocate a strong emphasis on the elimination or minimisation of emissions at source. As contemplated in the National Environmental Management: Air Quality Act (39 of 2004) and in terms of GN 495 in Government Gazette No. 35435, the Waterberg was declared a national priority area in June 2012 (DEA 2012). This declaration is likely in reaction to the proposed development plans for the region and will hopefully function as a mechanism to monitor and regulate future industrial development. This will be important given the UNESCO status of the Waterberg and the various other land-use activities and livelihoods which exist in the region.

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6 Appendices

Appendix A: Exploratory data analysis (EDA).

Introduction

As described in Helsel and Hirsch (2002), hydrological data (*i.e.* constituent concentration and discharge) are continuous, often characterised by outliers, have no negative values and tend to follow lognormal rather than normal distributions. Outliers in the data often result in distributions exhibiting positive skewness (where median values are less than the mean values) and as a result, the mean is generally not a resistant and robust statistical measure of location. The median is preferred as it is minimally affected by the magnitude of a single observation or event (Helsel and Hirsch 2002).

Logarithmic transformations are often useful for making data more symmetric, linear and/or homoscedastic. Seasonal patterns and trends are often revealed in hydrological data with the associated variables tending to exhibit varying levels of autocorrelation (also known as serial correlation). Consecutive observations tend to be positively autocorrelated with high values following high values and low values following low values. Water quality is also highly dependent on exogenous catchment variables such as discharge, hydraulic conductivity and sediment grain size, and exhibits strong co-variation with these variables (Helsel and Hirsch 2002).

Methods

Correlation tests

Spearman's correlation tests were computed to determine the degree of relation (correlation) between total monthly flow volumes and monthly rainfall figures. Computed p-values and correlation coefficients provided some indication whether discharge alone as an explanatory catchment variable (and as a proxy to rainfall) could be modelled with WQ variables in the trend analyses. Discharge data in the form of total monthly flow volumes were plotted against monthly rainfall figures (A1 and A2) to visualise the long-term relationship in both variables in parallel prior to further analysis.

Normality tests

All variables were tested for normality at a 5% significance level using four widely used normality tests: the Shapiro-Wilk (S-W), Anderson-Darling (A-D), Lilliefors (L) and Jarque-Bera (J-B) tests. Initial tests were run on the raw and log transformed data. The outputs were generated as summary

statistics, P-P Plots and Q-Q plots (for visualising the data distributions) and the respective hypothesis test interpretations. In all cases and for all tests, the null and alternate hypotheses were stated respectively as:

H_0: *The variable from which the sample was extracted follows a Normal distribution*

H_A: *The variable from which the sample was extracted does not follow a Normal distribution*

Autocorrelation tests

Autocorrelation arises when consecutive observations are strongly correlated with each other and in time series hydrological data, positive autocorrelation (AR) is most prevalent. This is where high values tend to follow high values or low values follow low values. Although parametric (linear) regression was ultimately not used for the final trend analyses, it can also be used in a mixed approach with the Mann-Kendall test in trend analyses (refer to Table 2.6 in Chapter two). However, the aim of linear regression in this EDA test was only to determine whether consecutive variable observations in the regression residuals were strongly correlated with each other. The main problem associated with AR occurring in the error terms (residuals) is that the values of the sample variance (s^2) may significantly underestimate the true variance (σ^2), thus invalidating subsequent hypothesis test results in future stages of the analysis (Helsel and Hirsh, 2002).

To evaluate the dependence of the residuals and test for AR [1] (first-order), discharge (explanatory variable) was linearly regressed with the respective WQ parameters (response variables). The regression computations were conducted iteratively on the raw data for all rivers to obtain the residuals, which were subsequently tested for AR [1] using the Durbin-Watson (D-W) statistic (Appendix C).

The null and alternative hypotheses for the D-W test are as follows:

H_0*: The residuals are not autocorrelated*

H_A*: The residuals are AR [1]*

where p-values < α indicate the presence of AR [1] between discharge and the variables in question (thus rejection of H_0).

Heteroscedasticity tests

Heteroscedasticity is another term for non-constant variance. In the case of heteroscedastic WQ data, as constituent concentrations increase the variance in those values also tends to increase. The relevance of testing for heteroscedasticity, in conjunction with normality testing was to further verify the appropriate data transformations and trend analysis procedures (parametric or non-

parametric). These tests required simple linear regression computations between the explanatory X variable (discharge) and all Y response (WQ) variables to obtain the residuals, which were then subjected to 2 hypothesis tests. Natural log transformations of discharge were calculated prior to performing the regression. Log transformed discharge values (ln Q) were then regressed against the monthly median WQ values. The Breusch-Pagan (B-P) and White (W) tests were used, both of which have null and alternative hypotheses as follows:

H_0*: Residuals are homoscedastic*

H_A*: Residuals are heteroscedastic*

Results

Correlation

There was a strong but not absolute relationship between local monthly rainfall and discharge in the study rivers (A1 and A2). A common trend to the river sites examined was increasing flow volumes over time. The 31-year increase in the Tambotie was slight (~0.4%) and greatest in the Middle Mokolo (~5%). The four stations from the other rivers had similar rates of increase in flow volume of ~1%. This may be attributable to the increased intensity and frequency of regional flood events in recent decades, a similar observation in other studies (e.g. Engelbrecht et al., 2009). The anomaly is that rainfall averages (shown as red lines) do not show a general upward trend but appear to remain relatively constant over time, with slight decreases for the Matlabas and Palala.

The respective correlation scatterplots are presented (A4) where all rivers, with the exception of the Tambotie, show significant positive correlation between rainfall and flow volume (A3). However, given that the highest rho coefficient is 0.313 (Sterkstroom), the correlations are not strong. Bearing in mind that correlation is a measure of co-variation and does not provide evidence for causality between variables (Helsel and Hirsch 2002), there could be a number of reasons for these relationships as explained in the EDA discussion.

Correlation results (A5) between Q and the WQ variables can be summarised as follows:

pH: negative correlation in Matlabas, Sterkstroom and Palala.

Electrical conductivity: negative correlation in all rivers, except the Tambotie and Palala.

Calcium (dissolved): negative correlation in Matlabas and Sterkstroom.

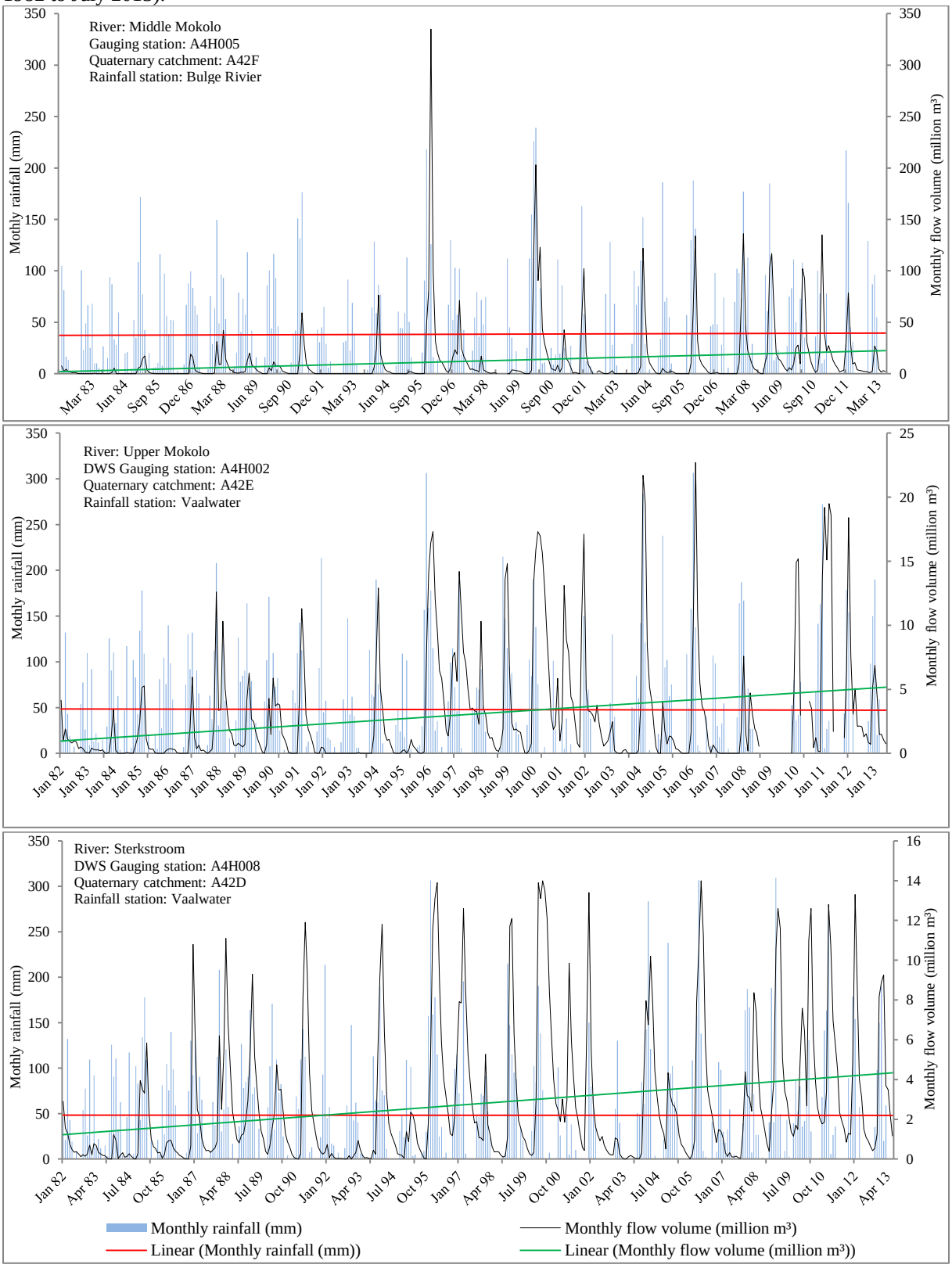
Nitrate + nitrite nitrogen (dissolved): positive correlation in all rivers, except the Matlabas.

Ammonium-nitrogen: negative correlation in Middle and Upper Mokolo and Sterkstroom.

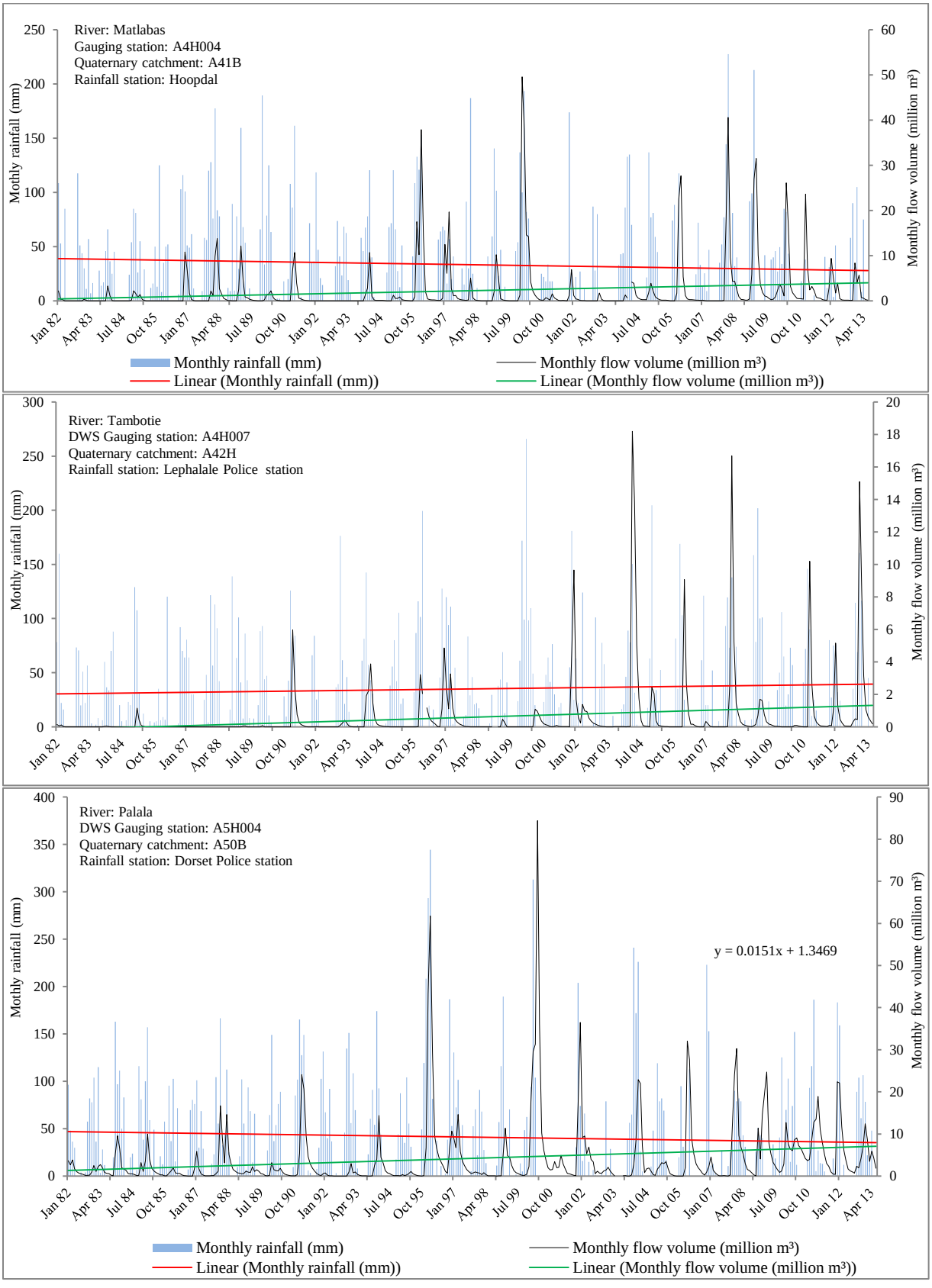
Phosphate-phosphorus (dissolved): positive correlation in the Middle Mokolo.

Sulphate (dissolved): negative correlation in the Sterkstroom and Palala.

A1: Long-term monthly flow volume and rainfall trends in 4°ry catchments A42F, A42D and A42E (January 1982 to July 2013).



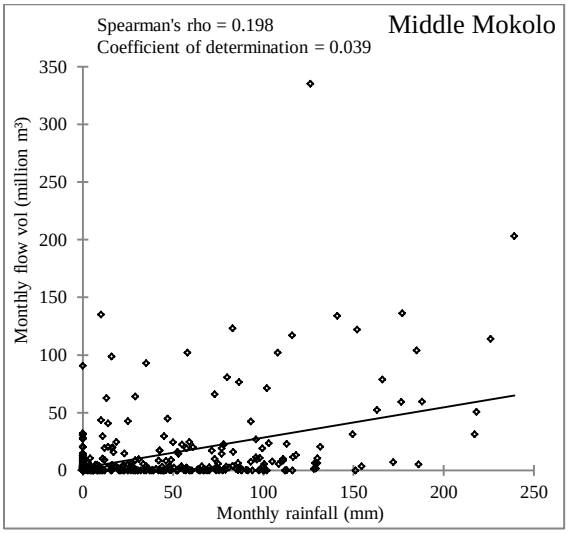
A2: Long-term monthly flow volume and rainfall trends in 4°ry catchments A41B, A42H and A50B (January 1982 to July 2013).

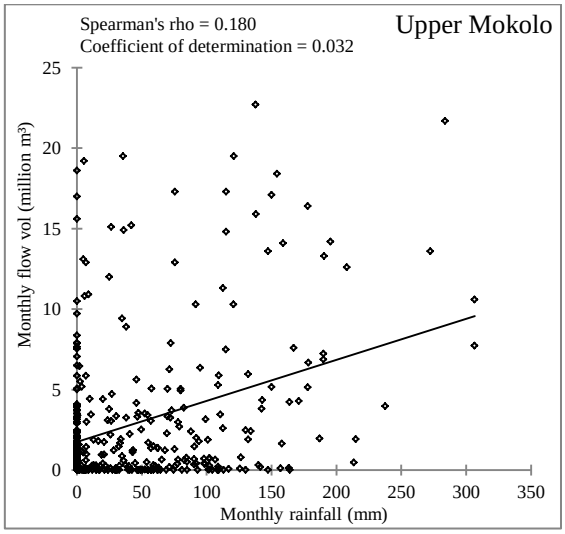


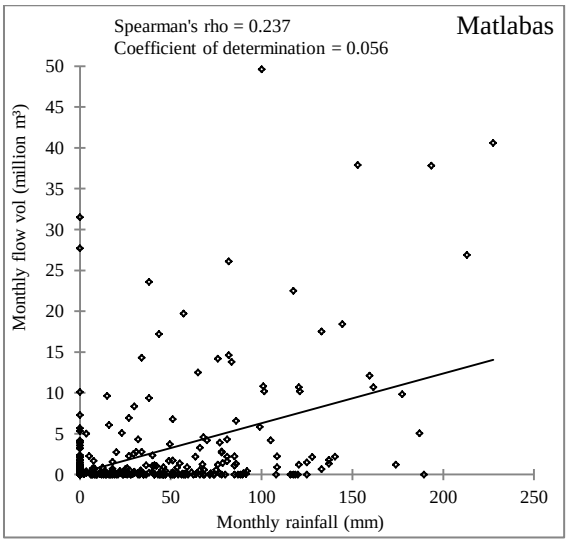
A3: Spearman's rho correlation summary statistics for monthly flow volumes vs monthly rainfall totals (January 1982 – July 2013).
p-values shown in bold are different from 0 with a significance level of $\alpha = 0.05$.

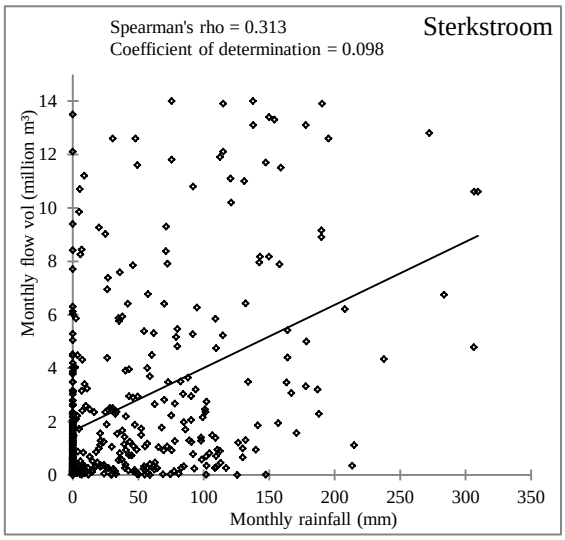
	Middle Mokolo		Matlabas		Palala		Sterkstroom		Tambotie		Upper Mokolo	
	Monthly flow volume (million m^3)	Monthly rainfall (mm)	Monthly flow volume (million m^3)	Monthly rainfall (mm)	Monthly flow volume (million m^3)	Monthly rainfall (mm)	Monthly flow volume (million m^3)	Monthly rainfall (mm)	Monthly flow volume (million m^3)	Monthly rainfall (mm)	Monthly flow volume (million m^3)	Monthly rainfall (mm)
Observations (n)	378	378	378	378	378	378	378	378	378	378	378	378
Missing values	5	0	2	0	3	0	0	0	3	0	21	0
Minimum	0	0	0	0	0	0	0	0	0	0	0	0
Maximum	335	239.1	49.6	227.5	84.4	344.5	14	309.5	18.2	266	22.7	306.6
Mean	12.322	38.321	2.206	32.965	4.206	41.198	2.782	48.198	0.573	34.903	2.972	47.933
Standard deviation	30.705	48.514	6.028	43.947	8.306	56.021	3.491	60.912	2.037	45.949	4.569	59.974
p-value	**0.00012**		**<0.0001**		**<0.0001**		**<0.0001**		0.269		**0.00067**	
Coefficient of determination	0.0393		0.056		0.05		0.098		0.003		0.0322	
Spearman's rho	**0.1983**		**0.237**		**0.223**		**0.313**		0.057		**0.180**	

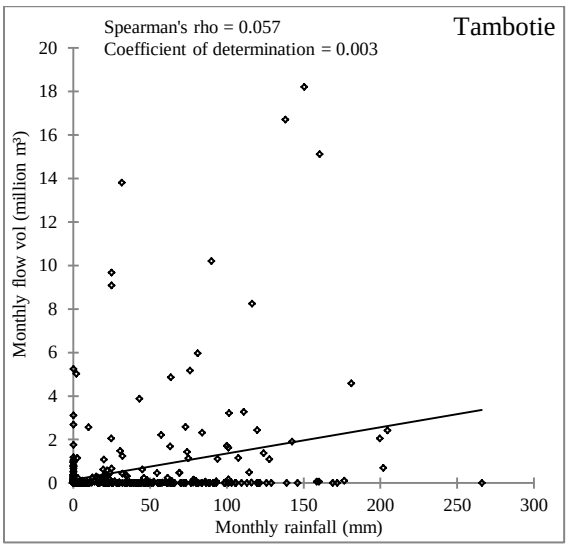
A4: Correlation scatterplots for monthly rainfall totals vs monthly stream flow volumes.

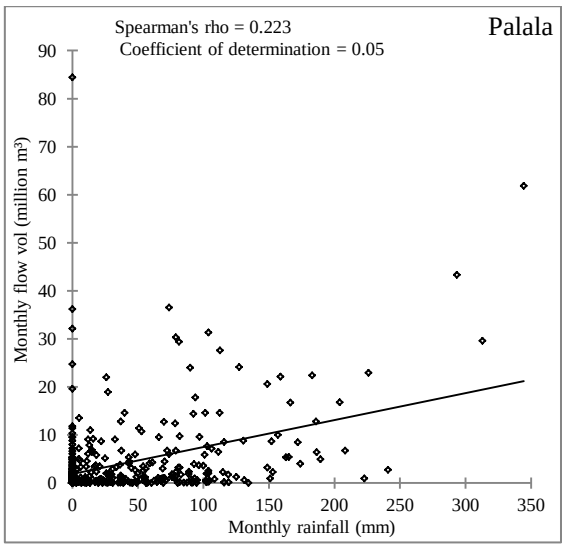












A5: Spearman's rho correlation matrix of discharge (Q) versus water quality response variables, based on raw untransformed data. The rho values shown in bold are where p-values were different from 0 with a significance level $\alpha = 0.05$.

Variables	Unit	Q (cumecs)					
		Middle Mokolo	Upper Mokolo	Matlabas	Sterkstroom	Tambotie	Palala
ln Q	cumecs	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
pH		-0.063	-0.085	**-0.465**	**-0.432**	0.202	**-0.266**
EC	$ms.m^{-1}$	**-0.322**	**-0.316**	**-0.768**	**-0.670**	-0.106	-0.136
Ca^{2+}	mg/l	-0.111	-0.138	**-0.631**	**-0.560**	0.095	-0.037
SO_4^{2-}	mg/l	0.112	0.108	-0.116	**-0.146**	0.140	**-0.197**
PO_4^{3-}	mg/l	**0.191**	0.106	0.009	-0.001	0.145	-0.126
$NO_3^-+NO_2^-$-N	mg/l	**0.314**	**0.230**	0.124	**0.122**	**0.397**	**0.281**
NH_4^+-N	mg/l	**-0.176**	**-0.181**	0.061	**-0.116**	-0.211	0.173

Normality

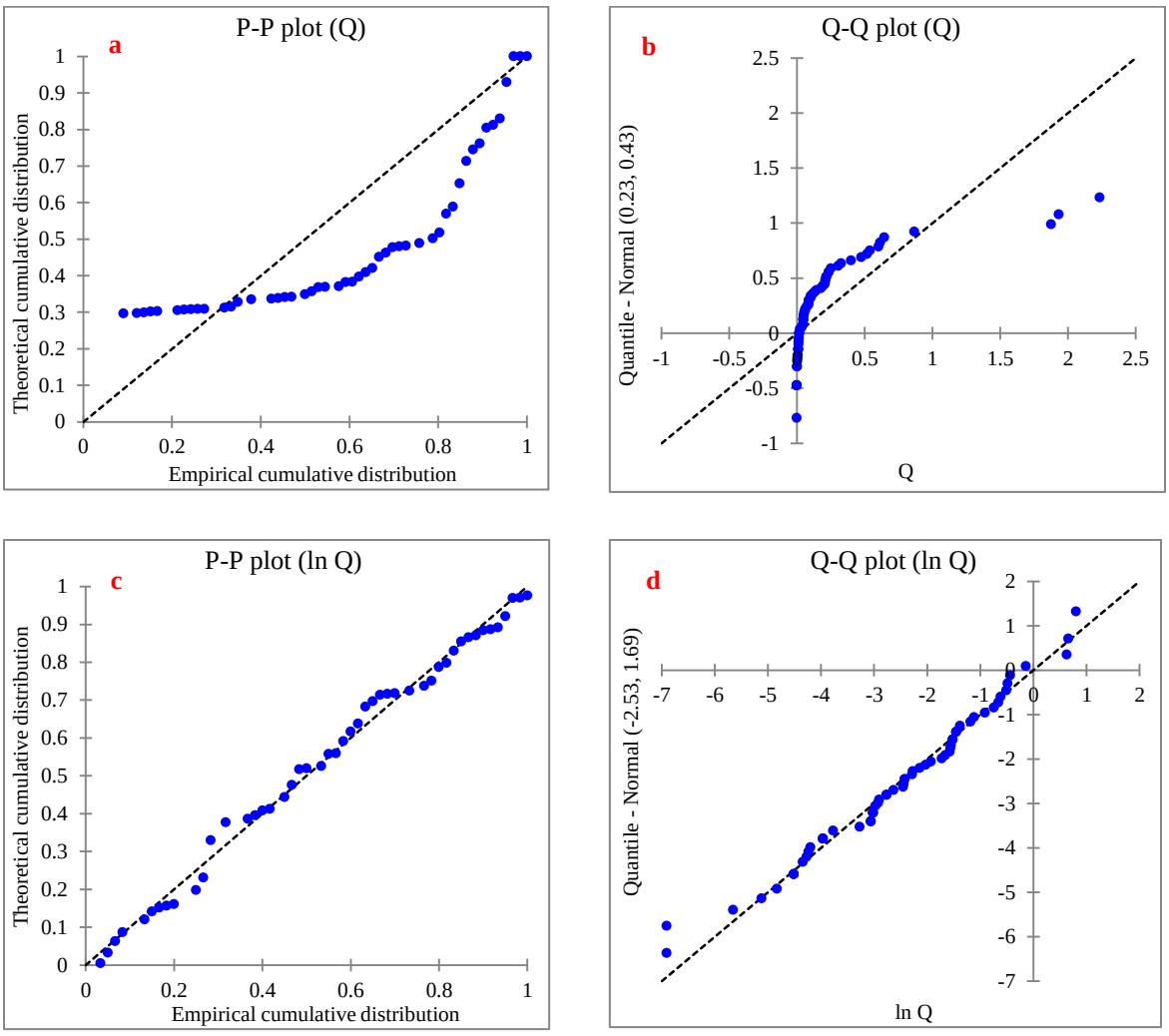
The majority of the variables in the original, raw form did not follow normal distributions (A6). H_0 was therefore rejected in most cases. Albeit to a lesser degree, this was also true for log transformed data. Repeating the tests on both monthly and seasonal averages (medians and means) yielded mostly similar results, where the majority of the variables again exhibited non-parametric distributions. The general tendency when calculating averages over progressively longer time periods and log transforming these averages is that the distributions gradually start to exhibit parametric characteristics (Helsel and Hirsh 2002) which was evident in the Q-Q plots, P-P plots and the hypothesis test results. This trend is also reflected and summarised by the decreasing percentages reflected in parentheses (A6).

A6: Summary of normality tests for all rivers and all variables. Percentages in parentheses represent the ln transformed data.

	% Non-normal distribution					
River	Middle Mokolo	Upper Mokolo	Sterkstroom	Matlabas	Tambotie	Palala
Raw data (ln transformed)	100 (78)	100 (100)	100 (100)	100 (85)	91 (66)	100 (88)
Monthly means (ln transformed)	100 (78)	100 (100)	100 (97)	100 (81)	91 (69)	97 (91)
Monthly medians (ln transformed)	100 (60)	100 (94)	100 (97)	100 (84)	84 (88)	97 (84)
Seasonal means (ln transformed)	72 (66)	97 (81)	97 (81)	94 (56)	69 (47)	97 (75)
Seasonal medians (ln transformed)	72 (50)	94 (66)	100 (88)	100 (66)	78 (84)	88 (72)

A7 depicts how a natural log transformation of median discharge in the Tambotie River rendered the distribution from non-parametric (in the case of Q) to parametric (in the case of ln Q). As illustrated in the P-P and Q-Q plots, the data are not linearly distributed along both axes (a and b) but are so in the log transformed plots (c and d).

A7: Median discharge data distributions for the Tambotie River. a and b illustrate the non-normal distribution of Q in untransformed form whilst c and d illustrate how a natural log transformation of Q renders the data to follow a normal distribution.



Autocorrelation

There were mixed results of first-order autocorrelation depending on river and variable (Appendix D). pH and EC residuals were autocorrelated with Q in all rivers. Dissolved Ca^{2+} residuals were autocorrelated with Q in all rivers except for the Tambotie and Palala whilst dissolved SO_4^{2-} residuals were autocorrelated with Q in all rivers except the Tambotie. With only a few exceptions,

the remaining variables (NO_3^-+NO_2^-, NH_4^+ and PO_4^{3-}) were predominantly not autocorrelated with Q.

Heteroscedasticity

Heteroscedasticity between discharge and the respective WQ variables was confirmed when the computed p-values were less than the 5% level of significance ($\alpha = 0.05$) and have been indicated as such in bold (Appendix D). The mixed results reveal that some WQ variables exhibited a higher degree of heteroscedasticity in all the rivers when compared to other variables. For example the NO_3+NO_2^--N, PO_4^{3-}-P and Ca^{2+} residuals were homoscedastic in all rivers, where the data points appeared as random scatter on the X-Y scatterplots. The other 4 variables showed both constant and non-constant variance in the different rivers. The heteroscedasticity results are summarised as follows:

pH: heteroscedastic in 2 rivers, namely the Middle Mokolo and Sterkstroom. Homoscedastic in the other 4 rivers.
Electrical conductivity: heteroscedastic in the Upper Mokolo and Matlabas. Homoscedastic in the other 4 rivers.
Calcium (dissolved): homoscedastic in all rivers.
Nitrate + nitrite nitrogen (dissolved): homoscedastic in all rivers.
Ammonium nitrogen (dissolved): both tests confirmed homoscedasticity in the Sterkstroom alone, whilst in every other river there were conflicting tests result. In all cases where either the W test or B-P test indicated hetersocedasticity, the other test found the opposite to be true.
Phosphate phosphorus (dissolved): homoscedastic in all rivers.
Sulphate (dissolved): both tests indicate homoscedasticity in most rivers, except for the Matlabas (both tests) and Tambotie (W test) where sulphate was found to be heteroscedastic.

Discussion

Correlation

The relationship between flow volume and rainfall was weaker than expected (A3), as co-variation between these variables is typically strong and positive where rainfall ultimately causes runoff. Inputs or outputs from groundwater (sub-surface base flow) could have affected and skewed the observed relationship. The permeability of the catchment is also a factor where stronger correlation relationships would be expected in less permeable catchments, where more of the water from rainfall does not infiltrate the soils but translates directly into streamflow.

Another possible explanation is that the rainfall data derived from regional weather stations (at different locations to the stream gauging stations) did not translate accurately with the in-stream measured flow volumes. In other words, regional rainfall patchiness is a possible factor which could have confounded the observations in this study where some regions in the catchment may have experienced significantly more rain, on average, than other nearby regions (e.g. see Krajewski et al., 1991, Smakhtin 2001, Baldassarre and Montanari 2009). Although unlikely, stream water abstraction in the past could have led to flow volumes being lower in previous decades due to such abstraction, a pattern that is reflected (A1 and A2). However and with respect to the study site catchments, the extent of human settlements and agricultural activities were relatively low in the past (c. 1982) and both have shown minimal growth since then.

In terms of the relationship between Q and each WQ variable (A5), the mixed correlation results probably owe to the local catchment characteristics. Although insightful, it may be erroneous to use the correlation results to provide evidence to explain reasons for causation in any of the observed relationships. Such evidence is better derived from theoretical knowledge of the catchment processes involved (Helsel and Hirsh 2002). One such process is that of 'dilution', where a solute may be delivered to a stream at a reasonably constant rate, for example from a point source or from groundwater inputs into the stream. If this delivery remains at a constant rate whilst stream discharge increases over time, due to increased rainfall for example, the result is a decrease in solute concentration with increasing Q. There appears some evidence to suggest that this process may have influenced some of the correlation results in this study. At points in time where Q was high (*i.e.* during episodic flood events), negative correlation was identified for many of the WQ variables (A5). In such cases this would have been as a result of dilution (decreasing concentrations with increasing flows). The Sterkstroom, Palala and Matlabas exhibited more significant negative correlation relationships than the other study rivers.

The second major conceptual catchment process driving patterns in constituent concentrations is 'wash-off', where a solute is transported into a stream mainly from overland flow. Overland flow can come from cultivated lands, impermeable surfaces in urban areas or purely from erosion of the stream channel surfaces and associated banks. In reality, a combination of all these examples of overland flow at a catchment scale is likely. In such a scenario, when increased rainfall and flooding results in increasing Q, so increases the rate of solute delivery to the stream. A continuous supply of solute available to increase in parallel with Q gives rise to positive correlation. Nitrate, phosphate and sulphate concentrations in several of the study site streams seem to follow the conceptual scenario of 'wash-off', where concentrations of these ions increased as Q increased.

Nitrate concentration increased with increasing Q in all rivers (A5). Similar (positive) relationships were true for phosphate and sulphate in three of the study rivers, but not the Sterkstroom and Palala.

A possible explanation for the decrease in pH (*i.e.* increasing acidity) with increasing Q in most of the rivers is that there may have been more acidic anions (and not cations) in solution during past flood events. Erosion of the stream bank and channel would theoretically also accelerate at higher flows. If the surrounding landscape (geology and soils) is prone to acid generation as some studies suggest (Vermeulen et al., 2011, ISRIC 2013), this may also have contributed to slightly acidic overland and subsurface flow ending up as stream-flow. Sandstone and quartzites are prominent in regions of the Waterberg, where higher stream-flows would lead to decreasing pH due to the acidic properties of these rock types (Waterberg EMF 2010). Further analysis of the catchments' ANC and the ratio of acidic anions to basic cations would yield more substantive evidence and may be warranted in future studies.

EC also decreases with increasing Q (albeit weakly) and with the exception of the Tambotie, exhibits significant strong positive correlation with pH in the study-site rivers. However, in a scenario where pH may decrease as a result of more anions in solution, EC will not necessarily increase due to these extra acidic anions being in solution. It is worth noting that EC measures a solution's ability to conduct electric current resulting from the concentration of all ions (cations and anions) in solution (Stumm and Morgan 1981). Comparing the relationship between pH and EC (and hence both with Q) is not advisable as there may be cases when significant concentrations of non-pH-determining ions also contribute to EC, which would render its observed relationships with pH (and with respect to Q) less relevant (Stumm and Morgan 1981).

A number of other variables not included in this study may have provided a more detailed explanation about EC. Stream cations not analysed in this study include magnesium, potassium, sodium and the amphoteric species aluminium. However the strong positive correlation between EC and Ca^{2+} provides some indication that as a surrogate to other stream cations, levels of calcium in solution can to some extent also explain the relationship of other cations with respect to EC.

Normality

For the majority of all normality tests, the data were predominantly non-normal (A6). Non-parametric tests were therefore selected for subsequent trend testing. And as with most time-series hydrological data, outliers in the data often results in the distributions exhibiting positive skewness

(Appendix B). For this reason the mean is generally not a resistant and robust statistical measure of location (Helsel and Hirsh 2002, Malan et al., 2003). Median values were calculated and used in all subsequent trend analyses in Chapter two.

Autocorrelation

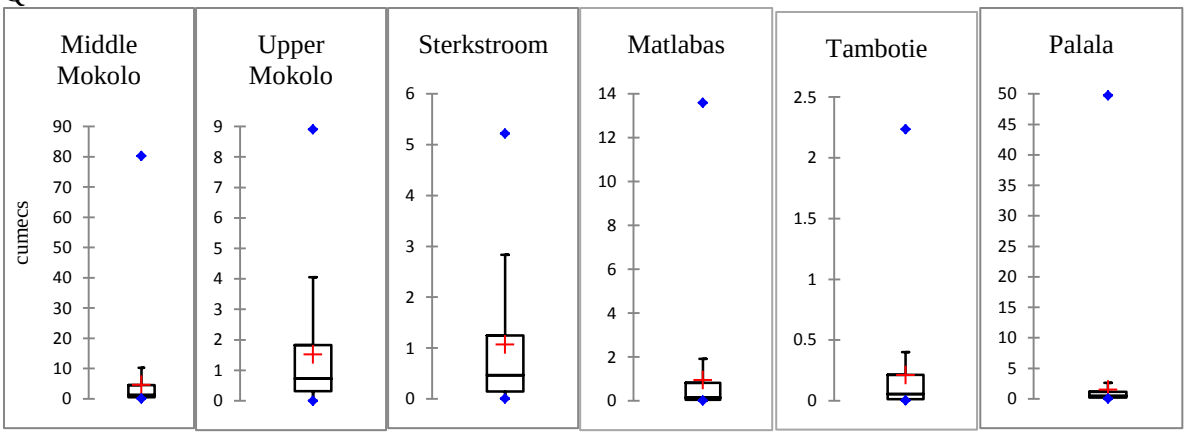
In theory, there are several options to correct for AR [1] from the data prior to trend analysis. The most suitable option in this case was to group the raw data into monthly time periods and calculate median values. There are more advanced estimation methods which can be employed such as transfer function models after Box and Jenkins (1976) or autoregressive moving average (ARMA) models, however, these approaches are based on parametric regression requiring minimal gaps in the record (Helsel and Hirsh 2002). The data record gaps as well as the nature of the data used in this study compromised the use of such methods. The trend tests that were applied were able to further account for and minimise the effects of autocorrelation in the time series.

Heteroscedasticity

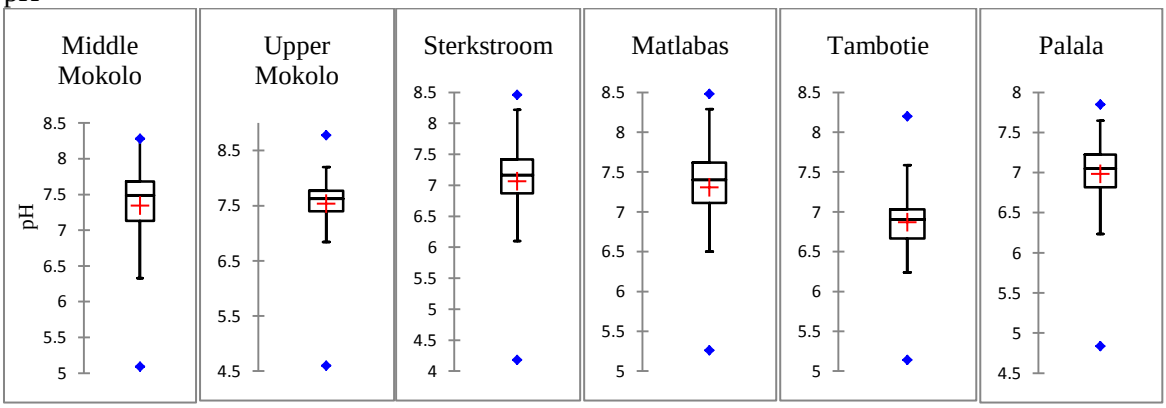
Given the mixed results in the various 'ladder of powers' regression scenarios that I carried out for the data, the median WQ variables displayed both heteroscedasticity and homoscedasticity with ln discharge (Appendix D). The B-P and W tests formed part of the overall EDA phase and were carried out as a formality in conjunction with the normality tests. By exploring and testing the data in different formats and searching for normality throughout, the intention was to demonstrate that a logical, systematic and empirical process was followed in determining the fundamental characteristics of the data. If predominantly parametric and homoscedastic characteristics were possible to generate through correct data manipulation methods, this would have justified the application of parametric tests in the latter stages of the trend analyses. As such, this was not the case. Chapter two explains in more detail which non-parametric techniques were applied.

Appendix B: Summary statistics represented graphically using boxplots, created using XLSTAT-Pro, version 2014.5.

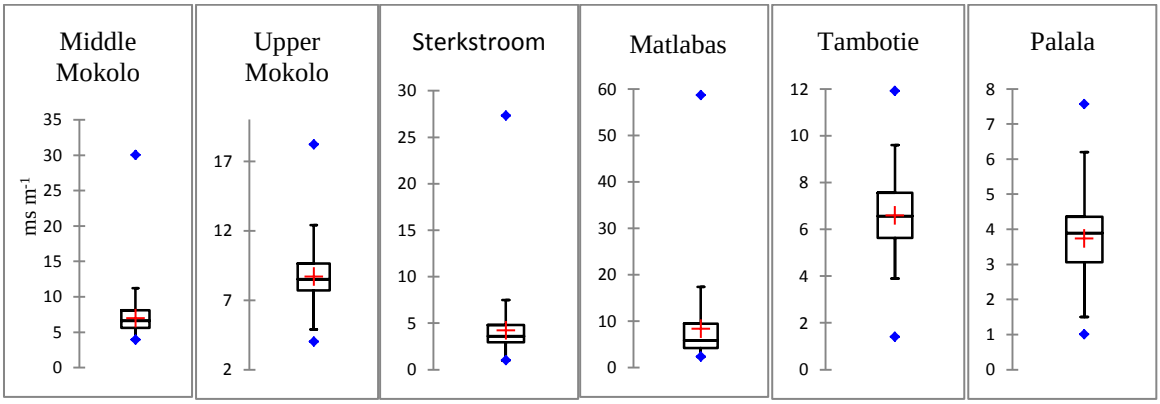
Q



pH

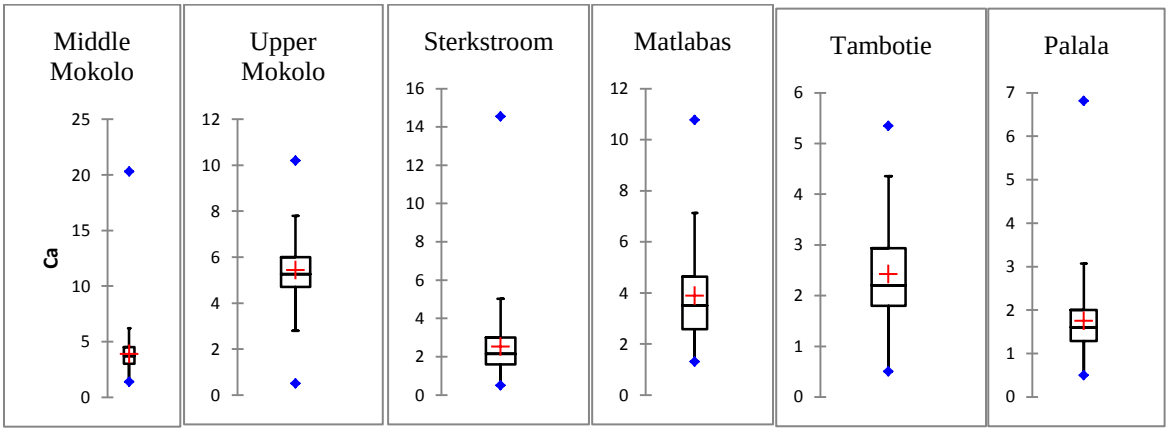


EC

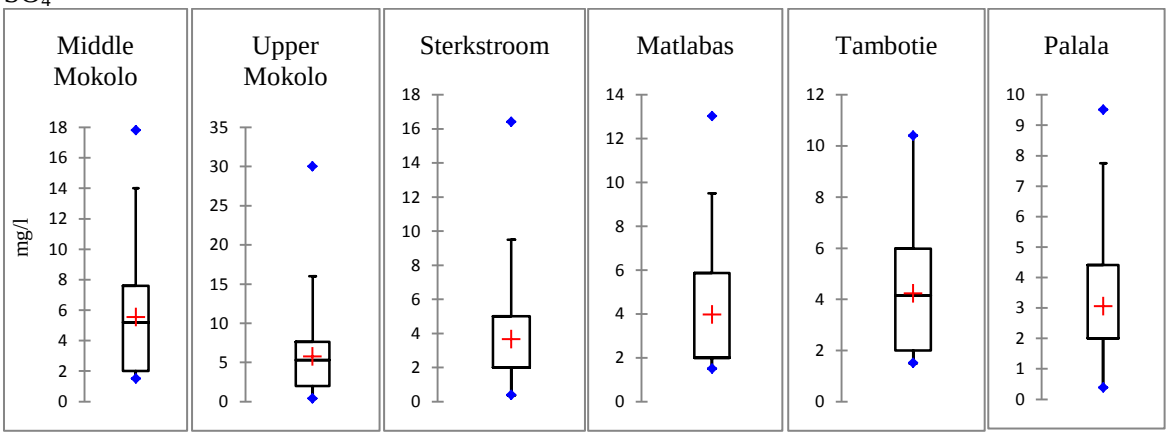


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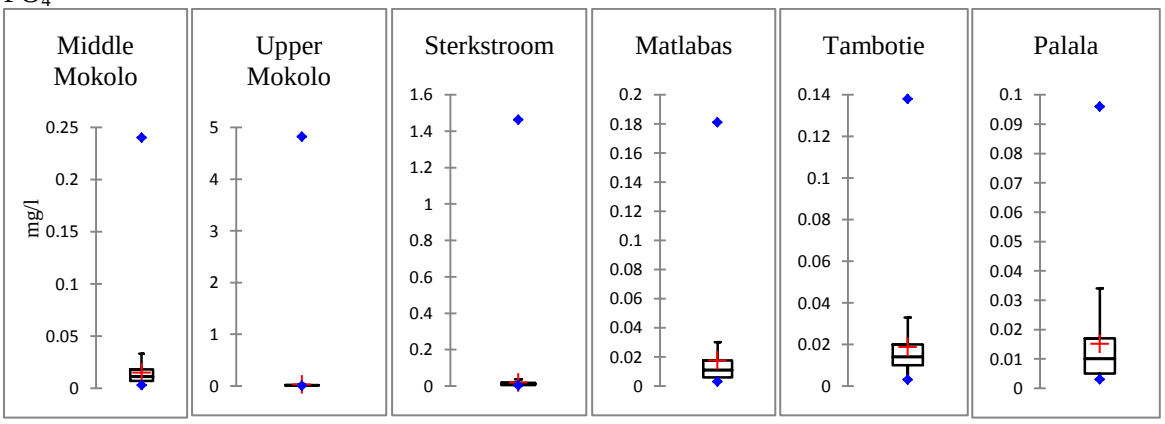
Ca^{2+}



SO_4^{2-}

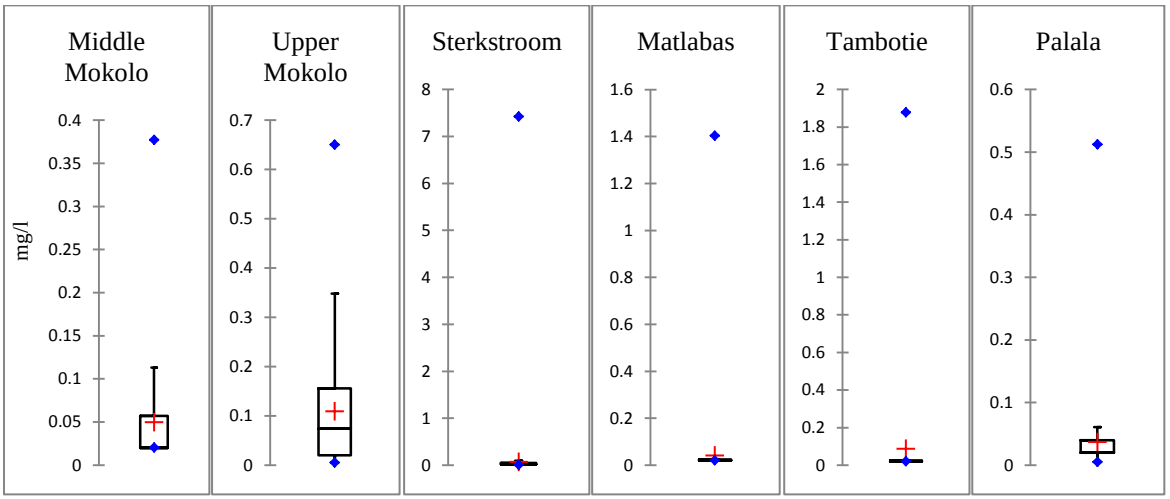


PO_4^{2-}

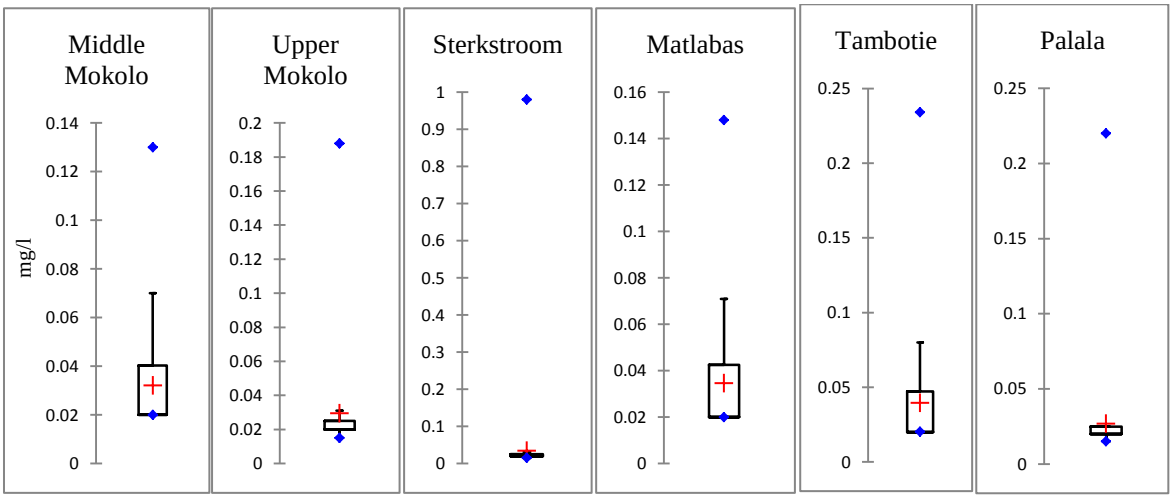


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$NO_3^- + NO_2^-$


Middle Mokolo
Upper Mokolo
Sterkstroom
Matlabas
Tambotie
Palala
mg/l

NH_4^+


Middle Mokolo
Upper Mokolo
Sterkstroom
Matlabas
Tambotie
Palala
mg/l

Appendix C: Durbin-Watson test statistic.

$$d = \sum_{i=2}^{n} [e_i - e(_i - 1)]^2 \; / \; \sum_{i=1}^{n} e_i^2$$

Where: n = number of observations
e_i = estimated residuals for the i^{th} observation
d = Durbin-Watson statistic

Appendix D: First-order autocorrelation and heteroscedasticity test results on residuals after linear regression. D-W p-values shown in bold indicate AR[1]. W and B-P p-values shown in bold indicate heteroscedasticity (W-test p-values are shown in parentheses).

pH

	River	Lower Mokolo	Upper Mokolo	Sterkstroom	Matlabas	Tambotie	Palala
Summary statistics	Obs (n)	210	100	465	138	59	125
	Minimum	-2.258	-2.556	-2.724	-1.819	-1.459	-2.147
	Maximum	0.934	1.557	1.128	1.227	1.266	0.858
	Mean	$1.4x10^{-16}$	$-1.8x10^{-15}$	$-8.2x10^{-16}$	$5.1x10^{-16}$	$8x10^{-16}$	$-2.73x10^{-15}$
	SD	0.501	0.602	0.573	0.521	0.416	0.416
Heteroscedasticity (Breusch-Pagan and White test)	LM (Observed value)	11.5 (11.5)	1.209 (2.38)	17.261 (20.181)	1.5 (1.569)	2.412 (4.132)	4.011 (4.011)
	LM (Critical value)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)
	DF	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)
	p-value	**0.001 (0.003)**	0.272 (0.304)	**<0.0001 (<0.0001)**	0.221 (0.456)	0.12 (0.127)	0.045 (0.135)
Autocorrelation (Durbin Watson)	U	0.461	0.837	0.564	0.747	1.421	1.478
	p-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.008**	**0.002**

EC

	River	Lower Mokolo	Upper Mokolo	Sterkstroom	Matlabas	Tambotie	Palala
Summary statistics	Obs (n)	241	144	514	151	70	150
	Minimum	-3.198	-4.453	-3.557	-11.803	-4.262	3.699
	Maximum	23.283	9.807	23.866	46.49	5.288	3.726
	Mean	$-1.65x10^{-15}$	$4x10^{-16}$	$-7.1x10^{-16}$	$-2.37x10^{-15}$	$7x10^{-16}$	3.713
	SD	2.271	2.2	1.924	6.418	1.657	0.005
Heteroscedasticity (Breusch-Pagan and White test)	LM (Observed value)	2.277 (4.887)	9.54 (11.146)	0.479 (0.498)	6.327 (7.657)	1.526 (1.682)	2.343 (3.854)
	LM (Critical value)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)
	DF	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)
	p-value	0.131 (0.087)	**0.002 (0.004)**	0.489 (0.78)	**0.012 (0.022)**	0.217 (0.431)	0.126 (0.146)
Autocorrelation (Durbin Watson)	U	1.363	1.026	1.386	0.995	1.06	0.000001
	p-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

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Ca^{2+}

	River	Lower Mokolo	Upper Mokolo	Sterkstroom	Matlabas	Tambotie	Palala
Summary statistics	Obs (n)	210	98	455	138	58	124
	Minimum	-2.542	-5.82	-2.697	-2.282	-1.942	-1.325
	Maximum	16.259	3.95	12.534	5.874	2.949	5.039
	Mean	$4.29x10^{-15}$	$4.7x10^{-15}$	$-5.9x10^{-16}$	$-7.1x10^{-16}$	$2.9x10^{-16}$	$-2.52x10^{-16}$
	SD	1.615	1.4	1.402	1.552	0.915	0.946
Heteroscedasticity (Breusch-Pagan and White test)	LM (Observed value)	0.0001 (0.364)	0.011 (1.208)	0.638 (5.871)	0.633 (0.842)	0.565 (0.573)	0.029 (2.698)
	LM (Critical value)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)
	DF	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)
	p-value	0.992 (0.834)	0.916 (0.547)	0.424 (0.053)	0.426 (0.657)	0.452 (0.751)	0.866 (0.26)
Autocorrelation (Durbin Watson)	U	1.576	1.067	1.39	1.284	1.65	1.734
	p-value	**0.001**	**<0.0001**	**<0.0001**	**<0.0001**	0.07	0.071

SO_4^{2-}

	River	Lower Mokolo	Upper Mokolo	Sterkstroom	Matlabas	Tambotie	Palala
Summary statistics	Obs (n)	210	98	455	138	59	124
	Minimum	-5.006	-3.909	-3.279	-2.717	-2.92	-2.801
	Maximum	12.067	24.792	12.155	8.723	6.034	6.049
	Mean	$-4.28x10^{-15}$	$-1x10^{-15}$	$-2.8x10^{-16}$	$1.3x10^{-15}$	$-2x10^{-16}$	$-2.93x10^{-16}$
	SD	3.351	4.363	2.455	2.688	2.541	2.009
Heteroscedasticity (Breusch-Pagan and White test)	LM (Observed value)	1.009 (2.162)	0.118 (0.182)	0.38	5.054 (7.259)	2.172 (7.444)	1.462 (1.819)
	LM (Critical value)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)
	DF	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)
	p-value	0.315 (0.339)	0.731 (0.913)	0.537 (0.702)	**0.025 (0.027)**	0.141 **(0.024)**	0.227 (0.403)
Autocorrelation (Durbin Watson)	U	1.2	1.539	1.366	1.101	1.67	1.137
	p-value	**<0.0001**	**0.011**	**<0.0001**	**<0.0001**	0.08	**<0.0001**

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PO_4^{3-}-P

	River	Lower Mokolo	Upper Mokolo	Sterkstroom	Matlabas	Tambotie	Palala
Summary statistics	Obs (n)	210	98	463	138	59	122
	Minimum	-0.018	-0.012	-0.03	-0.015	-0.017	-0.018
	Maximum	0.224	0.047	1.434	0.164	0.116	0.075
	Mean	$3.6x10^{-18}$	$-3.7x10^{-18}$	$2.7x10^{-18}$	$-4.8x10^{-19}$	$-4x10^{-19}$	$6.9x10^{-18}$
	SD	0.019	0.011	0.068	0.028	0.019	0.017
Heteroscedasticity (Breusch-Pagan and White test)	LM (Observed value)	1.646 (1.91)	1.128 (1.146)	0.018 (0.528)	3.019 (3.04)	0.182 (0.421)	0.046 (3.703)
	LM (Critical value)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)
	DF	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)
	p-value	0.199 (0.385)	0.288 (0.564)	0.894 (0.768)	0.082 (0.219)	0.669 (0.81)	0.831 (0.157)
Autocorrelation (Durbin Watson)	U	1.981	1.817	2.012	1.531	1.804	1.615
	p-value	0.449	0.186	0.555	**0.003**	0.189	**0.017**

$NO_3^-+NO_2^-$-N

	River	Lower Mokolo	Upper Mokolo	Sterkstroom	Matlabas	Tambotie	Palala
Summary statistics	Obs (n)	210	100	463	137	59	123
	Minimum	-0.059	-0.057	-0.092	-0.07	-0.243	-0.044
	Maximum	0.344	0.589	7.321	1.306	1.658	0.459
	Mean	$3.4x10^{-17}$	$-2.5x10^{-17}$	$-1.2x10^{-17}$	$-5.3x10^{-18}$	$-4.8x10^{-17}$	$-1.2x10^{-17}$
	SD	0.052	0.086	0.384	0.117	0.286	0.051
Heteroscedasticity (Breusch-Pagan and White test)	LM (Observed value)	2.561 (2.615)	0.005 (0.666)	0.066 (0.537)	0.347 (0.594)	1.263 (1.319)	1.624 (1.76)
	LM (Critical value)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)
	DF	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)
	p-value	0.11 (0.271)	0.946 (0.717)	0.797 (0.764)	0.556 (0.743)	0.261 (0.517)	0.203 (0.415)
Autocorrelation (Durbin Watson)	U	1.376	1.611	2.013	2.018	2.011	2.021
	p-value	**<0.0001**	**0.025**	0.558	0.551	0.467	0.555

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NH_4^+-N

	River	Lower Mokolo	Upper Mokolo	Sterkstroom	Matlabas	Tambotie	Palala
Summary statistics	Obs (n)	210	98	463	138	59	122
	Minimum	-0.021	-0.02	-0.042	-0.018	-0.024	-0.012
	Maximum	0.096	0.093	0.934	0.113	0.193	0.193
	Mean	$-4.69x10^{-18}$	$1.43x10^{-17}$	$1.7x10^{-17}$	$5.48x10^{-18}$	$1.42x10^{-17}$	$1.02x10^{-17}$
	SD	0.023	0.024	0.065	0.025	0.042	0.021
Heteroscedasticity (Breusch-Pagan and White test)	LM (Observed value)	4.631 (5.566)	0.095 (9.501)	0.766 (0.766)	0.002 (14.368)	4.409 (5.441)	5.116 (9.512)
	LM (Critical value)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)	3.84 (5.99)
	DF	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)
	p-value	**0.031** (0.062)	0.758 **(0.009)**	0.382 (0.682)	0.966 **(0.001)**	**0.036** (0.066)	0.024 **(0.009)**
Autocorrelation (Durbin Watson)	U	1.495	1.307	1.928	1.666	1.945	1.776
	p-value	**0.0001**	**0.0002**	0.22	**0.025**	0.369	0.111

Appendix E: M-K trend test and Seasonal M-K test results for monthly medians (with the exception of rainfall where the monthly totals were used). Wherever significant non-null trends were identified in the median series, the p-values are shown in bold. If either increasing or decreasing trends were identified, symbols of up (↑) or down (↓) arrows are shown respectively next to the p-values.

Q

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13
	Obs (n)	72	235	72	212	72	236	72	234	72	235	72	233
	Minimum	0	0	0	0	0	0	0	0	0	0	0	0
	Maximum	11.686	133.701	4.704	8.475	4.032	5.228	4.032	18.519	0.475	65.586	6.235	32.562
	Mean	0.79	8.141	0.345	1.575	0.397	1.347	0.188	1.168	0.011	1.145	0.639	2.156
	SD	1.991	16.662	0.713	2.038	0.680	1.465	0.595	2.811	0.059	7.245	1.024	3.81
Mann-Kendall trend test	Kendall's tau	-0.095	0.066	-0.139	-0.053	0.088	0.037	-0.021	0.107	-0.241	0.099	-0.229	0.141
	S_1	-221	1796	-350	-1172	225	1029	-41	2873	-328	2592	-586	3817
	Variance	39174.33	1444781.33	42047.33	1065008	42315	1469677	30425	1426858.3	16492.7	1403316	42311.3	1414431
	p-value	0.266	0.135	0.089	0.257	0.276	0.396	0.819	**0.016**↑	**0.011**↓	**0.029**↑	**0.004**↓	**0.001**↑
	Sen's slope	0	0.0015	-0.0008	-0.0006	0.001	0.0006	0	0.00034	0	0	-0.005	0.003
Seasonal Mann-Kendall test	Kendall's tau	-0.088	0.069	-0.138	-0.051	0.094	0.049	-0.022	0.112	-0.240	0.100	-0.229	0.148
	S_2	-101	922	-172	-562	119	671	-15	1505	-162	1292	-289	1972
	p-value	0.379	0.198	0.156	0.361	0.321	0.350	0.889	**0.018**↑	**0.016**↓	**0.033**↑	**0.009**↓	**0.003**↑

Rainfall

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13
	Obs (n)	72	235	72	236	72	234	72	234	72	234	72	234
	Minimum	0	0	0	0	0	0	0	0	0	0	0	0
	Maximum	172	239.1	208	309.5	208	309.5	128	227.5	160	266	166.5	344.5
	Mean	37.715	39.777	50.831	47.476	50.831	47.476	33.93	31.831	29.342	38.297	44.351	39.012
	SD	40.914	52.425	51.288	67.014	51.288	67.014	37.748	45.218	37.952	50.291	45.093	61.039
Mann-Kendall trend test	Kendall's tau	0.064	-0.044	0.057	-0.035	0.057	-0.035	0.1	-0.064	-0.019	-0.039	0.013	-0.011
	S_1	159	-1091	144	-750	144	-750	241	-1538	-47	-974	32	-264
	Variance	93946	2758684.2	96941.2	2264755	96941.2	2264755	104330	3146172	87378.5	2843094	87440.3	3248759
	p-value	0.606	0.512	0.646	0.619	0.646	0.619	0.457	0.389	0.876	0.564	0.917	0.884
	Sen's slope	0.011	0	0.065	0	0.065	0	0	0	0	0	0	0
Seasonal Mann-Kendall test	Kendall's tau	0.068	-0.043	0.071	-0.028	0.071	-0.005	0.102	-0.059	-0.006	-0.039	0.007	-0.013
	S_2	83	-533	88	-294	88	-60	121	-711	-7	-490	7	-160
	p-value	0.467	0.408	0.434	0.616	0.434	0.928	0.279	0.267	0.958	0.452	0.957	0.803

Continues overleaf

pH

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	W82-D87	W94-D13	1982 only	94-13
	Obs (n)	20	62	29	146	36	169	9	97	6	17	10	104
	Minimum	6.22	7.135	4.6	6.417	4.5	5.078	5.75	6.343	5.14	0.354	6	4.835
	Maximum	7.17	7.976	7.26	8.671	6.91	7.966	7.2	8.251	6.6	7.584	6.8	7.848
	Mean	6.759	7.569	6.808	7.605	6.184	7.184	6.434	7.458	6.135	6.561	6.309	7.022
	SD	0.298	0.204	0.527	0.263	0.579	0.407	0.510	0.369	0.52	1.663	0.216	0.404
Mann-Kendall trend test	Kendall's tau	-0.423	0.313	-0.388	-0.107	-0.441	-0.045	-0.423	0.014	-0.276	-0.176	0.322	-0.15
	S_1	-88	590	-177	-1131	-274	-642	-15	65	-4	-24	14	-805
	Variance	2257	27096.667	7261	349265	11865.2	541005.7	91	102941.3	27.33	0	121.3	126751
	p-value	**0.034**↓	**0.0007**↑	**0.019**↓	0.056	**0.006**↓	0.383	0.142	0.842	0.566	0.349	0.238	**0.024**
	Sen's slope	-0.028	0.005	-0.021	-0.00079	-0.026	-0.0005	-0.08	0.0002	-0.113	-0.019	0.022	-0.002
Seasonal Mann-Kendall test	Kendall's tau	-0.324	0.336	-0.406	-0.116	-0.440	-0.045	-0.441	0.004	-0.092	-0.214	0.253	-0.152
	S_2	-29	312	-84	-607	-133	-313	-5	9	-1	-12	5	-404
	p-value	0.132	**0.0005**↑	**0.004**↓	**0.03**↓	**0.001**↓	0.433	0.285	0.482	1	0.310	0.439	**0.016**↓

EC

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	1982 only	94-13
	Obs (n)	20	62	29	145	36	167	15	96	6	17	13	102
	Minimum	4.5	4.25	4.3	6.6	2	2.222	2.6	2.3	4.45	0.354	1	2.86
	Maximum	10.9	11.2	12	18.2	7.5	17.9	6.6	49	11.9	9.43	3	7.56
	Mean	6.475	7.551	7.659	9.144	4.481	4.262	4.593	8.687	7.175	6.584	1.942	4.297
	SD	1.789	1.718	1.922	1.662	1.473	2.12	1.149	7.367	2.647	1.974	0.498	0.908
Mann-Kendall trend test	Kendall's tau	0.259	0.376	0.174	0.089	0.290	0.131	0.295	0.117	0.2	-0.059	0.232	0.177
	S_1	49	708	70	928	411	1809	31	531	3	-8	17	910
	Variance	949	27088.667	2834	342132.7	45016.5	521981.7	91	99804.7	27.33	0	256.3	304343
	p-value	0.119	**0.002**↑	0.195	0.113	**0.027**↑	**0.012**	0.139	0.093	0.719	0.766	0.318	**0.009**↑
	Sen's slope	0.08	0.057	0.061	0.004	0.044	0.005	0.114	0.02	0.47	-0.031	0.022	0.0497
Seasonal Mann-Kendall test	Kendall's tau	0.2	0.385	0.181	0.11	0.286	0.121	0.333	0.122	0.533	-0.107	0.635	0.183
	S_2	18	357	68	578	199	824	14	274	16	-6	17	464
	p-value	0.321	**<0.0001**↑	0.163	**0.045**↑	**0.006**↑	**0.02**↑	0.165	0.06	0.07	0.679	**0.019**↑	**0.007**↑

Continues overleaf

Ca^{2+}

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	1982 only	94-13
	Obs (n)	20	62	29	146	36	166	9	97	6	17	9	104
	Minimum	2.1	2.15	0.5	2.849	0.5	0.5	1.6	1.371	1	0.354	0.5	0.5
	Maximum	5.2	7.263	8.4	10.2	4.4	9.953	18.234	10.5	3.5	4.358	4.1	6.805
	Mean	3.405	4.108	4.697	5.572	2.214	2.643	5.408	3.951	2.2	2.356	1.1	1.837
	SD	0.843	1.003	1.414	1.151	1.035	1.682	5.111	1.787	0.974	0.978	1.273	0.958
Mann-Kendall trend test	Kendall's tau	0	0.275	-0.202	0.00019	0.058	0.009	0.254	-0.068	0.2	0.147	0.129	-0.105
	S_1	0	534	-81	2	36	119	9	-316	3	20	3	-557
	Variance	946	27079.33	2830.33	349210.3	5374.7	512737.7	91	102934	27.33	588.3	47.667	126617.7
	p-value	1	**0.019**↑	0.133	0.999	0.633	0.869	0.402	0.326	0.719	0.440	0.772	0.118
	Sen's slope	0	0.019	-0.038	0	0.011	0.0001	0.2	-0.006	0.175	0.028	0	-0.003
Seasonal Mann-Kendall test	Kendall's tau	-0.012	0.293	-0.216	0.016	0.072	0.005	0.5	-0.091	0.333	0.036	0.236	-0.105
	S_2	-1	271	-39	83	22	37	6	-206	2	2	2	-277
	p-value	1	**0.002**↑	0.195	0.803	0.590	0.924	0.230	0.881	0.763	0.924	0.398	0.133

$NO_3^-+NO_2^-$-N

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	1982 only	94-13
	Obs (n)	20	62	29	143	36	167	8	96	6	17	10	102
	Minimum	0.02	0.02	0.02	0.005	0.02	0.005	0.02	0.02	0.02	0.02	0.02	0.005
	Maximum	0.08	0.29	0.65	0.451	7.42	3.399	0.1	1.402	0.1	0.354	0.11	0.512
	Mean	0.029	0.06	0.07	0.123	0.256	0.067	0.033	0.046	0.045	0.05	0.029	0.037
	SD	0.017	0.066	0.127	0.1	1.231	0.268	0.028	0.141	0.039	0.082	0.028	0.055
Mann-Kendall trend test	Kendall's tau	0.364	0.24	0.046	0.104	0.31	0.105	0.157	0.328	0.086	0.319	0.348	0.1
	S_1	46	393	16	1049	154	1342	3	1268	1	37	7	460
	Variance	540.667	23633	2428	327286.3	4122.7	497587.3	37	200983.7	19.667	496.3	33	111276.7
	p-value	0.053	**0.011**	0.761	0.067	**0.017**	0.057	0.742	**0.002**↑	1	0.106	0.296	0.169
	Sen's slope	0	0	0	0.00027	0	0	0	0	0	0.00008	0	0
Seasonal Mann-Kendall test	Kendall's tau	0.257	0.220	-0.049	0.115	0.154	0.096	0.236	0.331	0	0.258	n/a	0.096
	S_2	15	177	-8	581	-43	604	2	633	0	11	n/a	220
	p-value	0.167	**0.019**↑	0.782	0.074	0.234	0.116	0.729	**<0.0001**↑	1	0.341	n/a	0.236

Continues overleaf

NH_4^+-N

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	1982 only	94-13
	Obs (n)	20	62	29	144	36	167	10	97	6	17	9	102
	Minimum	0.02	0.02	0.02	0.015	0.02	0.015	0.02	2	0.02	0.02	0.02	0.015
	Maximum	0.11	0.113	0.09	0.174	0.98	0.155	48.469	123.771	0.08	0.354	0.04	0.22
	Mean	0.043	0.026	0.04	0.027	0.074	0.028	4.885	24.424	0.053	0.054	0.022	0.028
	SD	0.029	0.016	0.023	0.019	0.158	0.02	15.314	18.01	0.022	0.088	0.007	0.023
Mann-Kendall trend test	Kendall's tau	0.429	0.337	0.52	0.405	0.469	0.265	0.298	0.439	0.067	0.421	0.471	0.473
	S_1	70	439	182	3054	271	2886	12	1551	1	276	8	1883
	Variance	819.333	17203	5386.8	1235733.5	11809	1791510	112.7	254265	19.667	423.3	26.667	293499
	p-value	**0.016**	**0.001**	**0.007↑**	**0.003↑**	**0.006↑**	**0.016↑**	0.3	**0.001↑**	1	**0.026↑**	0.175	**0.0003↑**
	Sen's slope	0.002	0	0.002	0	0.001	0	0.003	0	0.003	0	0	0
Seasonal Mann-Kendall test	Kendall's tau	0.396	0.341	0.614	0.398	0.477	0.264	0.416	0.437	0	0.503	n/a	0.483
	S_2	31	220	98	1474	135	1407	7	742	0	21	n/a	947
	p-value	0.062	**0.001↑**	**0.0003↑**	**<0.0001↑**	**0.0003↑**	**<0.0001↑**	0.341	**<0.0001↑**	1	**0.02↑**	n/a	**<0.0001↑**

SO_4^{2-}

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	1982 only	94-13
	Obs (n)	20	62	29	146	36	166	10	97	6	17	9	104
	Minimum	2	1.5	2	0.375	2	0.375	2	1.5	2	0.354	2	0.375
	Maximum	12.2	16.7	16.9	15.618	12.2	10.664	36.375	12.6	8.5	8	6.5	9.144
	Mean	4.36	5.583	5.281	5.304	4.608	3.046	7.538	3.988	6.05	3.305	3.433	3.01
	SD	2.963	3.085	4.266	3.089	2.965	1.778	10.392	2.717	2.552	2.198	1.812	2.064
Mann-Kendall trend test	Kendall's tau	0.066	-0.366	-0.019	-0.333	-0.162	-0.208	0.024	-0.564	0.2	-0.363	0	-0.405
	S_1	11	-702	-7	-3521	-93	-2546	1	-2440	3	-46	0	-1966
	Variance	825	68440	2572.33	1098300	4979.7	1746801	116.3	227288	27.33	557.3	75.33	489797
	p-value	0.728	**0.004↓**	0.906	**0.0004↓**	0.192	**0.027↓**	1	**<0.0001↓**	0.719	0.057	1	**0.002↓**
	Sen's slope	0	-0.077	0	-0.031	0	0	0	-0.051	0.4	-0.077	0	-0.009
Seasonal Mann-Kendall test	Kendall's tau	0.005	-0.376	-0.046	-0.331	-0.154	-0.207	0.160	-0.562	0.333	-0.313	-0.392	-0.406
	S_2	1	-343	-8	-1716	-43	-1262	3	-1184	2	-16	-4	-978
	p-value	1	**0.0002↓**	0.767	**<0.0001↓**	0.234	**0.0005↓**	0.75	**<0.0001↓**	0.674	0.217	0.326	**<0.0001↓**

Continues overleaf

PO_4^{3-}-P

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	1982 only	94-13
	Obs (n)	20	62	29	144	36	167	9	97	6	17	9	102
	Minimum	0.003	0.005	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.005	0.003	0.003
	Maximum	0.033	0.24	0.051	4.815	1.463	0.076	42.422	0.181	0.029	0.354	0.032	0.096
	Mean	0.008	0.019	0.012	0.051	0.051	0.015	4.723	0.02	0.016	0.037	0.009	0.016
	SD	0.007	0.031	0.011	0.4	0.242	0.012	14.173	0.033	0.009	0.083	0.011	0.018
Mann-Kendall trend test	Kendall's tau	0.236	-0.272	0.091	-0.066	-0.140	-0.097	0.057	-0.193	0.467	-0.194	-0.109	-0.260
	S_1	41	-521	36	-665	-82	-1315	2	-867	7	-26	-3	-1286
	Variance	881.667	62650	2810	334178.3	5114.7	519373.7	90	232853	27.33	585.3	63.667	288022
	p-value	0.178	**0.019**↓	0.509	0.251	0.257	0.068	0.916	**0.036**↓	0.272	0.301	0.802	**0.008**↓
	Sen's slope	0.00015	-0.00013	0.00007	-0.00002	0	-0.00002	0.0002	-0.00006	0.002	-0.0003	0	-0.00005
Seasonal Mann-Kendall test	Kendall's tau	0.209	-0.276	0.124	-0.079	-0.130	-0.09	0.333	-0.183	0.667	-0.4	-0.471	-0.270
	S_2	17	-252	22	-398	-38	-601	4	-399	4	-22	-4	-662
	p-value	0.255	**0.0003**↓	0.411	0.201	0.296	0.121	0.399	**0.01**↓	0.317	0.073	0.299	**0.0003**↓

Appendix F: M-K trend test and Seasonal M-K test results for monthly and seasonal residuals, after LOWESS.

pH residuals

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	W82-D87	W94-D13	1982 only	94-13
	Obs (n)	20	62	29	146	36	169	9	97	6	16	10	104
	Minimum	-0.72	-0.506	-2.806	-0.943	-1.990	-1.842	-0.549	-0.888	Insufficient data for LOWESS and trend analysis on residuals	-1.610	-1.530	-2.257
	Maximum	0.608	0.571	0.755	0.409	0.847	0.916	0.624	0.675		0.932	0.767	0.865
	Mean	-0.008	-0.0005	-0.080	-0.001	0.010	0.002	0.113	-0.009		-0.016	-0.197	0.006
	SD	0.336	0.216	0.632	0.226	0.562	0.355	0.408	0.301		0.585	0.767	0.419
Mann-Kendall trend test	Kendall's tau	0.343	0.324	-0.399	-0.177	-0.429	-0.102	-0.343	0.038		-0.05	0.222	-0.103
	S_1	-72	413	-162	-1417	-270	-1325	-72	171		-6	8	-432
	Variance	0	15158.3	5903	230249	11952.7	476721	2383.4	96737.7		1004.8	0	87906
	p-value	**0.031**	**0.012**↑	**0.018**↓	**0.047**↓	**0.007**↓	0.055	0.275	0.585		0.875	0.477	0.146
	Sen's slope	-0.02	-0.02	-0.024	-0.001	-0.028	-0.0008	-0.092	0.0006		-0.007	0.04	-0.002
Seasonal Mann-Kendall test	Kendall's tau	-0.311	0.327	-0.407	-0.196	-0.431	-0.105	-0.667	0.038		-0.071	0	-0.104
	S_2	-28	196	-74	-766	-132	-678	-8	82		-4	0	-216
	p-value	0.104	0.998	**0.004**↓	**0.002**↓	**0.0006** ↓	**0.034** ↓	0.124	0.638		0.791	1	0.152

EC residuals

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	W82-D87	W94-D13	1982 only	94-13
	Obs (n)	20	62	29	145	36	167	15	96	6	16	13	102
	Minimum	-2.821	-3.841	-3.657	-2.426	-2.347	-2.673	-1.748	-17.456	Insufficient data for LOWESS and trend analysis on residuals	-1.610	-2.452	-1.409
	Maximum	4.259	3.224	3.767	5.819	3.074	11.717	11.172	34.081		0.932	2.233	3.516
	Mean	0.075	-0.029	-0.041	-0.019	-0.001	-0.014	1.321	-0.226		-0.017	-0.019	0.009
	SD	2.035	1.503	2.061	1.377	1.497	1.816	4.156	6.288		1.152	1.412	0.979
Mann-Kendall trend test	Kendall's tau	0.248	-0.032	0.163	0.118	0.397	0.278	0.214	0.151		0	0	0.247
	S_1	52	-41	66	944	250	3628	6	673		0	0	1036
	Variance	2342	31413.4	6177.2	230248	12904.5	476720	2342	96739.7		1121.9	194.3	220029
	p-value	0.126	0.745	0.225	**0.049**	**0.014**↑	**0.0002**↑	0.548	**0.031**		1	0.5	**0.014**↑
	Sen's slope	0.123	-0.00001	0.061	0.006	0.09	0.008	0.327	0.028		-0.0005	0.049	0.011
Seasonal Mann-Kendall test	Kendall's tau	0.222	0.222	0.143	0.101	0.379	0.275	0.167	0.152		0.036	-0.5	0.248
	S_2	20	20	26	396	116	1779	2	328		2	-6	514
	p-value	0.259	0.130	0.372	0.133	**0.002** ↑	**< 0.0001**↑	0.803	**0.027**↑		0.925	0.307	**0.001**↑

Continues overleaf

Ca^{2+} residuals

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	W82-D87	W94-D13	82-87	94-13
	Obs (n)	20	62	29	146	36	166	9	97	6	16	9	104
	Minimum	-1.683	-2.065	-6.399	-2.188	-1.871	-3.692	-1.366	-3.857	Insufficient data for LOWESS and trend analysis on residuals	-1.677	-6.574	-1.425
	Maximum	1.933	3.614	3.418	4.664	1.596	6.153	2.522	5.260		1.802	2.479	5.255
	Mean	0.004	-0.012	-0.141	-0.010	-0.007	-0.013	0.073	-0.038		-0.046	-0.301	-0.001
	SD	0.941	1.074	1.789	1.086	0.818	1.393	1.371	1.549		0.816	2.577	0.969
Mann-Kendall trend test	Kendall's tau	-0.067	0.384	-0.158	0.075	0.190	0.131	-0.067	-0.066		0.317	-0.065	-0.075
	S_1	-14	489	-64	599	120	1711	-14	-293		38	-2	-314
	Variance	2405	15158.3	24	230249	91.3	476721	2404.95	96739.7		1039.3	158.9	87906
	p-value	0.699	**0.003**↑	0.240	0.213	0.106	**0.013**	0.399	0.348		0.251	0.468	0.291
	Sen's slope	-0.016	0.036	-0.049	0.003	0.022	0.004	-0.016	-0.004		0.065	0	-0.002
Seasonal Mann-Kendall test	Kendall's tau	-0.133	0.360	-0.264	0.089	0.190	0.141	0.333	-0.08		0.357	-0.365	-0.088
	S_2	-12	216	-48	348	58	914	4	-172		20	-4	-182
	p-value	0.507	**0.001**↑	0.109	0.184	0.122	**< 0.007**↑	0.509	0.274		0.107	0.448	0.237

SO_4^{2-} residuals

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	W82-D87	W94-D13	82-87	94-13
	Obs (n)	20	62	29	146	36	166	10	97	6	16	9	104
	Minimum	-4.092	-5.136	-6.167	-5.643	-5.565	-3.468	-25.777	-9.485	Insufficient data for LOWESS and trend analysis on residuals	-3.522	-5.305	-3.084
	Maximum	7.911	10.395	12.373	9.650	9.543	7.659	3.000	6.994		5.215	4.500	6.500
	Mean	0.215	0.059	-0.030	-0.023	-0.067	-0.008	-3.440	-0.165		0.042	-0.066	-0.003
	SD	3.242	2.901	4.844	2.950	3.360	1.835	9.194	2.916		2.566	3.374	2.109
Mann-Kendall trend test	Kendall's tau	0.276	-0.087	-0.190	-0.198	-0.083	-0.127	0.276	-0.465		-0.2	0.056	-0.303
	S_1	58	-111	-77	-1582	-52	-1653	58	-2074		-24	2	-1268
	Variance	2330.4	15158.3	2841	627587.6	46	476666	2330	96732		1069	15.7	87904
	p-value	0.086	0.372	0.154	**0.023**↓	0.490	**0.017**	0.803	**<0.0001**↓		0.482	0.919	**0.004**↓
	Sen's slope	0.182	-0.02	-0.111	-0.025	-0.032	-0.003	0.182	-0.059		-0.148	0.136	-0.022
Seasonal Mann-Kendall test	Kendall's tau	0.244	-0.133	-0.275	-0.193	-0.052	-0.125	-0.107	-0.465		-0.179	-0.333	-0.307
	S_2	22	-80	-50	-755	-16	-810	-1	-1004		-10	-4	-636
	p-value	0.215	0.876	0.043	**0.003**↓	0.688	**0.017**↓	1	**<0.0001**↓		0.379	0.556	**<0.0001** ↓

Continues overleaf

PO_4^{3-} residuals

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	W82-D87	W94-D13	82-87	94-13
	Obs (n)	20	62	29	144	36	167	9	97	6	16	9	102
	Minimum	-0.01	-0.031	-0.054	-0.139	-0.336	-0.013	-0.028	-0.034	Insufficient data for LOWESS and trend analysis on residuals	-0.018	-0.014	-0.031
	Maximum	0.027	0.22	0.038	4.798	1.448	0.060	0.015	0.169		0.039	0.075	0.086
	Mean	0.00002	0.0004	-0.001	-0.001	-0.004	0.000	-0.003	0.000		-0.0002	0.010	0.000
	SD	0.008	0.034	0.015	0.433	0.269	0.012	0.013	0.034		0.013	0.027	0.020
Mann-Kendall trend test	Kendall's tau	0.253	0.07	0.02	0.02	-0.025	-0.067	0.253	-0.130		-0.283	-0.243	-0.142
	S_1	53	89	8	158	-16	-871	53	-582		-34	-8	-595
	Variance	1095.7	15158.3	14	230248	46	476719	2504.7	96738.7		1086.3	83.3	87905
	p-value	0.116	0.475	0.897	0.744	0.839	0.208	0.851	0.062		0.317	0.443	**0.045**
	Sen's slope	0.0005	0.00009	0.00003	0.00002	-0.00008	-0.00002	0.0005	-0.00007		-0.0007	-0.002	-0.00006
Seasonal Mann-Kendall test	Kendall's tau	0.378	0.063	0.110	0.0003	-0.026	-0.065	0.5	-0.114		-0.286	-0.548	-0.145
	S_2	34	38	20	1	-8	-419	6	-246		-16	-6	-300
	p-value	**0.017**↑	0.571	0.441	1	0.819	0.269	0.307	0.129		0.243	0.251	**0.032**↓

$NO_3^-+NO_2^-$ residuals

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	W82-D87	W94-D13	82-87	94-13
	Obs (n)	20	62	29	143	36	167	8	96	6	16	10	102
	Minimum	-0.024	-0.07	-0.111	-0.138	-1.673	-0.209	-0.064	-0.272	Insufficient data for LOWESS and trend analysis on residuals	-0.036	-0.072	-0.074
	Maximum	0.057	0.174	0.610	0.311	7.370	3.345	0.173	1.355		0.082	0.090	0.465
	Mean	0.001	-0.002	0.007	0.001	-0.019	-0.003	0.022	-0.001		0.001	0.002	-0.001
	SD	0.022	0.048	0.143	0.100	1.373	0.278	0.074	0.152		0.025	0.041	0.058
Mann-Kendall trend test	Kendall's tau	0.152	0.091	-0.158	0.134	0.267	0.047	-0.182	0.213		0.217	0.043	0.002
	S_1	32	116	-64	1069	168	610	-5	949		26	1	10
	Variance	2349.3	15157.3	2840	230249	5388	476720	64.3	96719.7		1066.7	47.7	87902
	p-value	0.355	0.350	0.237	**0.026**	**0.023**	0.378	0.618	**0.023**↑		0.444	1	0.976
	Sen's slope	0.0007	0.0003	-0.001	0.0005	0.002	0.00006	-0.012	0.00009		0.0006	0	$5.9x10^{-7}$
Seasonal Mann-Kendall test	Kendall's tau	0.156	0.075	-0.226	0.123	0.262	0.044	-0.167	0.216		0.25	n/a	-0.007
	S_2	14	45	-41	480	80	286	-2	466		14	n/a	-14
	p-value	0.368	0.249	0.07	0.077	0.057	0.450	0.810	**0.002**↑		0.282	n/a	0.932

Continues overleaf

NH_4^+ residuals

	River	Middle Mokolo		Upper Mokolo		Sterkstroom		Matlabas		Tambotie		Palala	
Summary statistics	Period	82-87	94-13	82-87	94-13	82-87	94-13	82-87	94-13	W82-D87	W94-D13	82-87	94-13
	Obs (n)	20	62	29	144	36	167	10	97	6	16	9	102
	Minimum	-0.05	-0.014	-0.032	-0.013	-0.232	-0.018	-0.696	-0.022	Insufficient data for LOWESS and trend analysis on residuals	-0.081	-0.016	-0.024
	Maximum	0.088	0.058	0.059	0.148	0.949	0.126	0.065	0.119		0.174	0.020	0.195
	Mean	0.0004	-0.0001	0.000	0.000	-0.002	0.000	-0.084	0.001		-0.003	0.000	0.000
	SD	0.043	0.014	0.025	0.018	0.179	0.021	0.250	0.025		0.055	0.009	0.025
Mann-Kendall trend test	Kendall's tau	0.438	-0.032	0.387	0.173	0.352	0.144	0.438	0.170		0.05	0.043	0.182
	S_1	92	-41	157	1383	222	1875	92	755		6	1	762
	Variance	2297.5	15158.3	2841	230224	91.3	476623	2297.5	96691.7		1058	47.7	212044
	p-value	**0.029**↑	0.745	**0.023**↑	**0.004**	**0.022**↑	**0.007**	**0.029**↑	**0.015**		0.878	1	**0.049**↑
	Sen's slope	0.003	-0.00001	0.002	0.00004	0.002	0.00003	0.003	0.00008		0.0003	0	0.00006
Seasonal Mann-Kendall test	Kendall's tau	0.422	-0.033	0.407	0.166	0.346	0.152	$-9.3x10^{-18}$	0.149		0.143	n/a	0.188
	S_2	38	-20	74	647	106	983	0	320		8	n/a	388
	p-value	**0.014**↑	0.620	**0.007** ↑	**0.006**↑	**0.003** ↑	**0.005**↑	**0.034**↑	**0.029**↑		0.49	n/a	**0.009**↑

Appendix G: NDS bioassay preparation, deployment and retrieval procedure.
(Adapted from Biggs and Kilroy 2000).

1. The NDS trays constituted rigid, heavy-duty PVC boxes comprised of compartments for each of the four nutrient treatments.
2. The plastic jars which fit in the box compartments should be pre-labelled appropriately for each nutrient treatment and arranged using one configuration for all trays.
3. After the nutrient-enriched agar solutions have been prepared and brought to boiling point, they must be allowed to cool sufficiently before being poured into each of the pre-labelled plastic jars.
4. The nutrient-agar solution must be poured to above the lip of each jar to allow for contraction which occurs during cooling. The reason for this is to ensure the filter paper growth surfaces are in contact with the agar, which ensures more effective diffusion during the experiment.
5. Plastic or tinfoil is to be secured over the surfaces of the jars which are refrigerated overnight.
6. Prior to deployment to the field, the refrigerated jars are placed into the boxes and elastic bands are used to secure the filter papers to the nutrient-agar surfaces.
7. Lids with raised edges between each treatment are fitted to each box. These function to prevent cross contamination of the diffusing nutrients from each treatment and to secure the jars inside each box.
8. Each tray unit is deployed to the pre-identified river site and secured to the stream bed using steel pegs that fit through holes on each of the four corners.
9. Rocks and bricks can be also used to provide additional support around the base of the box to prevent movement due to stream currents. This is necessary when the bed substrate is rocky and difficult to penetrate using pegs. If rocks are used, it should be ensured they do not impede or alter the natural stream flow at the surfaces of the trays (*i.e.* over the lids).
10. Depending on climate and stream conditions, trays are left for a period of between 7-14 days prior to retrieval.
11. During retrieval of the NDS trays, the filter papers are removed from the jars, any debris is brushed off the under surfaces, they are folded inwards in half and placed in pre-labelled tinfoil-lined zip-lock plastic bags.
12. Preservation of the samples to prevent degradation of the chlorophyll molecules is achieved by placing them in a coolbox containing dry ice for near instant freezing.
13. One prepared tray prior to deployment is shown (G1 and G2).
14. G3 and G4 respectively show a deployed and retrieved tray.

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G1: 1 x NDS tray showing jars in compartments (lid off).



G2: 1 x NDS tray (lid secured).



G3: NDS tray deployed in stream.



G4: Retrieved NDS tray showing periphyton growth.

Appendix H: Chl-*a* extraction procedure.
(After Sartory and Grobbelaar 1984 and Biggs and Kilroy 2000).

*To be carried out in a dimly lit laboratory.

1. Add 10ml 90% ethanol to 15ml centrifuge tubes.
2. Secure tinfoil around all centrifuge tubes to minimise penetration of light and subsequent break-down of chl-*a* molecules.
3. Cut the pre-frozen filter papers into thin slices before placing into centrifuge tubes.
4. Cap the tubes, shake vigorously to evenly distribute the ethanol and promote disintegration of the filters.
5. Place tubes in appropriate racks and refrigerate for a further 24hrs for extraction process to occur.
6. To improve the extraction, shake the tubes after 1hr and return to refrigerator.
7. After 24hrs, retrieve tubes and gently push the filter papers to the bottom using a glass rod.
8. Replace caps and place tubes in centrifuge for 15 minutes at 4500rpm.

Appendix I: Procedure for chl-*a* analysis via UV detection using spectrophotometry.
(After Sartory and Grobbelaar 1984 and Biggs and Kilroy 2000).

*To be carried out in a dimly lit laboratory.

1. Set the spectrophotometer (UV-1100 Mapada Instruments) wavelengths at the wavelength peak for chl-*a* and at 750nm.
2. Using 1cm path length cuvettes, zero the spectrophotometer with one cuvette (the blank) filled only with 90% ethanol. These blank readings should be repeated before each set of chl-*a* readings are taken.
3. Pipette 4ml from the 15ml centrifuged samples into the cuvettes and take absorbance readings at 665nm and 750nm. The 750 reading is the turbidity correction while the 665 reading is the absorption peak for chl-*a*.
4. Remove the cuvettes, pipette 0.1ml of 0.3M HCl into each, replace caps and gently invert cuvettes to allow acid to distribute throughout. The caps should be held firmly in place to prevent spillage.
5. After a 30-60 second delay to allow the acidification process to be entirely complete, replace the cuvettes and re-read absorbances at 665nm and 750nm.
6. The purpose of the acidification step is to convert the chl-*a* pigments to phaeopigments.
7. The chl-*a* calculation equations include corrections for the presence of turbidity and phaeopigments.
8. The final chl-*a* concentrations, in mg per m^2, are calculated using the formulae below:

Chl-*a* (mg per sample) = (Abs $_{665}$ before - Abs $_{665}$ after)*28.66*sample vol*extractant vol / filtered sub-sample

Phaeopigments (mg per sample) = [(1.72 x Abs $_{665}$ after) - (Abs $_{665}$ before)]*28.66*sample vol*extractant vol / filtered sub-sample

Chl-*a* (mg per m^2) = Chl-*a* (mg per sample) / area of sample (m^2)

Absorption co-efficient for chl-*a*	=	28.66
Extractant volume (ml)	=	10
Acid ratio for chl-*a* in ethanol	=	1.72
Radius of sample [*i.e.* jar radius/ exposed filter paper] (cm)	=	25mm
Area of sample (m^2)	=	1.964×10^{-3} (based on a jar diameter of 50mm)
Turbidity correction (for blanks)	=	Abs $_{750}$ nm
Absorption peak for chl-*a*	=	Abs $_{665}$ nm

Appendix J: Chl-*a* summary statistics for the 4 nutrient treatments in each of the 5 rivers.

River	Matlabas	Tambotie	Sterkstroom	Upper Mokolo	Palala
Treatment	Mean chl-*a* (mg/m^2)				
N	10.69	29.00	8.86	5.67	5.69
P	1.35	11.07	1.19	1.49	1.24
NP	8.65	22.03	3.10	6.94	3.84
C	3.47	25.13	0.57	1.30	0.75
	Standard deviation				
N	2.74	16.79	1.94	1.67	1.27
P	0.38	3.84	0.16	0.28	0.33
NP	1.52	6.32	2.11	0.41	2.24
C	2.36	12.44	0.28	0.26	0.28

Appendix K: Colour differences in the spectrophotometer cuvettes for one sample set of the Mokolo treatment post chl-*a* extraction (N sample not shown).



Appendix L: Average summer inorganic N concentrations (in mg/l). The summer season constitutes the six months from 1st October to 31st March of the following year.

Season	Middle Mokolo	Upper Mokolo	Matlabas	Sterkstroom	Tambotie	Palala
Summer 82′	0.067	0.153	0.040	0.040	0.060	0.040
Summer 83′	-	-	0.150	4.230		0.105
Summer 84′	0.110	0.070	0.085	0.070	0.150	-
Summer 85′	0.055	0.065	0.040	0.070	0.040	0.040
Summer 86′	-	0.130	-	0.083	-	-
Summer 87′	0.060	0.120	0.140	0.077	-	-
Summer 88′	0.104	0.094	0.083	0.114	-	-
Summer 89′	0.078	0.097	0.110	0.085	-	-
Summer 90′	0.105	0.129	0.088	0.244	-	-
Summer 91′	0.071	0.196	0.040	0.096	1.548	-
Summer 92′	-	0.070	-	0.065	-	-
Summer 93′	-	-	0.077	0.040	0.167	-
Summer 94′	0.077	0.072	0.040	0.064	0.664	-
Summer 95′	-	0.130	0.056	0.052	-	-
Summer 96′	0.067	0.086	0.040	0.049	-	-
Summer 97′	0.067	0.136	0.058	0.056	0.090	-
Summer 98′	0.060	0.068	0.062	0.059	-	0.051
Summer 99′	-	0.087	0.066	0.059	0.040	0.050
Summer 00′	0.091	0.118	0.076	0.093	-	0.054
Summer 01′	0.040	0.117	0.040	0.158	0.040	0.040
Summer 02′	-	0.120	0.054	0.065	0.069	0.051
Summer 03′	-	0.060	0.040	0.068	-	0.067
Summer 04′	-	-	-	0.074	-	-
Summer 05′	-	0.143	0.060	0.063	-	-
Summer 06′	-	0.076	1.422	1.256	-	0.114
Summer 07′	-	0.150	-	0.117	-	0.108
Summer 08′	-	0.130	-	0.110	-	0.110
Summer 09′	-	0.099	-	0.094	-	0.537
Summer 10′	-	0.116	0.050	0.073	-	0.030
Summer 11′	-	0.050	0.050	0.060	0.050	0.050
Summer 12′	0.050	0.081	0.050	0.050	0.063	0.050
Summer 13′	0.050	0.112	0.074	0.050	-	0.083
Overall 31-year average	**0.072**	**0.106**	**0.119**	**0.246**	**0.248**	**0.093**