

PROJECT TITLE

**SURFACE WATER AND GROUNDWATER RESOURCES  
MONITORING, CRADLE OF HUMANKIND WORLD  
HERITAGE SITE, GAUTENG PROVINCE,  
SOUTH AFRICA**

REPORT TITLE

**WATER RESOURCES STATUS REPORT FOR THE  
PERIOD APRIL 2023 TO MARCH 2024**

PROJECT No.

**GT/WHS/021/2022**

AUTHORS

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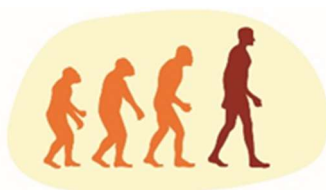
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PREPARED FOR

Management Authority  
Cradle of Humankind World Heritage Site  
Gauteng Department of Economic Development

PREPARED BY

Water Centre  
Smart Places Cluster  
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## SUMMARY

Following the conclusion of the bid GT/WHS/021/2022, the Management Authority (MA) of the Cradle of Humanity World Heritage Site (COHWHS) selected the CSIR as the preferred service provider to carry out the water resources monitoring programme of the site. The programme commenced in 2012 as a follow-up to project BIQ005/2008, which was commissioned to create a water resources monitoring programme for the COHWHS property. Since its commencement, nineteen (19) biannual *status quo* reports have been produced, including those compiled during the 2017 -2020 phase of the monitoring programme under the project GT/GDED/092/2017. This report, which spans the months of April 2023 to March 2024, is the twentieth (20<sup>th</sup>) of its kind. Notably, there exists a 2-year gap in the monitoring programme between the finalization and closure of project GT/GDED/092/2017 in September 2020 and the commencement of the monitoring programme under project GT/WHS/021/2022 in September 2022. Although efforts have been made to 'close the gap' by way of data collection from relevant stakeholders who continued their respective monitoring activities during this period, there remain gaps of permanently missing data which may compromise the holistic understanding of the COHWHS hydro(geo)logical environment and the various linkages between its surface water and groundwater systems.

The assessment of impacts on the water resources environment of the COHWHS property employs a comprehensive approach, referencing the conceptual hydrophysical and hydrochemical model developed in the Situation Assessment Report (Hobbs, 2011) and pays particular attention to those resources that are conceptually understood to be most at risk. In the context of the COHWHS, focus is placed on wastewater sources of which mine water (*aka* acid mine/rock drainage) rising in the Western Basin, and local municipal effluent discharged from Mogale City's Percy Stewart Wastewater Treatment Works, are of primary concern.

The outcome of monitoring activities as documented in this report reveal that the 2024 wet season's notably low rainfall has resulted in decreased discharge volumes compared to 2023, with the overall trend indicating reduced flow rates and potential impacts on water quality. Despite the generally lower salinity and acidity of rainwater, the surface water quality of the Tweelopiespruit and Rietspruit indicate the prevailing impact of acid mine drainage, exhibiting low pH, high salinity, and elevated concentrations of contaminants like manganese and aluminium, while the Blougatspruit and Bloubankspruit show relatively higher alkalinity. It is to be noted however that the values of the salinity and associated contaminants i.e. sulphate, manganese and aluminium, recorded in March 2024 remain considerably lower than the values recorded during the remediation period when AMD intervention measures were commissioned in the region, but suggest possible episodic and small-scale mine water discharge from the Western Basin. With regards to the microbiological health of the surface water resources, faecal contamination is widespread, with severe pollution noted in the Blougatspruit and Bloubankspruit systems. The deterioration of the surface water quality is further confirmed by the historical results of river health assessments conducted at various monitoring points. Groundwater resources remain largely at low risk of bacteriological pollution, with notable exceptions for the Sterkfontein, Zwartkrans and Kromdraai systems.

The summary of the current *status quo* of the water resources environment of the COHWHS is as follows:

- The 2024 wet season rainfall of 467 mm at station HDS is among the lowest in 16 years and 55% less than 2023, placing it in the 7<sup>th</sup> percentile of historical data, while station SC's 537 mm falls in the 42<sup>nd</sup> percentile, indicating a moderately drier period.
- The rainwater chemistry continues to confirm the low salinity and generally acidic nature of rainwater in the region.

- The wet season rainfall for the 2024 hydrological year has resulted in a discharge volume of 12.1 Mm<sup>3</sup> from October 2023 to December 2023. In comparison, the wet season rainfall for the 2023 hydrological year produced a total volume of 44.0 Mm<sup>3</sup>, with 12.8 Mm<sup>3</sup> recorded for the same months.
- The highest average monthly instantaneous discharge at station A2H049 was 4.9 m<sup>3</sup>/s in January 2023, while the flow rate between October 2023 and March 2024 averaged 1.5 m<sup>3</sup>/s with missing data for early 2024, but the overall trend over 52 years indicates an upward trajectory, potentially due to increased rainfall, hydrological changes, allogenic sources, or land use changes.
- The prevailing impact of acid mine drainage on downstream water resources of the Tweelopiespruit and Rietspruit is evidenced by the consistently low pH values (ranging from 4.0 to 6.2), and relatively elevated concentrations of EC, sulphate, manganese and aluminium recorded at the Pinocchio's Farm and MRd sampling sites. The EC and sulphate values recorded at these sites in March 2024 range from 285 mS/cm to 290 mS/cm and 1610 mg/L to 1764 mg/L, respectively. Despite this, the parameters recorded in March 2024 remain considerably lower than the values recorded during the period when AMD intervention measures were commissioned in the region, but suggest possible episodic and small-scale discharge of mine water.
- In contrast to the acidic Tweelopiespruit and Rietspruit, the Blougatspruit and Bloubankspruit exhibit relatively higher alkalinity, with pH values consistently ranging from 7.1 to 7.9.
- The most recent microbiological analyses of surface water samples collected at BG@N14, BB@M, BB@NOE, MRd and Pinocchio's Farm confirm the faecal contamination of surface water along the Blougatspruit, Bloubankspruit, Rietspruit and Tweelopiespruit, respectively. The most severe pollution is noted along the Blougatspruit and Bloubankspruit where the MPN count for E.coli consistently exceeds 2419.6 per 100mL.
- Groundwater level elevations are stable in the southern segment of the COHWHS property, while a slight decrease is evident in groundwater level elevations in the central and the northern segments.
- The March 2024 groundwater level of the Sterkfontein Cave lakes shows an apparent 1.5 m decrease since March 2023.
- The highest discharges were measured for the Zwartkrans, Nash and Nouklip springs, which respectively yielded ~29 ML/d, ~21ML/d and ~20ML/d in March 2023.
- The trendlines from September 2023 to March 2024 indicate that sulphate levels in ambient groundwater have generally reduced across the groundwater stations. There is however variability at individual stations and typically the sulphate values at the north-eastern end of the study area are lower than at the sites closer to the mine locations.
- The microbiological quality of the water from the Sterkfontein Cave lake is compromised by the increased total coliform bacteria and E.coli which exceeds the standard health-related limits across all sample results and thus reflects the impact of poorer quality surface water on the karst groundwater.
- The water from the major dolomitic springs is of excellent quality for all the parameters (i.e. pH, SEC, Ca, Mg, Na, K, Cl, SO<sub>4</sub>, HCO<sub>3</sub>, NO<sub>3</sub>+NO<sub>2</sub>, Si, Fe, Mn and Al) reported for March 2024 sampling results, with the exception of the Zwartkrans and Aquamine springs. The chemical results

from the Zwartkrans Spring show probable mine water impact as evidenced by the high sulphate content and the Aquamine spring show pronounced concentrations of manganese, iron and aluminium, which may reflect the interaction with the surrounding geology.

- Across all the spring sample results, the microbiological quality of the water is compromised by the total coliform bacteria and *E.coli* which far exceeds the standard health-related limits.
- The results of an ecotoxicological analysis of water samples collected from the Bloubankspruit confirm the deteriorating water quality of the system.

The reduced quantity and deteriorating quality of surface and groundwater resources, exacerbated by low rainfall, acid mine drainage, and faecal contamination, pose significant environmental and public health risks. The environmental impact extends to the fossil sites within the COHWHS, as altered water chemistry and contamination can threaten the preservation of these important paleontological resources, potentially leading to accelerated degradation and loss of critical fossil evidence. Therefore, the results of these monitoring activities provide crucial information for the State of Conservation (SOC) of the property, which remains a key concern for UNESCO's World Heritage Centre due to its implications for the Outstanding Universal Value (OUV) of the COHWHS.

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## ANNEXURES

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## SYMBOLS, ACRONYMS & ABBREVIATIONS

~	approximately
>	greater than
<	less than
%	per cent (parts per hundred)
%ile	percentile
°C	degree(s) Celsius
$\Delta$ h	change in head
a <sub>h</sub>	hydrological year
<i>aka</i>	also known as
AMD	acid mine drainage
amsl	above mean sea level
ASPT	average score per taxon
bc	below collar
bs	below surface
C <sub>5</sub>	concentration exceeded 95% of the time (5%ile)
C <sub>95</sub>	concentration exceeded 5% of the time (95%ile)
ca.	<i>Circa</i> (about)
COHWHS	Cradle of Humankind World Heritage Site ( <i>aka</i> 'the property')
CoV	coefficient of variation
CPOM	coarse particulate organic matter
CSIR	Council for Scientific and Industrial Research
DWS	Department of Water & Sanitation [formerly the Department of Water Affairs (DWA)]
EC	electrical conductivity
EoP	end-of-pipe
FFG	functional feeding group
FPOM	fine particulate organic matter
HDS	high density sludge
IHAS	integrated habitat assessment system
kg	kilogram(s)
km	kilometre(s)
L/d	litre(s) per day
L/s	litre(s) per second
L/s/km	litre(s) per second per kilometre
m	metre(s)
MA	Management Authority
meq/L	milliequivalent(s) per litre
mg/L	milligram(s) per litre
ML/d	megalitre(s) per day
mm	millimetre(s)

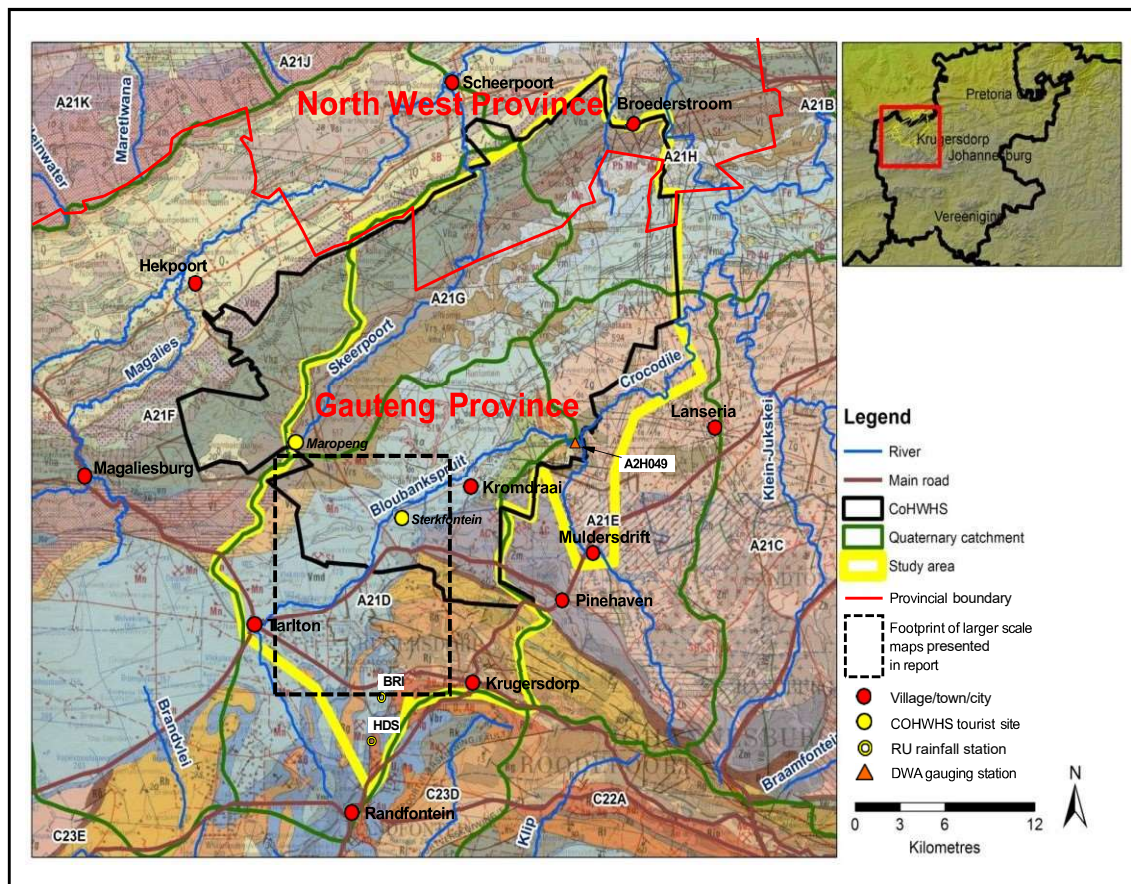
m <sup>3</sup> /s	cubic metre(s) per second
Mm <sup>3</sup>	million cubic metre(s)
Mm <sup>3</sup> /a	million cubic metres per annum
MPN	most probable number
mS/m	milli Siemens per metre
n	count
n.s.	not specified
pp	pages
Q <sub>50</sub>	discharge exceeded 50% of the time (50%ile or median)
REGM	Randfontein Estates Gold Mine
RU	Rand Uranium (earlier owner of the original REGM)
SASS	South African Scoring System
SD	standard deviation
SDM	synoptic discharge measurement
SEC	specific electrical conductance (electrical conductivity or EC @ 25°C)
SOC	State of Conservation
SRP	strategic research project
SS	Sibanye-Stillwater (formerly SibanyeGold and current owner of the original REGM)
TCTA	Trans-Caledon Tunnel Authority
TDS	total dissolved solids
USEPA	United States Environmental Protection Agency
UNESCO	United Nations Educational, Science and Cultural Organisation
WHC	World Heritage Committee (could also denote World Heritage Centre)
WWTW	wastewater treatment works

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# 1 INTRODUCTION, BACKGROUND & CONTEXT

The Management Authority (MA) of the Cradle of Humankind World Heritage Site (COHWS) appointed the CSIR to serve as service provider for the water resources monitoring programme of the property (**Figure 1**) following the outcome of bid GT/WHS/021/2022. Since its inception in 2012, the monitoring programme has, to date, generated nineteen (19) bi-annual *status quo* reports (i.e. Hobbs, 2012; 2013a; 2013b; 2014a; 2014b; 2015a; 2015b; 2016a; 2016b; 2017a; 2017b; Hobbs *et al.*, 2018; Bugan *et al.*, 2018 and Bugan *et al.*, 2019, Mvandaba *et al.* 2019, Mvandaba *et al.*, 2020a, Mvandaba *et al.*, 2020b, Mvandaba *et al.* 2023a, b). This document represents the twentieth (20<sup>th</sup>) such report and covers the period from April 2023 to March 2024.



**Figure 1** A map of the study area showing the regional geology, surface water drainages, quaternary catchments and other geographic locations for orientation.

## 2 TIMELINE OF KEY EVENTS

An updated timeline of key events since the start of mine water decanting in 2002, and incorporating the reporting period, is presented in **Figure 2**. The most recent landmark event on the timeline is the completion of a fourth State of Conservation (SOC) report (DEA, 2022) that was submitted to UNESCO's World Heritage Centre (WHC) for consideration by the World Heritage Committee. The outcome of this consideration sets out the concerns of the WHC for the property, which need to be addressed, and responded to, in the monitoring programme going forward. Progress with the resolution of the WHC's specific concerns will be documented in forthcoming State of Conservation reports.

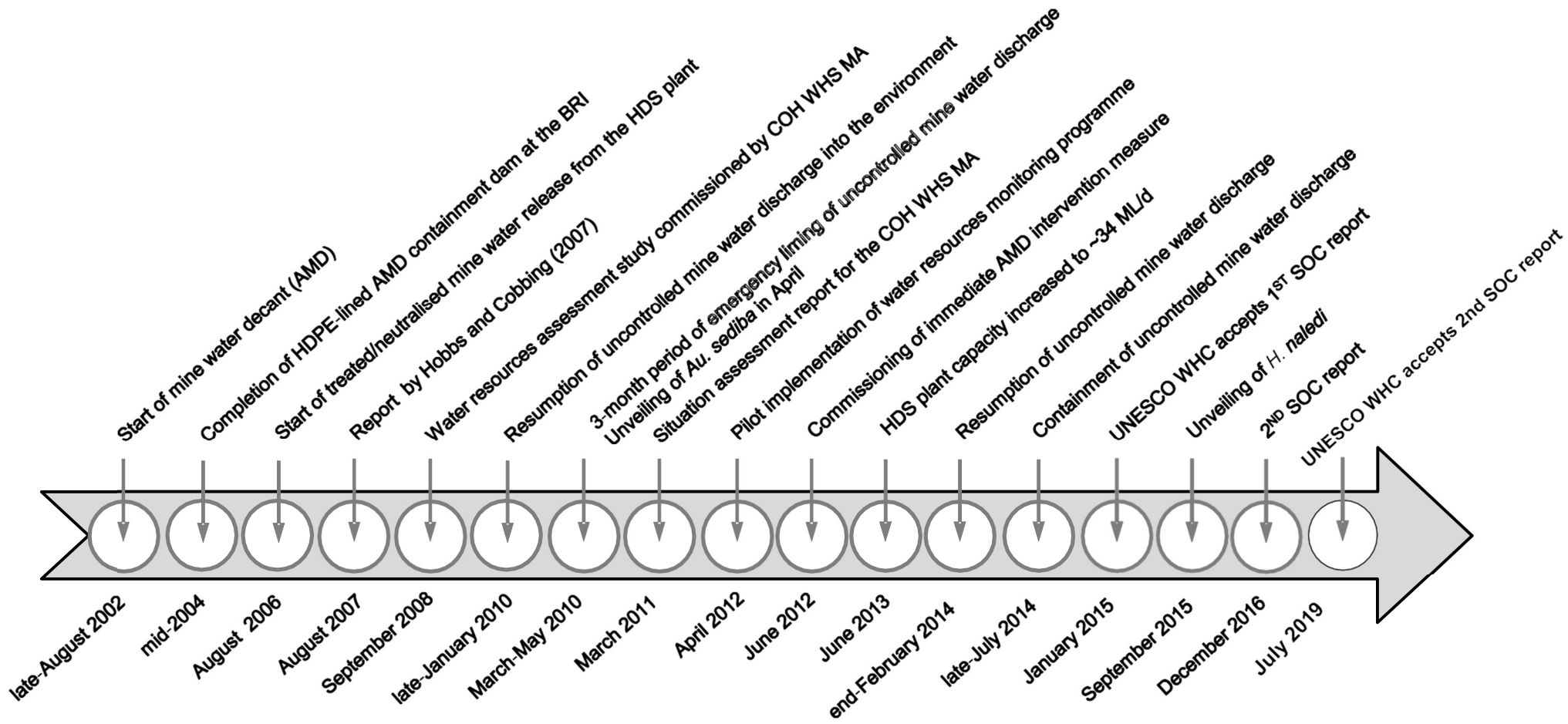


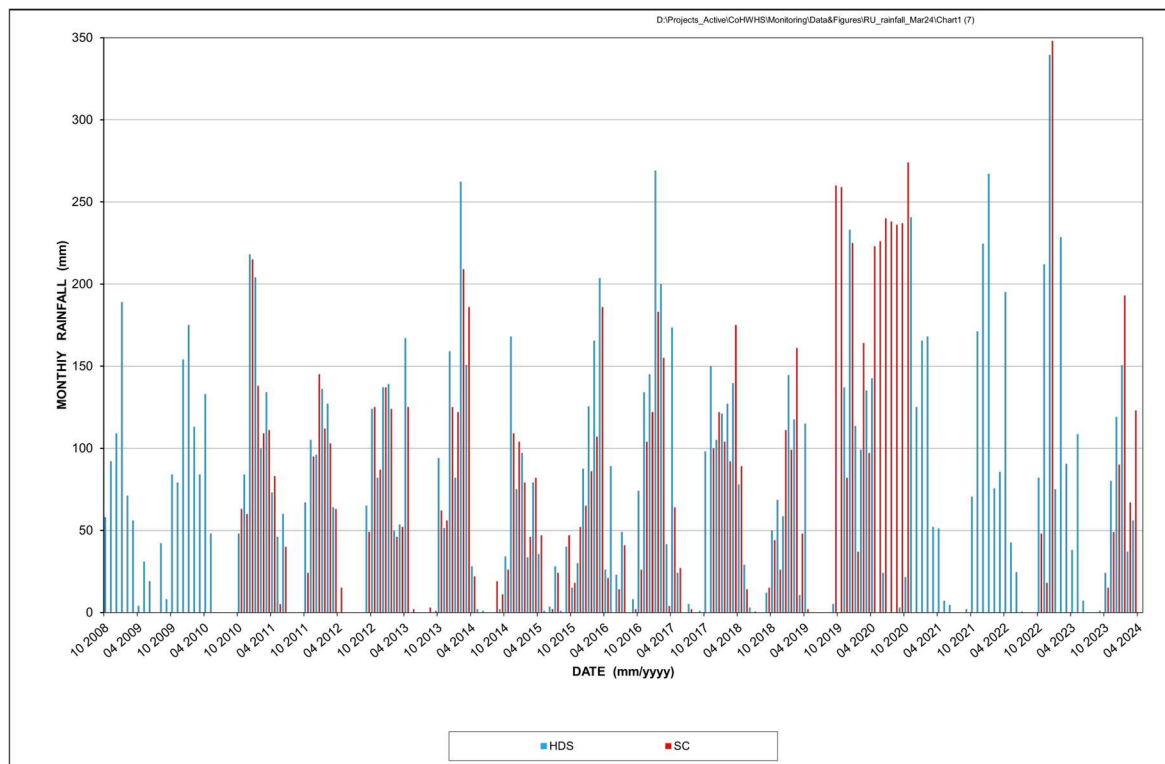
Figure 2 Timeline of key events relevant to the project and this report

### 3 RAINFALL

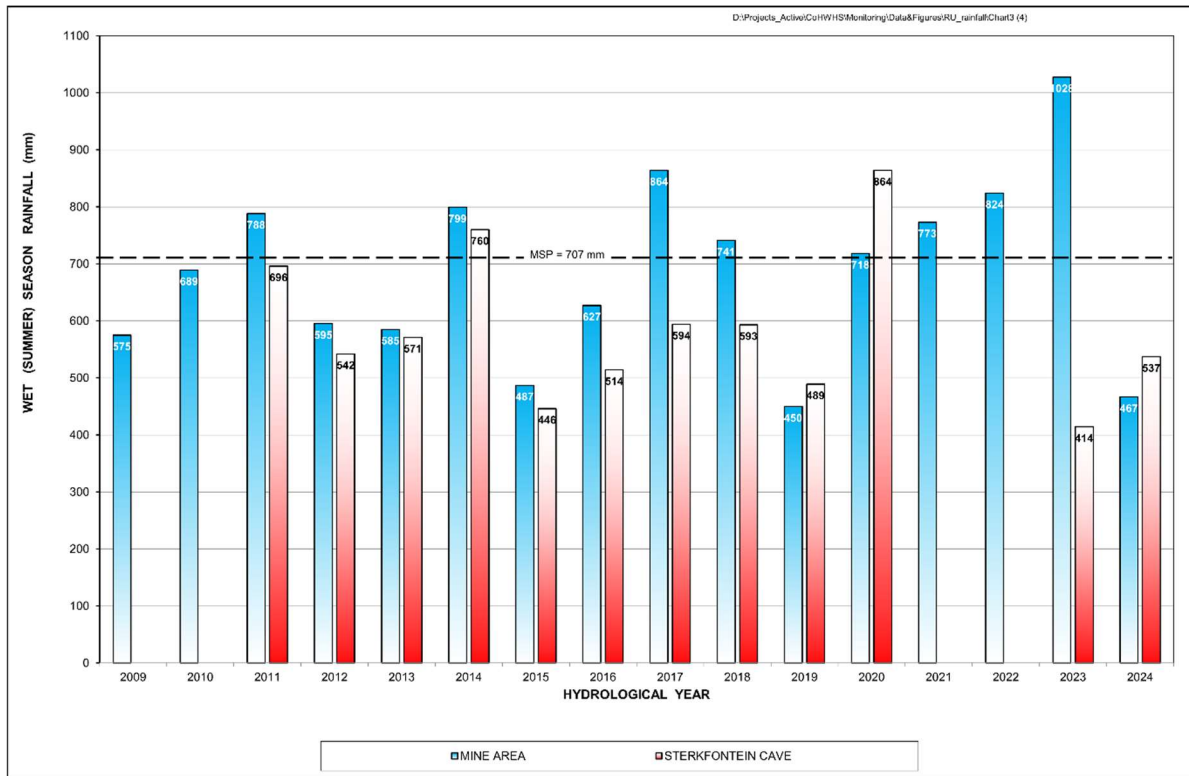
#### 3.1 Quantity

The monthly precipitation data covering the period from October 2008 to March 2024, observed at the Sibanye-Stillwater (SS) rainfall station HDS within the mine vicinity, and station SC positioned approximately 13 km northward at the Sterkfontein Cave (**Figure 1**) is presented in **Figure 3**. Available data records from November 2020 to December 2022, indicate an apparent gap of missing rainfall data for the SC station from November 2020 to August 2022 and January 2023 to September 2023. The Department of Water and Sanitation (DWS) has ascribed this data gap to instrument and software malfunction (De Meillon, January 2023; October 2023). The almost two-year data gap therefore renders it impossible to make a logical comparison of the rainfall record in the mine area to that of the Sterkfontein Cave. However, given the sixteen-year data record illustrated in **Figure 4**, some statistics can be considered for the region:

The 2024 wet season rainfall of 467 mm is among the lowest recorded at station HDS in the 16-year period from 2008 to 2024. This amount is approximately 55% lower than the wet season rainfall measured in 2023 and ranks at the 7th percentile in the historical dataset, indicating it as one of the driest periods on record. Similarly, the 537 mm of wet season rainfall recorded at station SC ranks at the 42nd percentile, highlighting a moderately drier period for the region.



**Figure 3** Monthly precipitation in the mine area (station HDS) from October 2008 to March 2024, and the concurrent record for the Sterkfontein Cave station from June 2010 to March 2024.



**Figure 4** Total wet season (summer) rainfall in the mine area (HDS station) in the past fifteen hydrological years, also showing the comparison with that for the available contemporaneous Sterkfontein Cave record; MSP denotes mean summer precipitation.

### 3.2 Quality

The chemical composition of rainwater in the southwest region of the COHWS property is assessed through samples collected from four rainfall stations managed by the Department of Water and Sanitation (DWS). These stations have a rainfall equivalent capacity of approximately 450 mm and are typically emptied every 2 to 4 months, depending on the season. Consequently, the chemistry of the collected rainwater represents a composite of multiple precipitation events over the collection period, which implies that the laboratory-determined pH values may not accurately reflect specific rainfall events.

No samples were collected beyond January 2024, and thus the water chemistry results from January 2024 are presented in **Table 1**, with the exception of station HDS, which includes data from the late dry season/early wet season as reported by Bujan et al. (2019). Efforts by the CSIR Project Team to acquire data for this site from either Sibanye-Stillwater or the DWS have been unsuccessful. The results from stations GP00303, GP00301, and SC pertain to late wet season/early dry season rainfall.

The observed rainwater chemistry at these monitoring stations consistently reaffirms the region's characteristic low salinity and predominantly acidic nature. However, the rainwater chemistry at the high-density sludge (HDS) station is relatively more saline than the other three stations. Due to insufficient data, the current understanding of temporal rainwater quality and distribution in the area cannot readily explain inter-station variances in sulphate, total alkalinity, and nitrate levels.

**Table 1** Composite rainwater chemistry in the south-western portion of the property

Variables	Unit	Rainfall Station			
		HDS <sup>1</sup>	GP00303 <sup>2</sup>	GP00301 <sup>3</sup>	SC <sup>4</sup>
Specific electrical conductance*	mS/m @ 25°C	18	3	2	2
pH*	$-\log_{10}a_{H^+}$	5.4	4.9	5.5	5.2
Calcium	mg Ca/L	6.7	0.6	0.5	0.5
Magnesium	mg Mg/L	1.4	<0.3	<0.3	<0.3
Sodium	mg Na/L	1.7	<0.6	<0.6	<0.6
Potassium	mg K/L	3.8	<0.6	<0.6	<0.6
Chloride	mg Cl/L	<2.0	<2.0	<2.0	<2.0
Sulphate	mg SO <sub>4</sub> /L	32	5.9	5.4	5.1
Total alkalinity	mg CaCO <sub>3</sub> /L	2.6	1.4	2.7	1.7
Nitrate + nitrite	mg N/L	11	0.71	0.63	0.42

\* Laboratory values

<sup>1</sup> At the high-density sludge plant in the mine area (Bugan *et al.*, 2019)

<sup>2</sup> At monitoring borehole GP00303, Vlakplaats 160IQ, Tarlton (sampled 15 January 2024)

<sup>3</sup> At monitoring borehole GP00301, Sterkfontein 173IQ (sampled 15 January 2024)

<sup>4</sup> At Sterkfontein Cave (sampled 15 January 2024)

## 4 SURFACE WATER HYDROLOGY

### 4.1 Physical Hydrology

#### 4.1.1 Surface Water Discharge

The discharge of the Bloubankspruit system is monitored at the DWS gauging station A2H049, strategically located approximately 700 meters upstream from the system's confluence with the Crocodile River (**Figure 1**). The extensive 52-year observed discharge record from 1972 to 2024 for this catchment (Quaternary A21D) provides the historical monthly statistics reported in **Table 2**. This record is updated as frequently as possible when new data are obtained from the station and processed by the DWS. As previously reported by Mvandaba *et al.* (2020b), data collection at the station faced interruptions during specific periods. Notably, no data were collected from June 2018 to May 2019 and again from December 2019 to January 2020. The absence of data between June 2018 and May 2019 was attributed to financial constraints, impeding regular data collection, updates, and essential maintenance of the data collection platforms at that time. Following the alleviation of national lockdown restrictions in South Africa, the DWS resumed the data update process for gauging station A2H049. In a subsequent update reported by Mvandaba *et al.* (2023), the data record was extended from September 2020 to March 2023. However, it revealed additional gaps in the data, specifically from December 2021 to January 2022, and June 2022 to July 2022. The latest updated data record encompasses information from August 2022 through to December 2023, providing a more comprehensive overview of the Bloubankspruit system's discharge patterns. The absence of data records for January 2024 to March 2024 has been attributed to financial constraints at the Department, which have impeded data collection (Setsome, 2024).

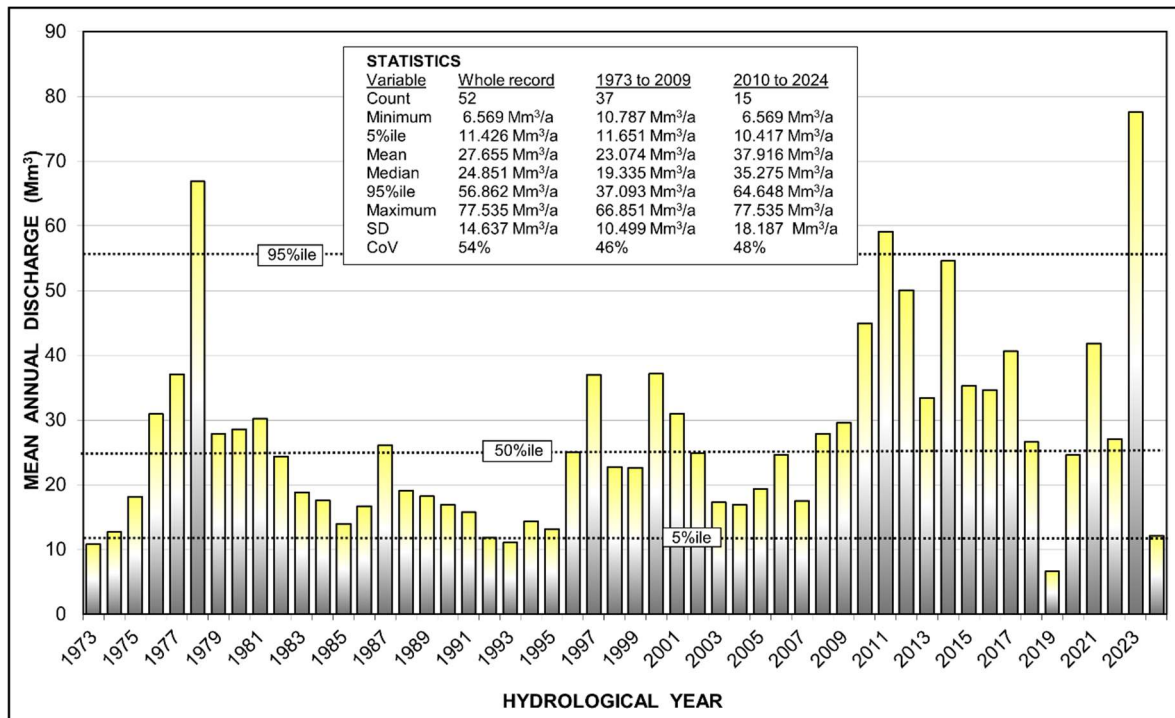
**Table 2** Statistical analysis of Bloubankspruit monthly discharge data gauged at station A2H049 in the period October 1972 (ah 1973) to March 2024 (ah 2024) (latest data as at March 2024).

Variable	Month											
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Count (n)	45	45	45	45	47	47	47	46	48	47	46	45
Minimum	0.682	0.269	0.711	0.721	0.706	0.828	0.886	0.847	0.521	0.939	0.89	0.77
5%ile	0.794	0.837	1.044	1.097	0.903	1.084	1.193	1.009	0.896	0.965	0.928	0.805
Mean	1.899	1.872	2.305	2.749	2.681	3.05	2.481	2.328	2.06	2.109	1.984	1.826
Median	1.691	1.896	2.092	2.513	2.222	2.566	2.132	1.993	1.772	1.879	1.804	1.587
95%ile	3.775	1.921	4.488	5.32	6.232	7.595	5.263	4.852	4.026	4.013	3.656	3.507
Maximum	4.211	1.941	5.9	12.079	10.619	11.351	6.081	5.373	5.166	4.754	4.055	4.342
SD	0.92	2.989	1.099	1.909	1.897	2.163	1.29	1.181	0.996	0.934	0.867	0.872
CoV (%)	48.45	2.95	47.68	69.47	70.75	70.91	52.02	50.72	48.33	44.28	43.71	47.78

- All units are Mm<sup>3</sup> unless otherwise indicated.

- Analysis excludes months with missing and station rating exceedance data, but includes unaudited (recent) and estimated data

The historical total annual discharge, measured in Mm<sup>3</sup>, for complete hydrological years up to September 2023 is shown in **Figure 5**. Notably, the 2023 hydrological year is the most recent complete year in the record. The hydrograph for the mean annual discharge reveals that the 2023 hydrological year recorded a discharge of approximately 77.5 Mm<sup>3</sup>, the highest discharge for the Bloubankspruit from October 1972 to September 2023. This significant discharge aligns with the occurrence of flash floods in the region between November 2022 and March 2023, following intense rainfall events (Krugersdorp News, December 2022). Noting the missing data, the wet season rainfall for the 2024 hydrological year has resulted in a discharge volume of 12.1 Mm<sup>3</sup> from October 2023 to December 2023. In comparison, the wet season rainfall for the 2023 hydrological year produced a total volume of 44.0 Mm<sup>3</sup>, with 12.8 Mm<sup>3</sup> recorded for the same months.

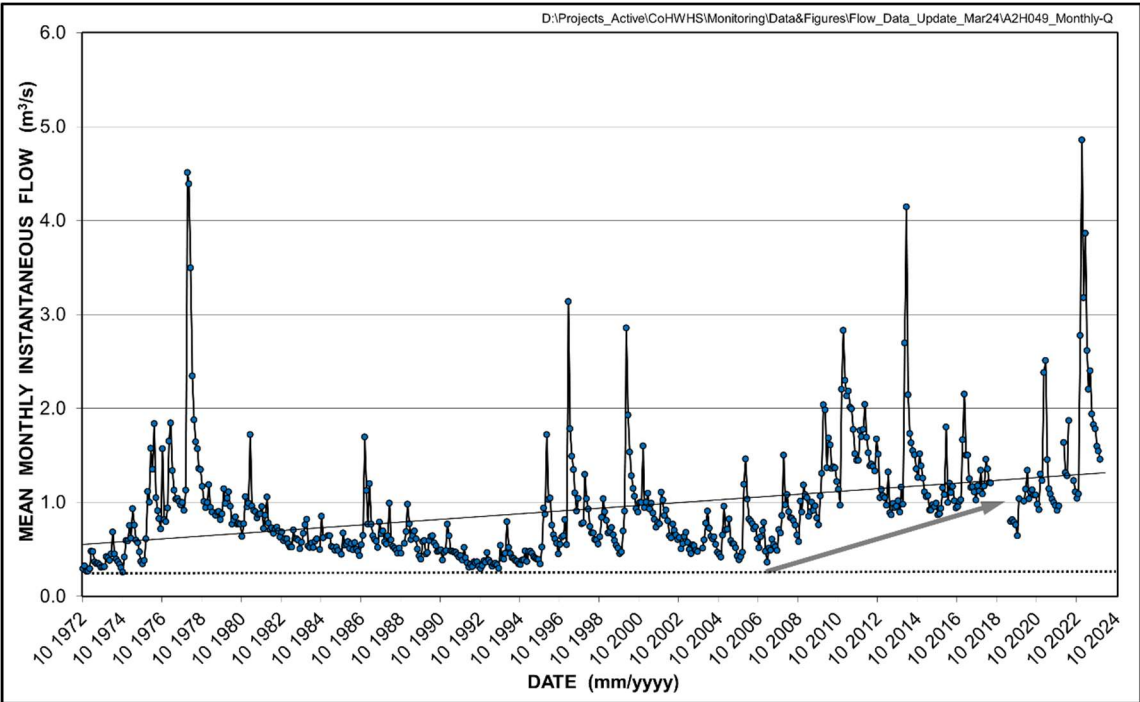


**Figure 5** Graph of Bloubankspruit annual discharge gauged at station A2H049 for the period October 1972 (ah 1973) to March 2024 (ah 2024)

**Figure 6** displays the average monthly instantaneous flow pattern at station A2H049 from October 1972 to

March 2024. According to the data, the highest average monthly instantaneous discharge during this period, from October 1972 to March 2024, is 4.9 m<sup>3</sup>/s. This flood peak corresponds to the mean monthly discharge of the Bloubankspruit in January 2023. In comparison, the average flow rate recorded between October 2023 and March 2024 is 1.5 m<sup>3</sup>/s however this period has missing data for the months January 2024 to March 2024. Despite the apparent lower flow rates for the 2024 hydrological year, the trendline for the average monthly flow rate exhibits a general upward trajectory over the 52-year dataset, suggesting several hydrological scenarios in the COHWHS:

- Increased rainfall: Increased rainfall during the wet season may be contributing to a higher average monthly flow;
- Hydrological changes: Broader hydrological changes in the watershed or catchment area may be occurring, potentially due to climate change or other environmental factors.
- Increased discharge: The trendline may indicate the contribution of allogenic sources, including treated/neutralized mine water from the Western Basin, with a secondary contribution from municipal wastewater effluent from the Percy Stewart Wastewater Treatment Works as well as contributions from dams or reservoirs, leading to an increase in water release or discharge.
- Potential changes in land use: Changes in land use, such as urbanization or deforestation, could lead to increased runoff entering the water system, thus elevating flow rates.



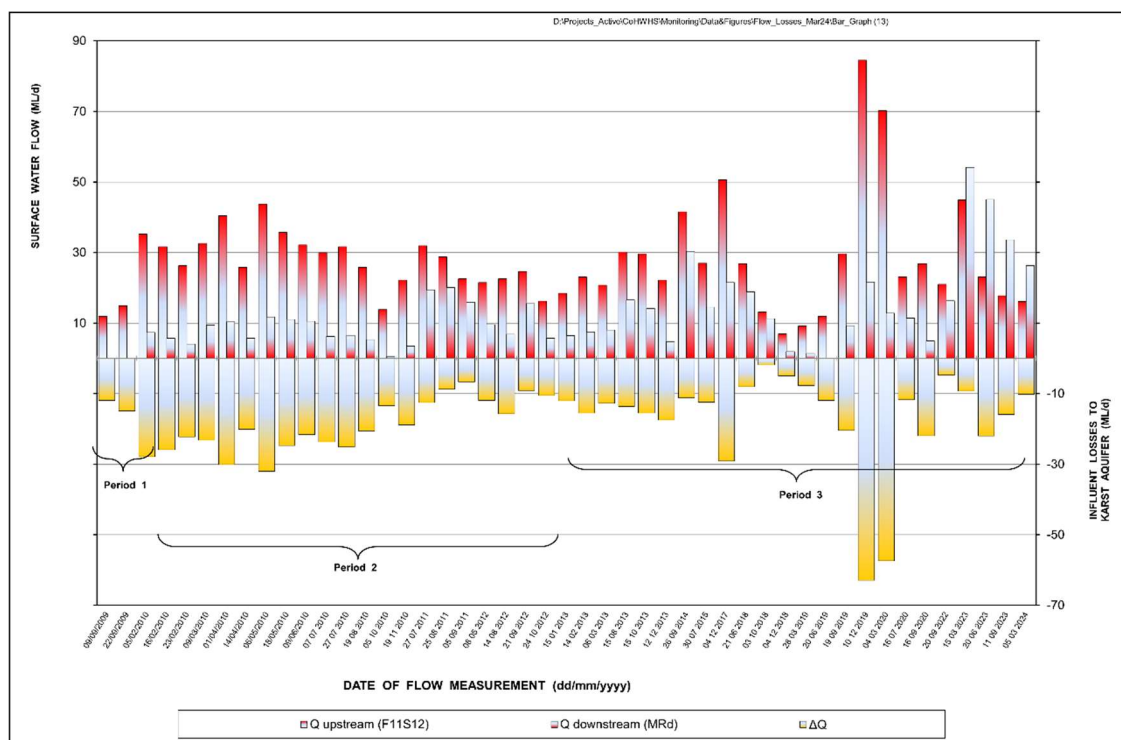
**Figure 6** Long-term monthly hydrograph of the Bloubankspruit at station A2H049 for the period October 1972 to March 2024 (latest data as of March 2024).

**4.1.2 Surface Water Fluxes**

The extent of surface water loss, primarily to the karst aquifer, is determined by calculating the difference in measured streamflow discharge at two key stations: F11S12 (Brickworks Dam) situated at the lower end of the Tweelopiespruit, and MRd, located approximately 3.9 km downstream along the Rietspruit (**Figure 7**). As reported by Mvandaba *et al.* (2023), during the September 2022 monitoring run, the gauging site was found to be permanently closed off by the property owner, citing security reasons. Consequently, a new

gauging site was established ~250m downstream from the station F11S12, on the property Pinocchio's Farm. Streamflow measurements at this site are conducted at a culvert through which the Tweelopiespruit flows, ensuring the continuity of data collection.

The latest data captured in March 2024 shows surface flow fluxes between the Pinocchio's Farm gauging site and MRd of magnitude 10.1 ML/d, respectively. This calculated flux falls between the 50<sup>th</sup> and 95<sup>th</sup> percentile of the 15-year period from September 2009 to March 2024 (**Figure 8**) and is less than the average and median losses recorded for the same 15-year period of 17.9 ML/d and 15.5 ML/d, respectively. The volume of surface flow flux measured in March 2024 is approximately 36.5% less than the 15.9 ML/d flux calculated in September 2023 indicating a notable reduction in surface water discharge of the Tweelopiespruit/ Rietspruit systems.



**Figure 7** Graph of streamflow and influent losses to the karst aquifer in the lower Riet Spruit valley.

## 4.2 Chemical Hydrology

### 4.2.1 Mine Water Impact

#### 4.2.1.1 Tweelopiespruit / Riet Spruit

From the point where it leaves the mine property down to its confluence with the Riet Spruit at Glen Almond north of the Krugersdorp Game Reserve (KGR), a distance of approximately 6.6 km, the chemistry of surface water in the Tweelopiespruit is still being monitored by Sibanye-Stillwater on a weekly basis at five localities (**Figure 8**). The monitoring of the variables pH, electrical conductivity (EC) and sulphate (SO<sub>4</sub>) dates to May 2004. The results for two of these stations, namely the (upstream) Hippo and (downstream) Brickworks (F11S12) dams, are presented in **Figure 9** (pH), **Figure 10** (SEC), **Figure 11** (SO<sub>4</sub>), **Figure 12** (Fe), **Figure 13** (Mn) and **Figure 14** (U). The 'upstream' and 'downstream' positions of these stations render the results of the other three stations superfluous for the purposes of this report.

The patterns revealed in **Figure 9** to **Figure 14** reflect the temporal variation and trend in the respective variable values in surface water as it flows through the KGR. The period(s) of most severe and sustained mine water impact have previously been discussed in Hobbs (2014b). Of relevance to the period covered by this report is the recovery of the mine water treatment and management measures to 'operationally optimal' levels (period E in the Figures). This follows the excursion in the 2017 summer of mine water discharges to poorer (suboptimal) levels because of copious decant volumes. Mvandaba and Shadung (2023) have previously discussed the trend of each respective parameter for the period from October 2022 to September 2023. Therefore, this report will focus on the emerging trends from October 2023 to March 2024:

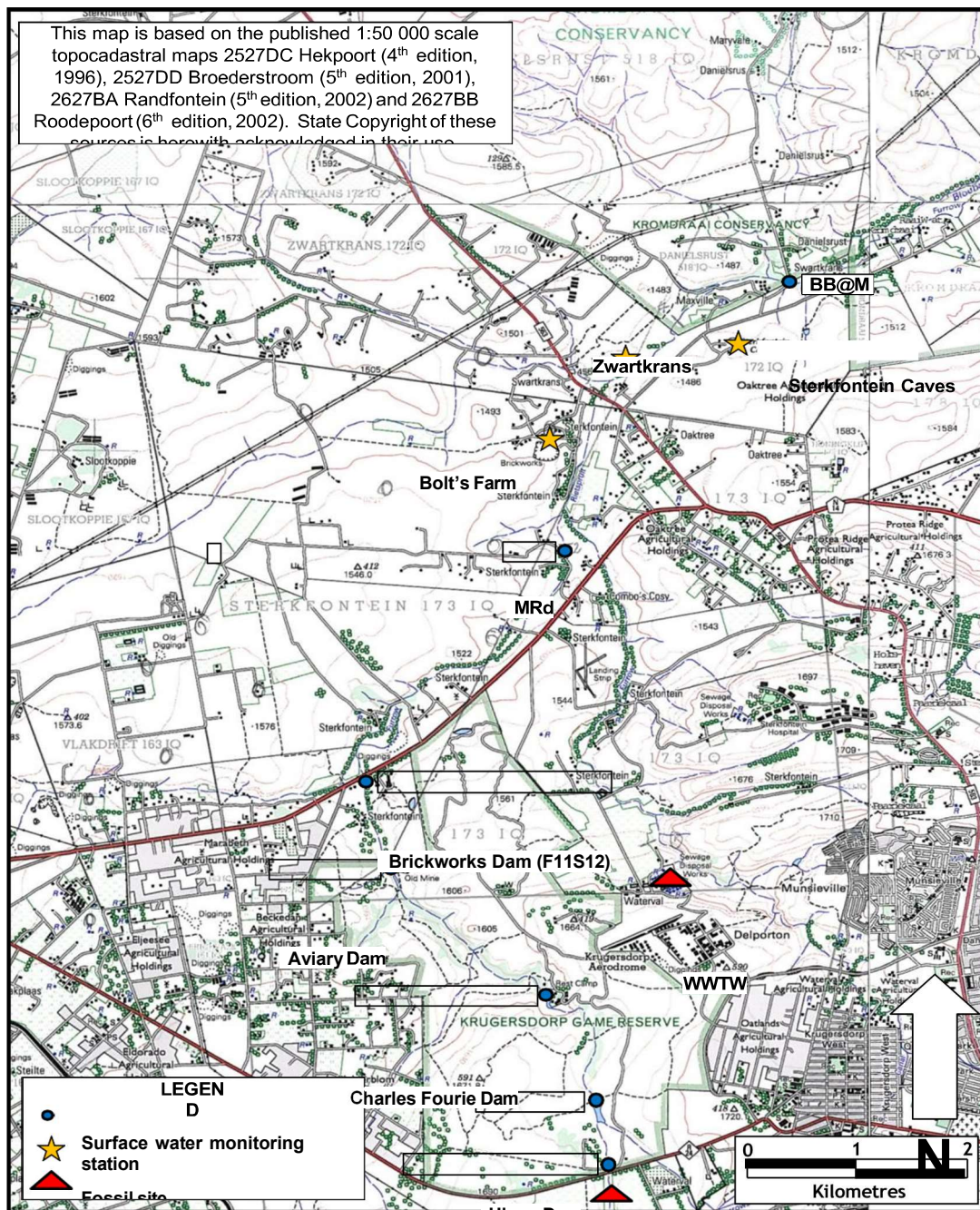
- The pattern of the pH values (**Figure 9**) recorded at the Hippo Dam and F11S12 stations between October 2023 and March 2024 illustrates a gradually decreasing trend for both stations, ranging from 7.4 to 8.4 and 4.5 to 6.3 respectively. The lower range of pH values (below 5) for the F11S12 station were recorded between November 2023 and March 2024, indicating acidic conditions.
- The SEC values (**Figure 10**) at both stations indicate a stable trend, however the concentrations have oscillated between 301 mS/m and 354 mS/m for the Hippo Dam station and 254 mS/m and 367 mS/m for the F11S12 station.
- Sulphate concentrations (**Figure 11**) appear to be stable at both stations, with values averaging 1850 mg/L at the Hippo Dam and 1777 mg/L at F11S12.
- The iron concentration (**Figure 12**) at the Hippo Dam station indicates generally decreasing levels with a range of 0.003 mg/L to 0.1 mg/L. At the F11S12 station, a gradually decreasing trend is noted with values ranging from 0.01 mg/L to 0.08 mg/L.
- Manganese concentrations (**Figure 13**) at both the Hippo Dam and F11S12 stations display a stable trend, averaging 0.9 mg/L and 1.34 mg/L, respectively.
- At both stations, uranium (**Figure 14**) has maintained a consistent concentration of 0.02 mg/l beyond March 2020.

A statistical analysis of the data associated with each of the periods of record A–B, B–C, C–D, D–E and E– defined by the divisions recognised in **Figure 9** to **Figure 14** is presented in **Table 3**. Period F in **Table 3** provides a more recent look at the statistics between October 2020 and March 2024, but may be considered to generally show the continuation of the recovery of mine water treatment and management measures. The outcome of the statistical analysis offers a quantitative assessment of the specific differences in variables between different periods at each station and across various stations. Notably, the deviations indicating poorer quality discharge during the B–C and D–E periods are evident in the median values of all variables, except possibly iron, at both stations. Hobbs et al. (2018) observed that this finding implies that the exceptionally wet hydrological year of 2017 did not have a similarly adverse impact on the quality of mine water discharges to the environment, unlike the situations observed in the 2010, 2011, and 2014 hydrological years. The primary reason behind these observations can be attributed to the mine water control and management measures that have been implemented in the Western Basin.

The effectiveness of these measures is clearly reflected in the median and 95<sup>th</sup> percentile values during the E–F and F- periods, which, in most cases, exhibit the lowest values across the five periods of analysis. This trend is comparable to the C-D period, which corresponds to the implementation of immediate Acid Mine

Drainage (AMD) intervention measures. The most recent results (March 2024) from hydrochemical and microbiological analyses of water samples collected by the CSIR along the Tweelopiespruit and Rietspruit are summarised in **Table 4**. At a more localised scale, the prevailing impact of acid mine drainage on downstream water resources of the Tweelopiespruit and Rietspruit is evidenced by the consistently low pH values (ranging from 4.0 to 6.2), and elevated concentrations of EC, sulphate, manganese and aluminium recorded at the Pinocchio's Farm and MRd sampling sites (**Figure 15**). The EC and sulphate values recorded at these sites in March 2024 range from 285 mS/cm to 290 mS/cm and 1610 mg/L to 1764 mg/L, respectively. These values are significantly lower than the average EC of 885 mS/cm reported in September 2023, indicating a seasonal variation possibly influenced by changes in discharge volume in the system. Barring the pH values, the parameters recorded in March 2024 remain considerably lower than the values recorded during the C-D period (Table 4), but suggest possible episodic discharge of mine water.

These findings underline the positive impact of the recent interventions on the quality of mine water discharges, providing compelling evidence of implemented control and management strategies.



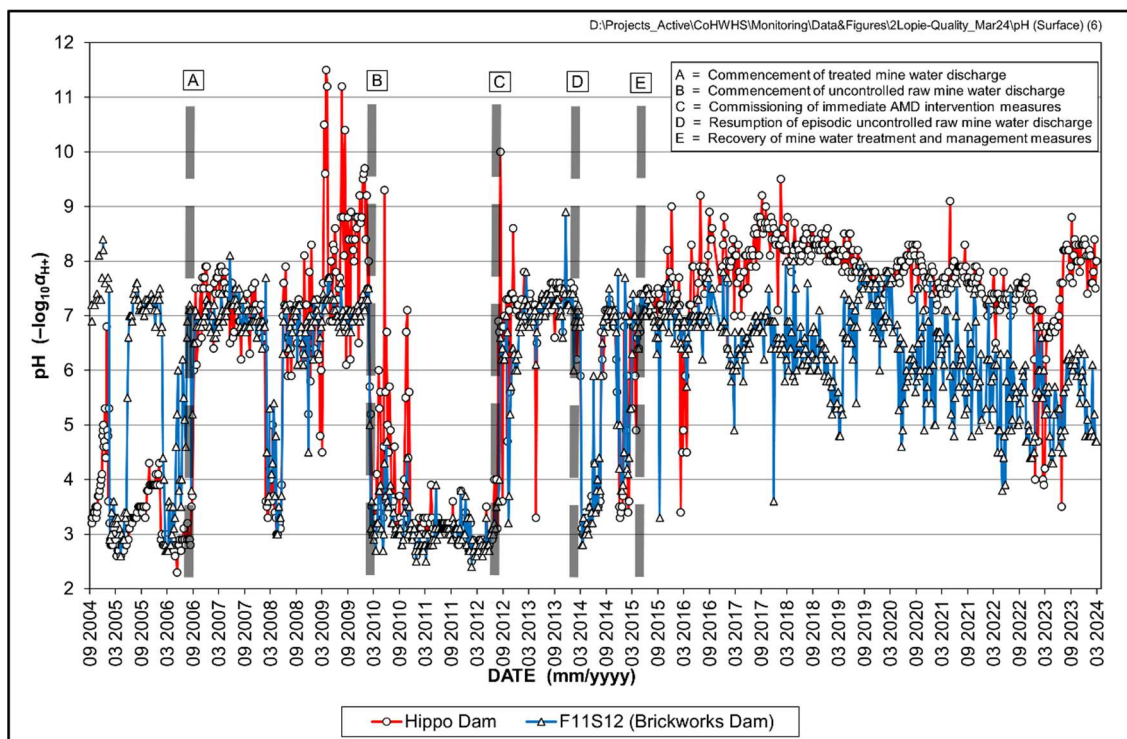
**Figure 8** Locality map of surface water quantity and quality monitoring stations

#### 4.2.1.2 Bloubankspruit

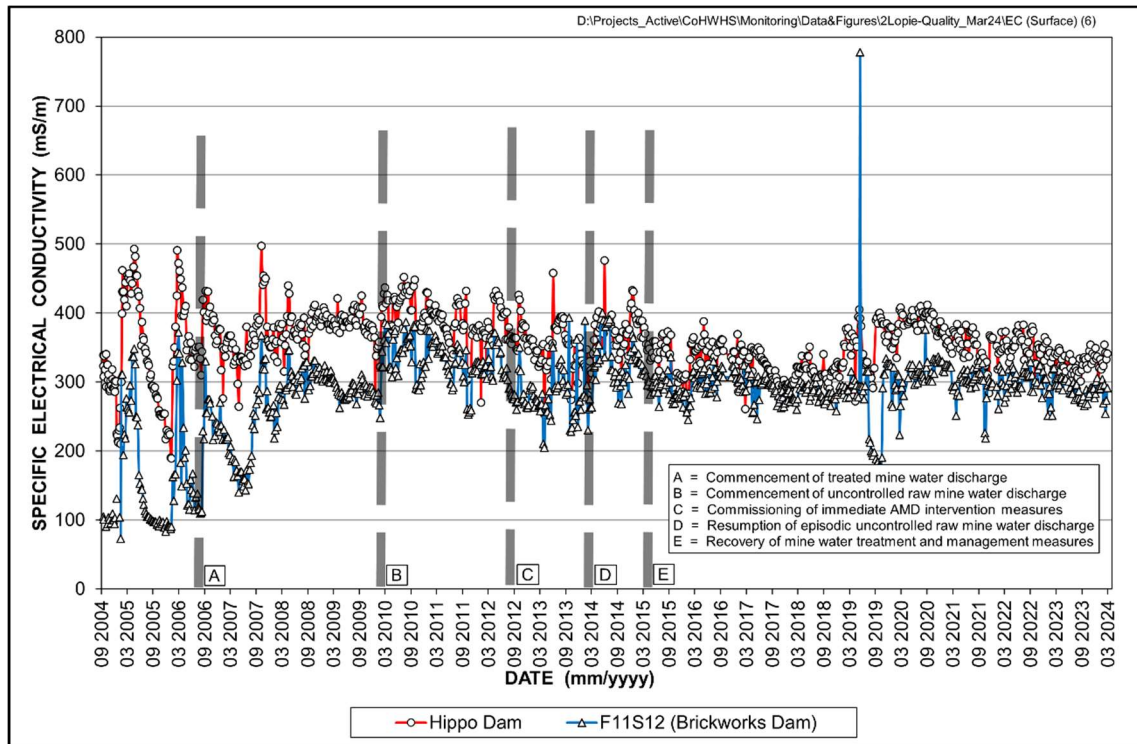
The latest available analysis of hydrochemical data from station A2H049 is as captured in the April 2023 to September 2023 edition of the Status Quo report (Mvandaba and Shadung, 2023). The DWS has reported an inability to update the data, citing system access issues caused by limited data connection, instrument failure as well as cost-cutting measures which reduced the frequency of data collection at the station (Botes, March 2024). The comprehensive analysis of synoptic surface water chemistry data for the DWS flow gauging station A2H049 (**Table 4**) at the lower reach of the Bloubankspruit system, as detailed in earlier reports, has been revised to highlight significant temporal variations in specific variable values. Noteworthy gaps existed in the dataset between May 2018 and July 2022 due to the absence of data collection activities at the site. The DWS attributed this hiatus to financial constraints and instrument failures.

The recent data update, spanning the timeframe from July 2022 to November 2022, provides a more current perspective, filling the gaps in the dataset. This update ensures a more recent portrayal of the surface water chemistry dynamics, allowing for a nuanced understanding of the current variable-specific changes.

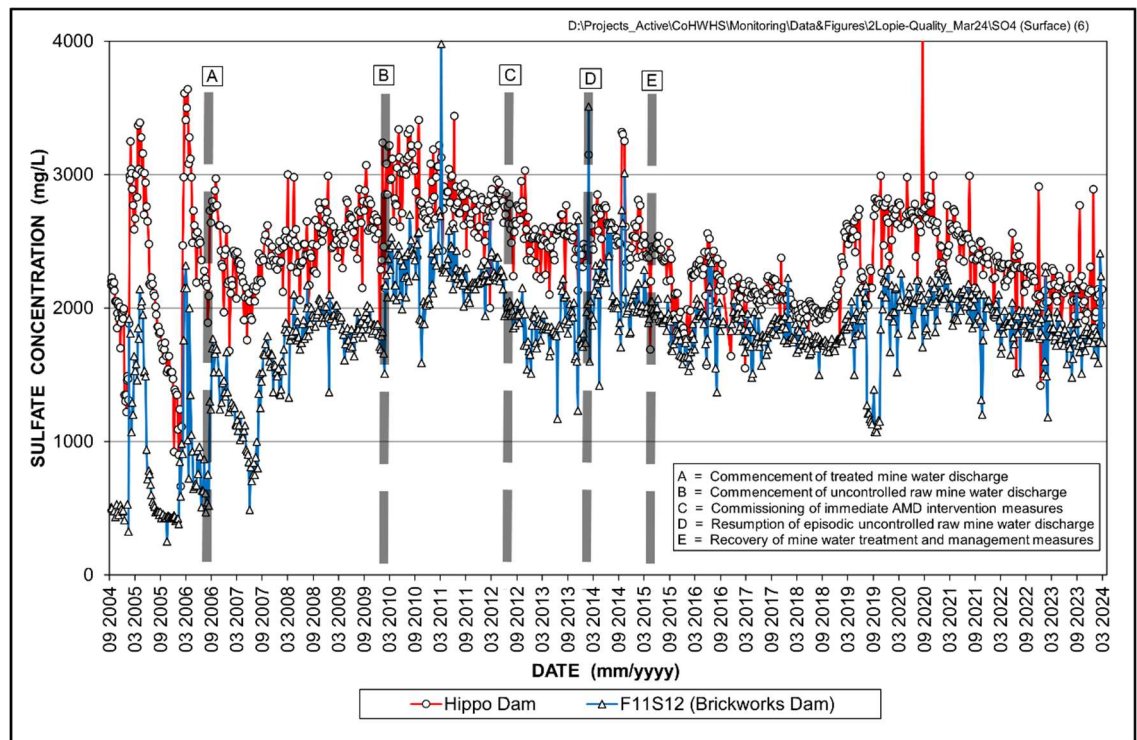
**Table 4** reflects statistics for a 'pre-impact' period, August 2002 to January 2010, and a 'post-impact' period February 2010 to May 2018, and July 2022 to November 2022. Barring sulphate concentrations at the 95<sup>th</sup> percentile (during the July 2022 to November 2022 period), none of the variables reported for either the 'pre-impact' or the 'post-impact' periods exceeded the respective SANS (2015a; 2015b) health-related limits for potable water, where specified, even at the C5 (95<sup>th</sup>ile) level and, in the case of pH, also at the C95 (5<sup>th</sup>ile) level.



**Figure 9** pH pattern of Tweelopiespruit surface water in the period September 2004 to March 2024



**Figure 10** Specific electrical conductivity pattern of Tweelopiespruit surface water in the period September 2004 to March 2024



**Figure 11** Sulphate pattern of Tweelopiespruit surface water in the period September 2004 to March 2024

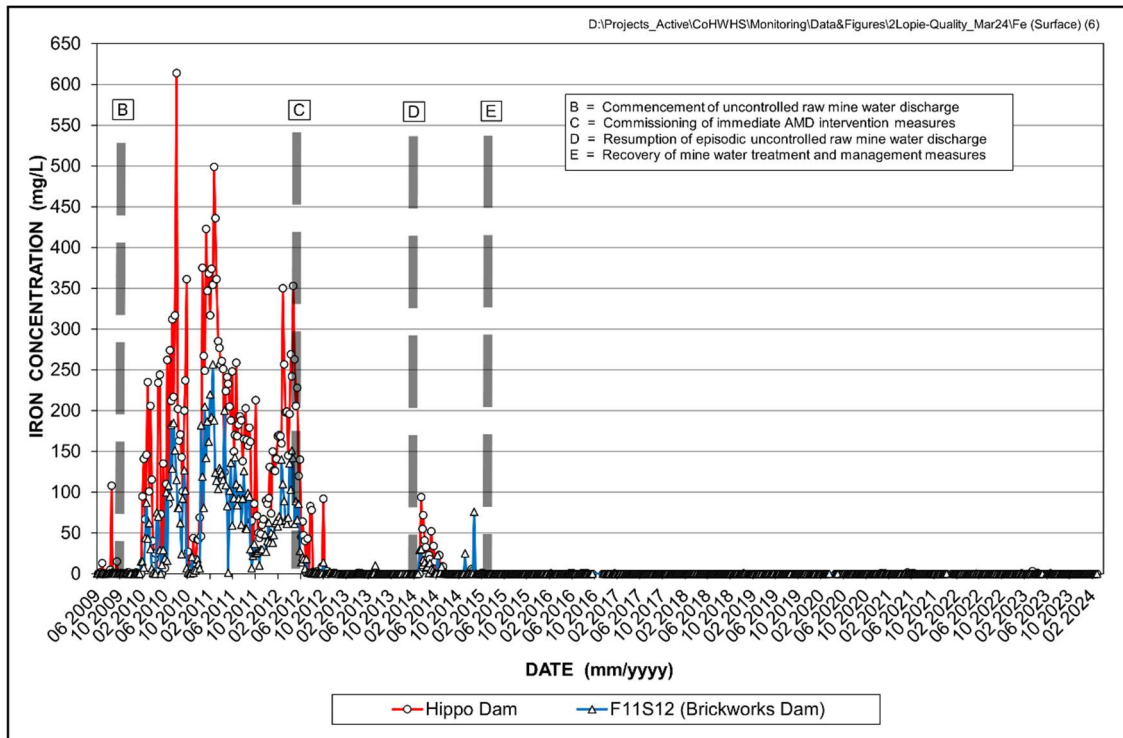


Figure 12 Iron pattern of Tweelopiespruit surface water in the period June 2009 to March 2024

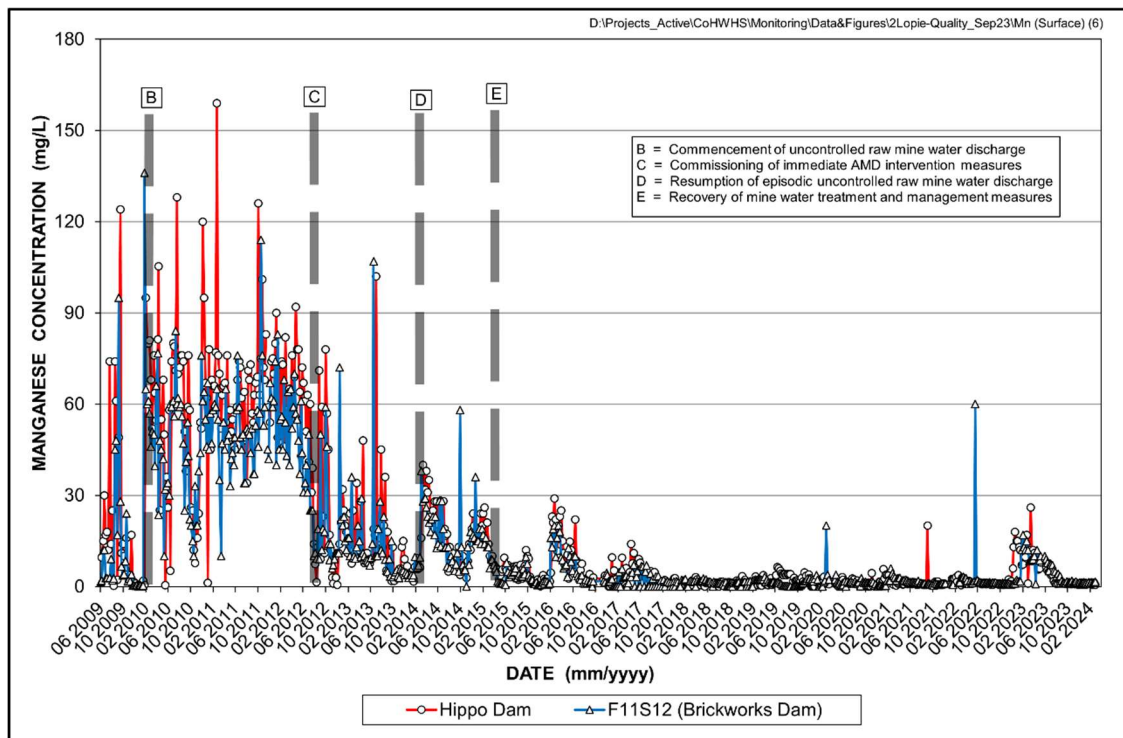
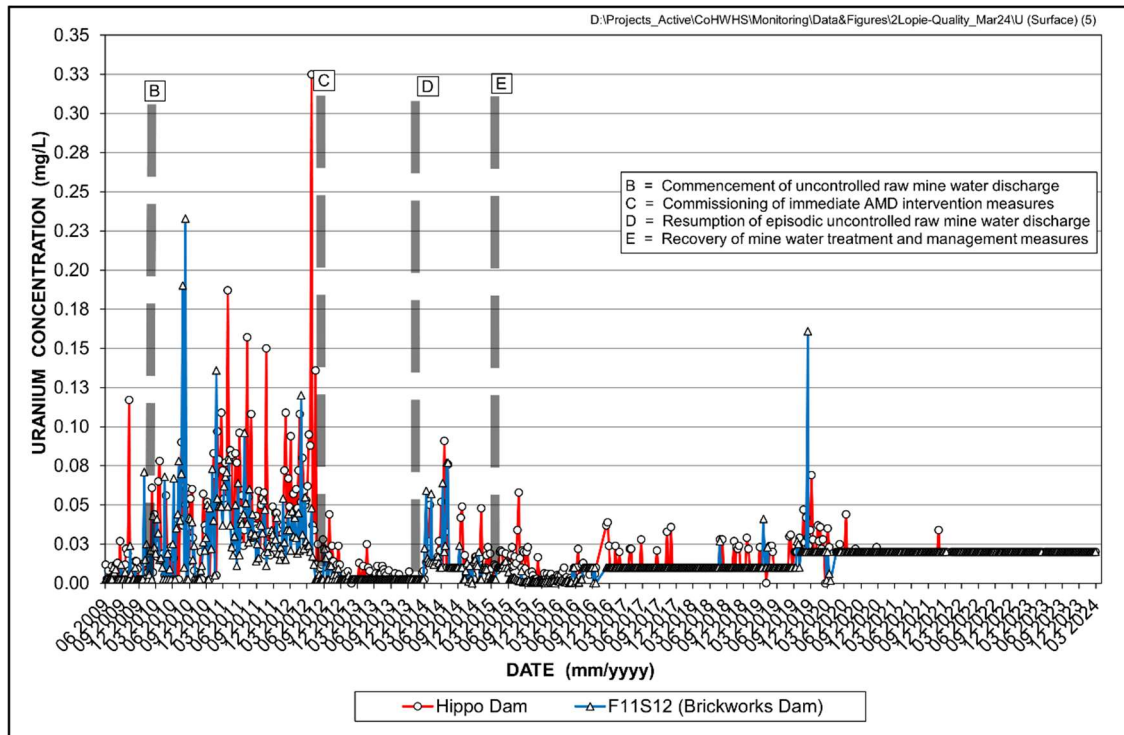


Figure 13 Manganese pattern in Tweelopiespruit surface water in the period June 2009 to March 2024



**Figure 14** Uranium pattern in Tweelopiespruit surface water in the period June 2009 to March 2024

**Table 3** Summary statistics of period-related surface water chemistry variability in the Tweelopiespruit (latest data as at March 2024).

Variable	Statistical Parameter	Hippo Dam						F11S12 (Brickworks Dam)					
		A—B <sup>(1)</sup>	B—C <sup>(2)</sup>	C—D <sup>(3)</sup>	D—E <sup>(4)</sup>	E—F <sup>(5)</sup>	F— <sup>(6)</sup>	A—B <sup>(1)</sup>	B—C <sup>(2)</sup>	C—D <sup>(3)</sup>	D—E <sup>(4)</sup>	E—F <sup>(5)</sup>	F— <sup>(6)</sup>
pH (-log <sub>10</sub> a <sub>H+</sub> )	n	176	129	83	57	269	182	173	128	83	57	276	173
	5%ile	3.6	2.8	5.9	3.2	6.8	6.2	3.9	2.7	5.3	3.0	5.4	4.5
	Mean	—	—	—	—	—	—	—	—	—	—	—	—
	Median	7.2	3.2	7.2	4.9	7.9	7.6	6.9	3.0	7.0	5.0	6.7	5.7
	95%ile	9.3	5.7	7.6	7.1	8.7	8.3	7.4	3.9	7.4	7.4	7.7	7.0
	SD	1.5	1.0	0.8	1.6	0.7	0.8	0.9	0.4	0.9	1.7	0.7	0.8
	CoV (%)	22.0	30	11	32	9.0	11.1	14	14	13	32	10	14.4
SEC (mS/m)	n	175	129	83	57	269	182	172	128	83	57	276	173
	Mean	374	391	350	376	348.7	351	268	332	281	329	291	298
	Median	379	393	354	377	347.5	350	283	330	276	323	293	296
	95%ile	426	438	395	417	393.9	396	329	378	350	391	323	325
	SD	32	33	34	28	24.2	25	48	29	34	34	41	20
	CoV (%)	9	8	10	7	7	7	18	9	12	10	14	6.7
SO <sub>4</sub> (mg/L)	n	176	128	82	56	269	182	171	128	83	56	276	173
	Mean	2448	2846	2520	2585	2305.4	2336	1636	2264	1879	2137	1832	1881
	Median	2460	2815	2525	2541	2300.0	2310	1760	2240	1870	2075	1840	1880
	95%ile	2828	3220	2770	2950	2770.0	2770	2015	2593	2148	2640	2160	2162
	SD	262	226	193	231	304.3	302	349	245	268	274	222	194
	CoV (%)	11	8	8	9	13	13	21	11	14	13	12	10.3
Fe (mg/L)	n	33	129	83	57	242	178	33	128	82	57	230	172
	Mean	4.7	168.4	2.5	8.9	0.0	0.1	0.3	72.9	0.47	4.9	0.0	0.0
	Median	0.4	163.0	0.03	0.10	0.0	0.0	0.2	64.0	0.08	0.04	0.0	0.0
	95%ile	13.8	365.2	3.1	52.6	0.0	0.2	0.8	186.3	1.00	25.7	0.1	0.1
	SD	18.8	116.2	13.10	19.5	0.0	0.3	0.3	57.7	1.9	12.2	0.0	0.0
	CoV (%)	399	69	528	220	5.3	336.9	94	79	407	2518	115	118.4
Mn (mg/L)	n	34	129	83	57	249	178	33	128	83	57	231	169
	Mean	18.1	62.7	16.5	17.3	3.8	2.7	10.3	50.3	14.4	16.1	3.0	2.9
	Median	9.8	65.0	11.0	16.0	2.2	1.0	2.7	50.0	10.0	14.0	1.8	1.3
	95%ile	74.0	95.0	56.1	32.6	11.9	12.0	46.2	76.0	45.0	30.4	9.8	10.0
	SD	27.6	23.5	18.0	9.1	4.6	4.1	19.4	17.6	15.8	9.9	3.6	5.4
	CoV (%)	153	38	109	53	120	149.9	188	35	110	61	117	184.5

(1) 09/2006 – 01/2010 (2) 02/2010 – 07/2012 (3) 08/2012 – 02/2014 (4) 03/2014 – 03/2015 (5) 04/2015 – 09/2020 (6) 10/2020 – 03/2024

**Table 4** Synoptic overview of Bloubankspruit water chemistry at station A2H049 in the periods August 2002 to January 2010 and February 2010 to May 2018 and July 2022 to November 2022 (latest data as of March 2024).

Variable	Statistical Parameter																					SANS (2015a)(1)
	Period August 2002 to January 2010							Period February 2010 to May 2018							Period July 2022 to November 2022							
	n	5%ile	Mean	Median	95%ile	SD	CoV (%)	n	5%ile	Mean	Median	95%ile	SD	CoV (%)	n	5%ile	Mean	Median	95%ile	SD	CoV (%)	
pH (-log <sub>10</sub> aH <sup>+</sup> )	251	7.7	—	8.1	8.4	0.2	2	202	7.5	8.2	8.2	8.5	0.3	3.7	5	7.0	7.2	7.2	7.3	0.1	1.7	5.0–9.7
SEC (mS/m)	232	51.1	61.2	62.3	66.8	5	8	199	61.5	93.4	90.1	126	23.2	24.8	5	81.5	81.5	124.1	137.7	26.3	32.3	<170
TDS (mg/L)	137	347.6	438.5	448.9	479.3	41.5	9	134	474.7	695.8	674.9	980.1	171.9	24.7	5	570.9	875.5	898.8	1153.8	250.6	28.6	<1200
Ca (mg/L)	172	40.1	51.3	52.1	57.7	5.31	10	191	54.3	96.3	90.7	153.7	33	34.3	5	47.9	147.0	152.2	281.5	105.2	71.6	n.s.
Mg (mg/L)	171	23.3	30.2	30.4	34.9	4.8	16	192	28.4	44.2	42.8	59.7	10.6	24	5	72.1	149.9	180.3	212.0	66.3	44.3	n.s.
Na (mg/L)	185	19.1	27.5	27.7	34	4.7	17	166	28.3	41.7	40.5	58.4	10.1	24.3	5	21.7	48.5	57.4	59.3	19.5	40.2	<200
K (mg/L)	173	1.4	2.4	2.4	3.4	0.7	27	169	2.9	4.1	4	5.7	1	24.4	5	5.5	5.8	5.8	6.3	0.3	5.8	n.s.
Cl (mg/L)	175	29.2	36.2	36.3	43.5	4.8	13	198	31.3	38.8	38.5	45.3	5.3	13.6	5	29.2	40.8	0.1	45.3	8.6	21.0	<300
SO <sub>4</sub> (mg/L)	191	63.4	85.8	83.9	110	15.1	18	189	96.8	284	247.8	475	132.7	46.7	5	290.2	398.3	412.3	501.4	91.2	22.9	<500
HCO <sub>3</sub> (mg/L)	185	146.1	188.1	190.2	216.1	21.1	11	189	242.4	422.2	404.4	621.6	121.6	28.8	5	0.0	134.7	142.9	159.0	32.0	23.8	n.s.
NO <sub>3</sub> +NO <sub>2</sub> (mg N/L)	214	3.294	4.74	4.414	7.085	1.19	25	193	3.5	5.6	5.4	8.3	1.6	27.7	5	2.6	5.8	5.6	8.8	2.8	47.6	<11
PO <sub>4</sub> (mg P/L)	247	0.043	0.189	0.158	0.451	0.131	69	197	0.005	0.097	0.048	0.261	0.124	127.8	5	0.262	0.524	0.400	0.925	0.300	57.3	n.s.
Si (mg/L)	247	4.93	5.84	5.83	6.69	0.6	10	198	4.95	5.64	5.6	6.59	0.61	10.86	5	4.7	5.6	5.7	6.3	0.7	12.8	n.s.
Fe (mg/L)	69	0.006	0.035	0.014	0.12	0.056	163	61	0.004	0.019	0.012	0.072	0.024	126.3	4	0.000	0.019	0.021	0.025	0.007	36.1	<2
Mn (mg/L)	69	0.001	0.049	0.002	0.146	0.226	459	61	0.001	0.162	0.003	0.05	0.855	527.8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	<0.5
Al (mg/L)	65	0.001	0.06	0.014	0.091	0.262	437	60	0.003	0.019	0.009	0.057	0.026	136.8	4	0.007	0.030	0.016	0.071	0.034	115.5	<0.3

(1) Standard health-related limit for consumption of 2 L/d over 70 years by a 60 kg person

#### 4.2.2 Municipal Wastewater Impact

The most recent results (March 2024) from hydrochemical and microbiological analyses of water samples collected by the CSIR along the Blougatspruit (both upstream and downstream of the Percy Stewart WWTW) and Bloubankspruit are summarised in **Table 5**. Unlike the acidic nature of the Tweelopiespruit and Rietspruit surface water, the hydrochemistry of the Blougatspruit and Bloubankspruit is characterised by relatively greater alkalinity, evidenced by higher bicarbonate values and pH values which consistently range between 7.1 to 7.9 (**Figure 15**). The moderately alkaline surface water reflects the continuing neutralising capacity of the carbonate strata and the impact of the municipal wastewater from the local WWTW.

The Blougatspruit is the conduit for the municipal wastewater effluent into the COHWHS property. The reticence of local government to provide water quality data for wastewater released to the Blougatspruit from the municipal wastewater treatment works (WWTW) has been documented previously (e.g. Hobbs, 2016a; 2016b; 2017a). Water samples collected ~5 km upstream (i.e. BG Spring) and ~5 km downstream of the WWTW end-of-pipe (EoP) (i.e. BG@N14 and BB@M ) provide a comparative measure of the bacteriological contamination in the Blougatspruit from this facility. This impact extends into the Bloubankspruit in its passage through the south-eastern portion of the property. The severity of the bacteriological contamination, more specifically of a faecal nature, is reflected in total coliform and *E. coli* values that routinely exceed a most probable number (MPN) count of 2419.6 per 100 mL at several sites along the Blougatspruit and Bloubankspruit, namely, BG@N14 and BB@M (**Table 5**). Furthermore, the CSIR project team has witnessed and received several accounts of obnoxious odours, murky water foam, and raw sewerage, in the Blougatspruit and Bloubankspruit rivers from local residents and reported to the MA, however the exact point of emergence of the water pollution has not been verified.

The South African Water Quality Guidelines (DWAf, 1996) do not have an applicable target water quality range for coliform in aquatic ecosystems, however, the drinking water guidelines (SANS 241:1, 2015) stipulate less than 10 MPN/100 mL (<10 MPN/100 mL) for total coliforms and zero detection for *E. coli*. According to the World Health Organization (2011), a count of 1–10 MPN/100 ml is regarded as low risk; 11–100 MPN/100 ml is moderate risk while an *E. coli* count greater than 100 MPN/100 ml is adjudged high risk. *E. coli* counts greater than 1000 MPN/100 ml are considered to be extreme high risk. Given the bulk volume of raw sewerage noted in these rivers, the surface water in these systems can be regarded as extreme high risk and it can also be argued that the municipal wastewater poses an equally dire threat to the quality and subsequent fitness for use of receiving surface water resources as does mine water. This threat extends into the Crocodile River as main stem of the Bloubankspruit.

**Table 5** Water chemistry and microbiological results of samples collected at monitoring sites along the Blougatspruit, Bloubankspruit, Rietspruit and Tweelopiespruit in June 2023, September 2023 and March 2024.

Variable	BG Spring (Blougatspruit)		BB @ N14 (Blougatspruit)			BB @ M (Bloubankspruit)			MRd (Rietspruit)			Pinocchio's Farm (Tweelopiespruit)			SANS 241  (2015a)(1)
	Sep 23	Mar 24	Jun 23	Sep 23	Mar 24	Jun 23	Sep 23	Mar 24	Jun 23	Sep 23	Mar 24	Jun 23	Sep 23	Mar 24	
pH (-log <sub>10</sub> αH <sup>+</sup> )	7.8	7.9	7.3	7.5	7.5	7.1	7.4	7.6	4.4	4.1	4	6.2	5.5	4.3	5.0–9.7
SEC (mS/m)	68	52	80	110	72	180	185	170	290	880	290	300	900	285	<170
Ca (mg/L)	73	59	29	32	34	237	223	210	491	481	535	527	495	540	n.s.
Mg (mg/L)	45	37	11	11	11	59	64	56	78	81	1.6	83	80	1.4	n.s.
Na (mg/L)	3.7	1.6	67	105	57	87	97	79	118	125	132	126	124	134	<200
K (mg/L)	<0.6	<0.6	15	14	11	8.8	8.7	9.4	11	11	13	11	11	12	n.s.
Cl (mg/L)	<2.0	3.3	65	98	68	57	64	57	52	50	52	55	52	52	<300
SO <sub>4</sub> (mg/L)	26	22	81	94	47	801	802	702	1610	1706	1753	1735	1725	1764	<500
HCO <sub>3</sub> (mg/L)	336	273	188	323	196	118	169	148	<1.0	<1.0	<1.0	5.1	2.9	<1.0	n.s.
NO <sub>3</sub> +NO <sub>2</sub> (mg N/L)	0.17	0.58	<0.05	<0.05	<0.05	0.43	<0.05	0.18	6.4	8.1	9.1	5.3	6.4	8.1	<11
Si (mg/L)	7.5	4.8	6.5	7.6	5.5	5.7	6.2	5.4	3.4	2.9	3	3.5	3.1	2.9	n.s.
Fe (mg/L)	0.03	0.09	1	2	3.3	0.43	0.81	1.3	0.18	0.11	0.16	0.1	0.04	0.13	<2
Mn (mg/L)	<0.01	<0.01	0.38	0.67	0.71	1.39	0.65	0.47	3.62	1.3	1.6	3.51	1.2	1.4	<0.5
Al (mg/L)	<0.02	<0.02	0.21	0.47	3.3	0.16	0.21	1.3	0.13	0.05	0.1	0.08	<0.02	0.06	<0.3
Total coliform bacteria (MPN/100 ml)	1553.1	123.6	>2419.6	>2419.6	>2419.6	>2419.6	>2419.6	>2419.6	>2419.6	>2419.6	>2419.6	>2419.6	>2419.6	>2419.6	<10
E. coli (MPN/100 ml)	(63.8)	3.0	>2419.6	>2419.6	>2419.6	>2419.6	>2419.6	>2419.6	547.5	298.7	686.7	106.7	201.4	79.4	n.d.



**Figure 15** During the March 2024 monitoring run, the water and rocky riverbed of the Tweelopiespruit (A) and Rietspruit (B) appeared to have a yellowish tint and field readings indicated low pH (<6.0). Further downstream, the opaque and foul-smelling Blougatspruit (C) and (D), had apparent settling of sludge along its banks.

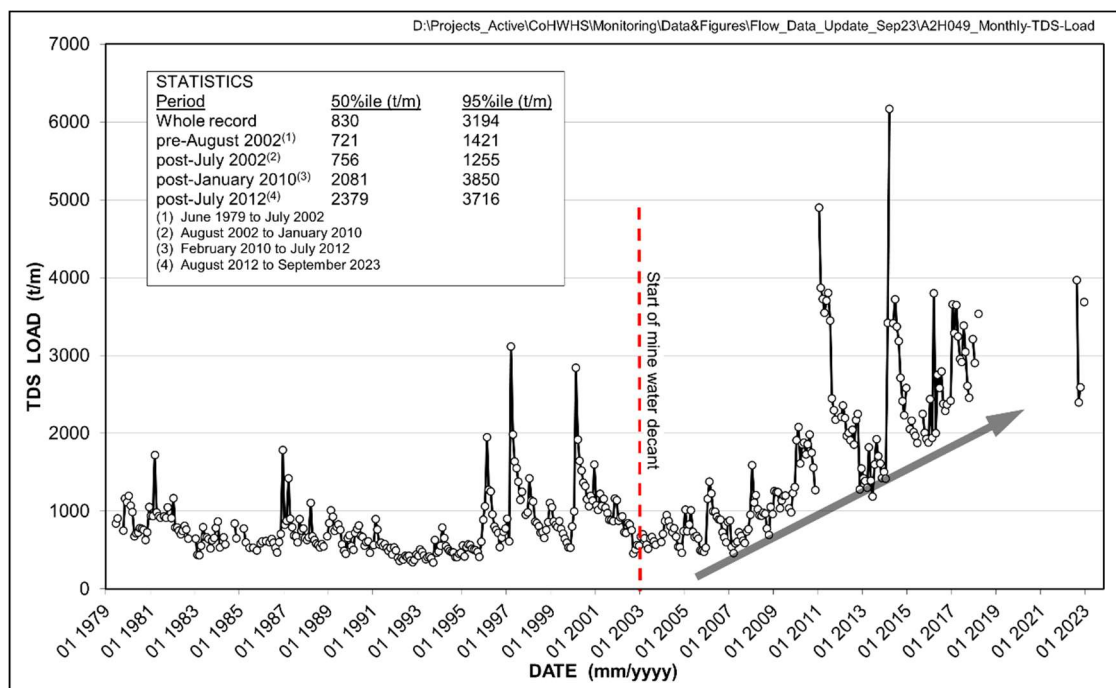
#### 4.4 Salt Load

The latest available analysis of hydrochemical data from station A2H049 is as captured in the April 2023 to September 2023 edition of the Status Quo report (Mvandaba and Shadung, 2023). The DWS has since reported an inability to update the data, citing data connection issues, instrument failure as well as cost-

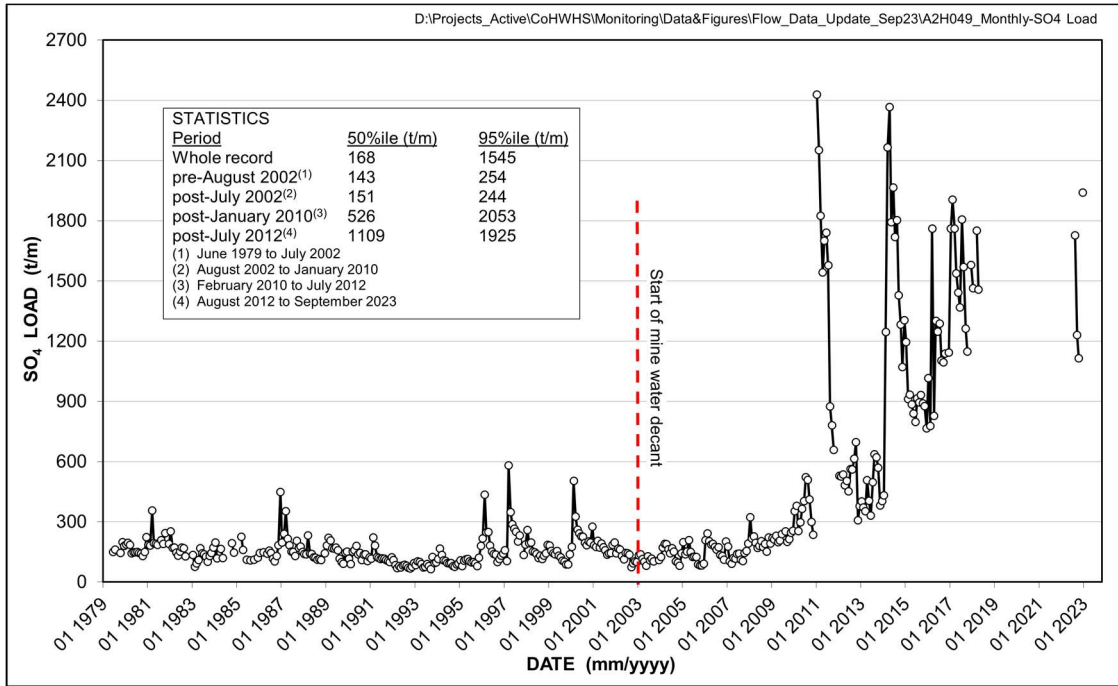
cutting measures which reduced the frequency of data collection at the station (Botes, March 2024).

The six years gap of missing data between May 2018 and July 2022 presents a challenge with comprehensively interpreting the long-term monthly trend in the TDS load delivered by the Bloubankspruit (**Figure 16**). However the statistics presented indicate an increasing salt load since early-2007. The text box in **Figure 16** lists the median and 95%ile values associated with different periods of record. The period February 2010 to July 2012 reveals a significant increase in the median and 95%ile values. This is readily attributable to the very high salt loads experienced in the 2011 hydrological year. Similar conditions prevailed in the subsequent period (August 2012 to March 2018) as indicated in **Figure 16** (text box). An evaluation of the sub-regional and regional temporal salt loads delivered to Hartbeespoort Dam is presented by Hobbs (2017c).

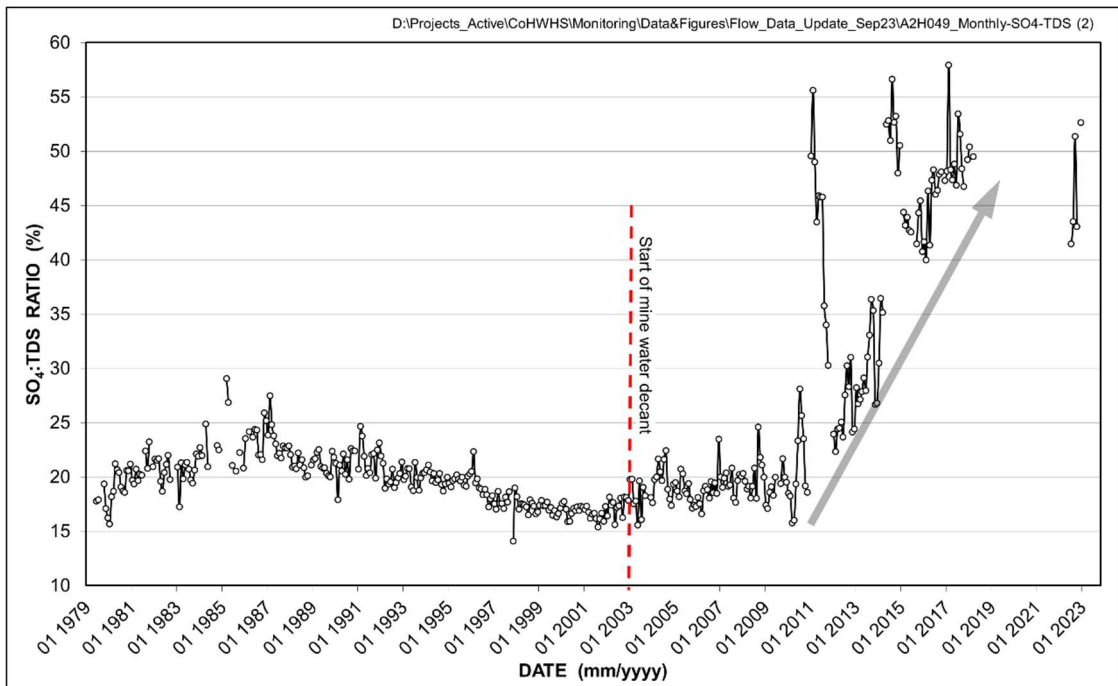
Similarly, the long-term monthly trend in the SO<sub>4</sub> load delivered by the Bloubankspruit (**Figure 17**) mimics the TDS load pattern (**Figure 16**) in the period since early-2010. The most recent period (August 2012 to September 2023) exhibits a lower median value of 1925 t/m compared to the 2053 t/m of the preceding period (February 2010 to July 2012). These circumstances indicate that the most recent period experienced lower sulphate loads than previously. **Figure 18** and **Figure 19** reflect more recent SO<sub>4</sub>: TDS ratio values in the range 41 to 52%.



**Figure 16** Long-term (June 1979 to September 2023) monthly TDS load pattern and trend in the Bloubankspruit at station A2H049



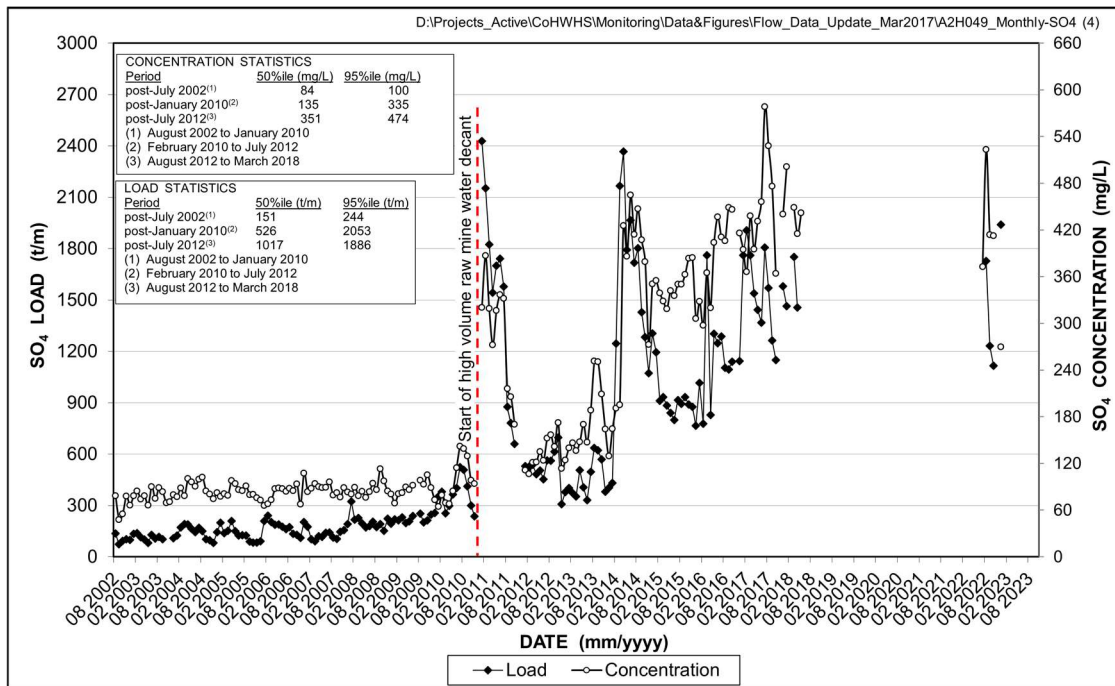
**Figure 17** Long-term (June 1979 to September 2023) monthly SO<sub>4</sub> load pattern and trend in the Bloubankspruit at station A2H049



**Figure 18** Long-term (June 1979 to March 2018) trend in the SO<sub>4</sub>: TDS ratio at station A2H049



**Figure 19** Pattern and trend of the SO<sub>4</sub>: TDS ratio at station A2H049 since the start of mine water decant in the Western Basin in mid-2002



**Figure 20** Monthly SO<sub>4</sub> concentration and load pattern and trend in the Bloubankspruit at station A2H049 since mid-2002

## 5 GROUNDWATER HYDROLOGY

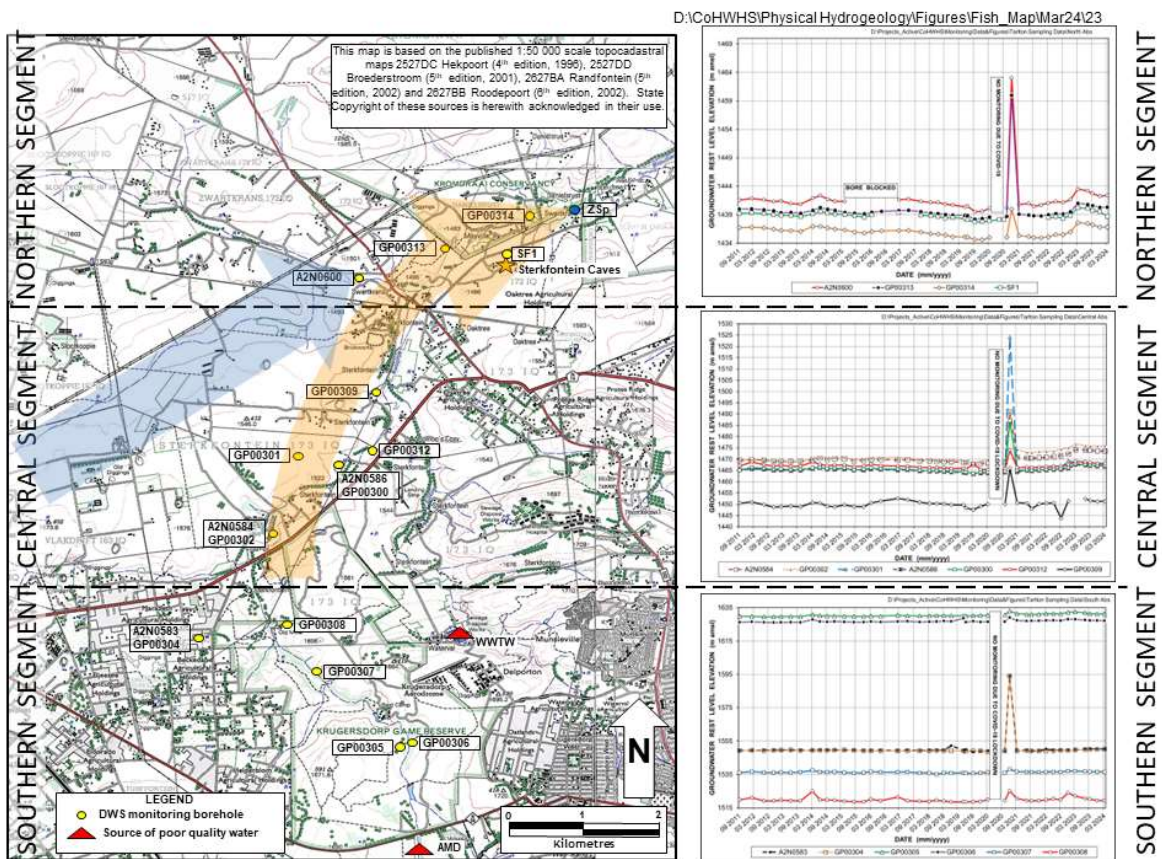
### 5.1 Physical Hydrogeology

#### 5.1.1 Monitoring Framework

An inspection of the more recent potentiometric response in DWS monitoring boreholes located downstream of the mine area is presented in **Figure 21**. The boreholes are grouped into a southern, a central and a northern segment to distinguish between their relative locations in the downstream receiving hydrogeologic environment. This distinction is brought out by using absolute groundwater level elevations that describe a decrease from south to north both within and between the respective segments. The most recent data presented in this report corresponds to monitoring conducted in March 2024.

#### 5.1.2 Sub-regional Groundwater levels

The groundwater hydrographs shown in **Figure 21** illustrate a slight decrease in the groundwater level elevations in the northern and central segments between March 2023 and March 2024. Barring the marked decrease in the groundwater level for station GP00308, the groundwater elevations in the southern segment display relatively stable values. The lake water level in Sterkfontein Cave at the north-eastern discharge end of the Zwartkrans Basin most clearly reflects the general decrease in groundwater levels, as shown in **Figure 22**.



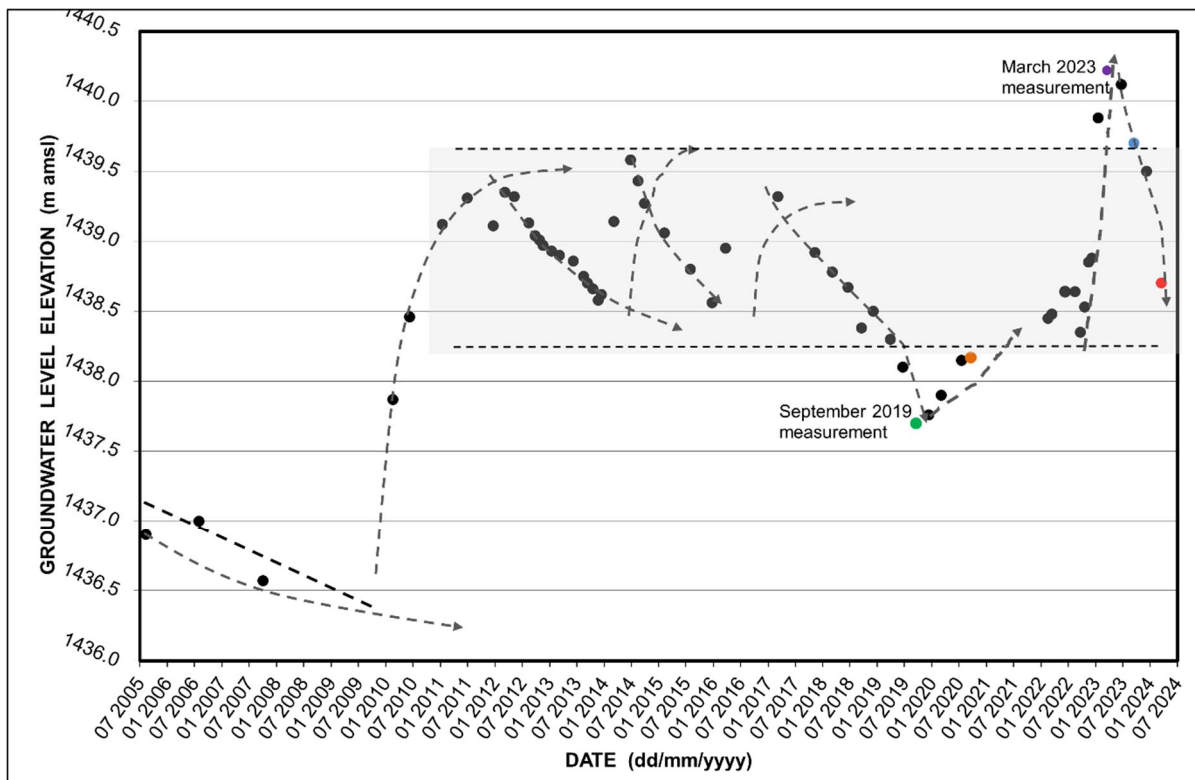
**Figure 21** Distribution of DWS monitoring boreholes with groundwater hydrographs (right); brown arrow denotes principal direction of impacted groundwater flow, and blue arrow direction of natural karst groundwater flow

### 5.1.3 Sterkfontein Cave Water Level

The global importance of Sterkfontein Cave (**Figure 23**), recognized as the primary fossil site within the property, places a spotlight on any potential impact affecting this site. An especially notable event occurred between 2010 and early 2012 when the cave water level experienced a substantial rise of approximately 3 m. This increase raised concerns and prompted a detailed exploration of the cave water level's hydrostatic behaviour, extensively examined by Hobbs and De Meillon (2017).

**Figure 22** visually represents the cave water level's response over the last 14 years. The hydrograph depicts fluctuations since mid-2010, totalling approximately 1 meter, with elevations varying within the range of 1439 ± 0.5 meters above mean sea level (amsl). It is hypothesized that the cave lake will persist at this level in the future due to continuous higher discharge rates in the upper tributaries of the Bloubankspruit, namely the Tweelopies/Rietspruit system and the Blougatspruit. These tributaries drive allogenic groundwater recharge from mine water and municipal wastewater into the Zwartkrans Basin (Hobbs et al., 2018).

Noteworthy deviations from the expected trend occurred in September 2018, December 2018, March 2019, and September 2019, when groundwater levels reached their lowest points since the end of 2010. In contrast, the Cave experienced its highest recorded groundwater level in March 2023 following heavy rainfall, which consequently caused some structural damage to the roof of the Cave. Since March 2023, the groundwater level has decreased by approximately 1.5 meters – the red data point on **Figure 22** illustrate the March 2024 elevation of 1438.7 m amsl. This decline aligns with the observed decrease in groundwater rest level elevation in the central and northern segments of the COHWHS property, as illustrated in **Figure 21**. These findings provide crucial insights into the evolving hydrodynamic conditions and groundwater dynamics within the property.



**Figure 22** Groundwater level response pattern and trend in borehole SF1 that serves as a proxy for the lake water level in Sterkfontein Cave; blue and red data points respectively represent September 2023 and March 2024 groundwater level and the shaded area denotes magnitude of fluctuation since mid-2010



**Figure 23** The groundwater level of the Sterkfontein Cave Lake as observed in March 2024.

#### **5.1.4 Discharge from the Dolomitic Springs**

Every six months, the discharge from the dolomitic springs in the COHWHS is measured, specifically targeting the end of the wet (March/April) and dry (September/October) seasons. This bi-annual measurement schedule aims to analyze variations in spring contributions to river flow, expand the limited spring discharge database, and identify any future impacts. Since March 2019, flow has been measured at eight of the ten identified springs (**Table 6**). The 'measured discharge' represents measurements taken upstream and downstream of the spring's source, with 'spring discharge' calculated from the difference between these two values. In some locations, such as the Tweefontein and Nash springs, only a portion of the stream's width is gauged due to the lack of suitable cross-sectional areas, necessitating an appropriate uncertainty factor.

For this report, discharge measurements from March 2023 and March 2024 are compared (**Table 6**). The discharge recorded for each spring in March 2023 is notably greater than in March 2024, corresponding to heavy rainfall and subsequent flooding in 2023. Significant discharges were recorded at the Zwartkrans,

Nash, and Nouklip springs, with approximately 157.85 ML/d, 19.88 ML/d, and 21.27 ML/d, respectively, in March 2023, compared to 8.67 ML/d, 16.92 ML/d, and 14.11 ML/d, respectively, in March 2024. This indicates a decrease of 4.35 ML/d (20%) for Nouklip Spring and 5.77 ML/d (29%) for Nash Spring.

However, there is significant uncertainty in these measurements due to continuous and direct extraction at the springs' sources and turbulence over rocky streambeds. The specific responses of these springs to rainfall and discharge variations within the COHWHS property have not been precisely quantified. A comprehensive analysis considering all factors affecting channel transmission losses, such as water usage and evapotranspiration rates, is necessary for a more precise understanding of the springs' behavior and improved management strategies.



**Figure 24** The concrete furrow near the eye of the Broederstroom spring where water collects as it flows downstream

**Table 6** Dolomitic spring discharge measured in March 2023 and March 2024

Spring	Compartment	Measured Discharge (Mar 23)		Spring Discharge (Mar 23)		Measured Discharge (Mar 24)		Spring Discharge (Mar 24)		Previous Discharge Volume <sup>1</sup>	
		(m <sup>3</sup> /s)	(ML/d)	(m <sup>3</sup> /s)	(ML/d)	(m <sup>3</sup> /s)	(ML/d)	(m <sup>3</sup> /s)	(ML/d)	(m <sup>3</sup> /s)	(ML/d)
Zwartkrans (upstream)	Zwartkrans	1.30	111.85	0.34	29.32	0.51	43.94	0.10	8.67	~0.1	~8.6
Zwartkrans (downstream)		1.63	141.17			0.61	52.61				
Plover's Lake	Krombank	0.06	5.54	0.06	5.54	0.046	3.96	0.046	3.96	~0.06	~5.2
Plover's Lake (culvert)											
Kromdraai (upstream)	Krombank	–	–	–	–	–	–	–	–	~0.28	~24.1
Kromdraai (downstream)		1.29	111.56			1.25	107.70				
Danielsrust	Danielsrust	0.07	5.75	0.07	5.75	0.05	4.10	0.05	4.10	~0.03	~2.4
Nouklip	Diepkloof	0.25	21.27	0.25	21.27	0.196	16.92	0.196	16.92	~0.14	~12.4
Tweefontein	Tweefontein	0.14	3.82	0.14	3.82	0.02	1.91	0.02	1.91		
Nash	Uitkomst	0.23	19.88	0.23	19.88	0.16	14.11	0.16	14.11		
Broederstroom	Broederstroom	0.04	3.28	0.04	3.28	0.03	2.4	0.03	2.4	~0.02	~1.8

<sup>(1)</sup> After Hobbs (2011)

<sup>(2)</sup> Measured at Plover's Lake 'control site'

## 5.2 Chemical Hydrogeology

### 5.2.1 DWS Monitoring Framework

The DWS groundwater monitoring programme in the south-western portion of the property was substantially expanded with the establishment of an additional 13 monitoring boreholes in late-2010. These stations (identified by the alpha-numeric code GP00####) supplement the four stations (identified by the alpha-numeric code A2N0####) that are the legacy of the mid-1980s DWAF study (Bredenkamp *et al.*, 1986) in the region. In 2022, the borehole near the Zwartkrans Spring (26.008220 S, 27.745 E) was added to the DWS monitoring programme as site A2N0811. The distribution of the monitoring network is shown in **Figure 25** and **Figure 26**. Whereas the older stations support a quasi-continuous monitoring record dating back to 2003, the record of the newer stations commences in March 2011. The outcome of this DWS groundwater monitoring programme (hereinafter referred to as simply ‘the monitoring programme’) forms the basis for evaluating the hydrochemical impact of mine water on the receiving karst environment (**Section 5.2.2**). The most recent chemical analyses of groundwater provided for the south-western portion of the property corresponds to monitoring conducted in July 2023, and September 2023.

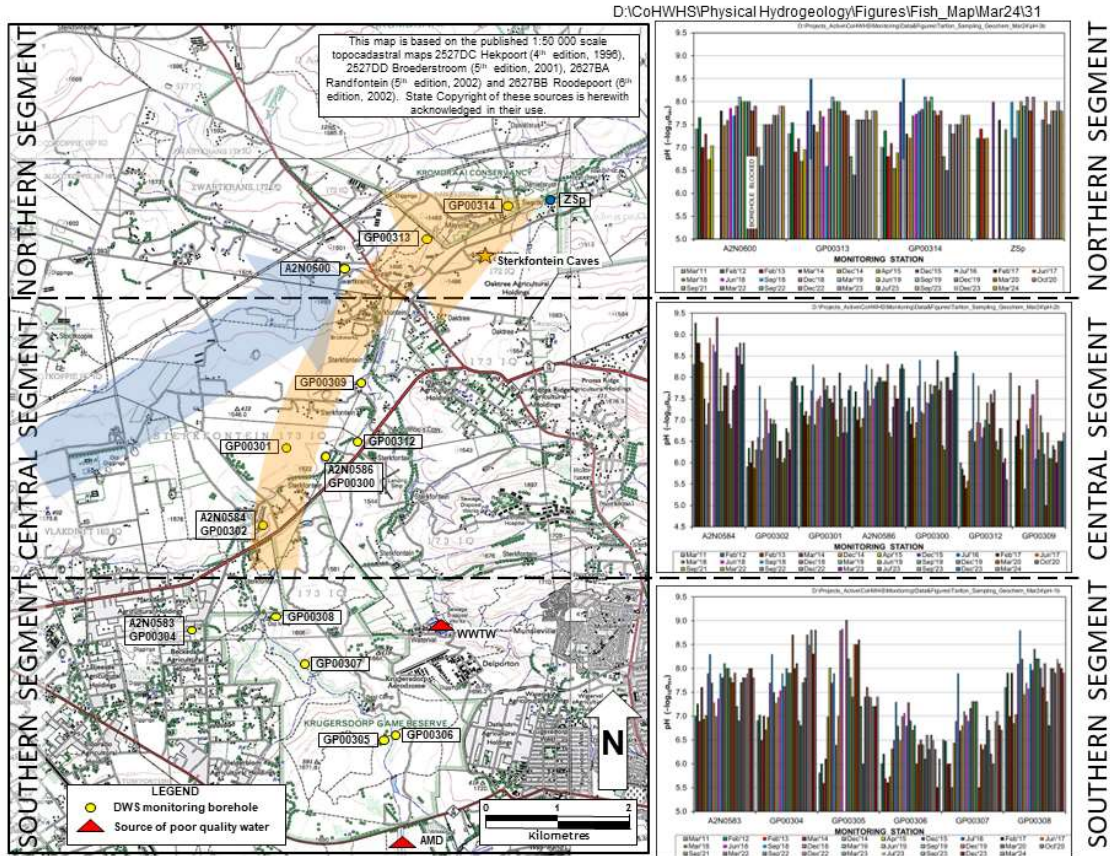
### 5.2.2 Mine Water Impact

The magnitude of the mine water impact on the karst aquifer in the Zwartkrans Basin is illustrated in **Figure 25** and **Figure 26** with the aid of bar graphs for the chemical variables pH and SEC respectively. Spatially, the bar graphs in **Figure 25** reflect a general progressive decrease in pH, from south to north within the southern, central and northern segments. The pH trendlines at individual stations however show seasonal variability, albeit marginal. Recent pH values in the southern segment bracket the range 6.1 to 8.8, and those in the central segment range from 6.7 to 8.8. In the northern segment, the most recent pH values are in the range 7.8 to 7.9. Due to the dissolution of carbonate rocks, the pH of karst groundwater is buffered and nearly neutral across all the groundwater sites.

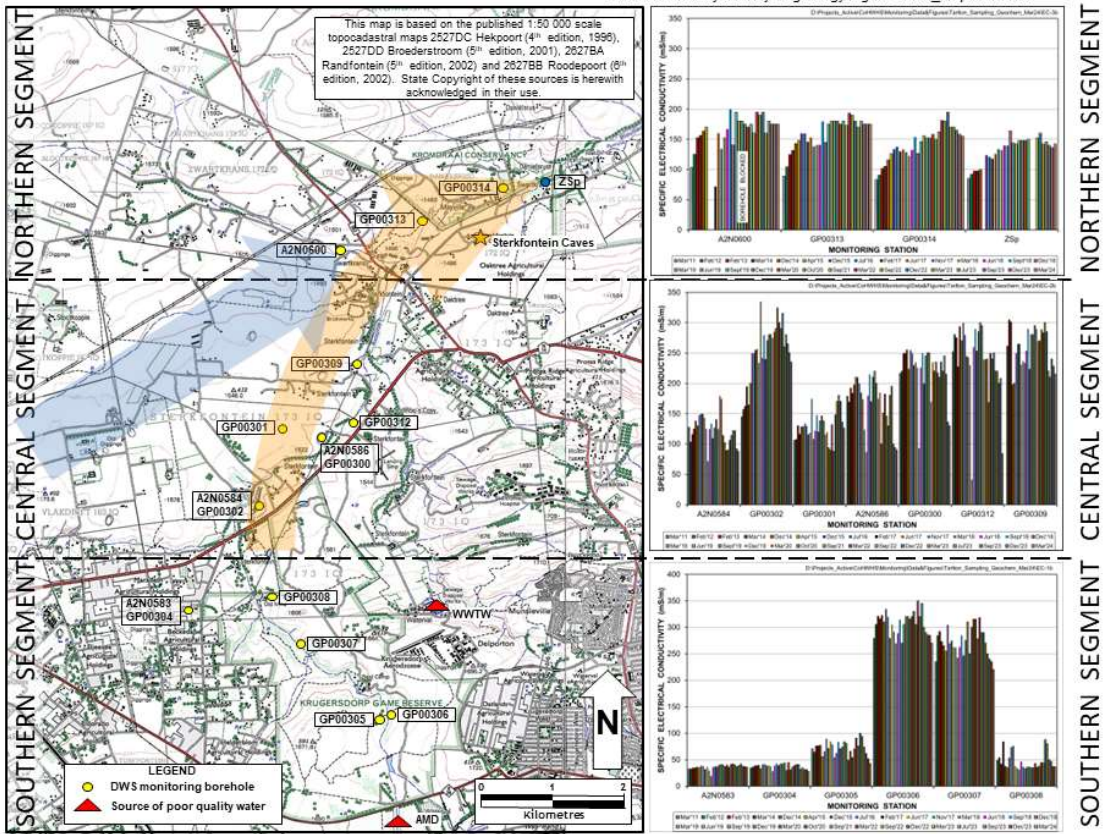
The specific electrical conductivity (salinity) of groundwater sampled in the southern and central segments of the study area demonstrates a general progressive increase in salinity from south to north. In all instances, the recent individual salinity values are either similar to or slightly higher than earlier results. The proximity of the Tweelopiesruit and Rietspruit to these sites appears to impact groundwater salinity in these segments. However, in the northern segment, the spatial salinity trend along the flow path shows a decline, with a decreasing trend in salinity at each station, except for the Zwartkrans Spring. The recent (March 2024) salinity values are constrained to the ranges of 142 to 175 mS/m in the northern segment, 84 to 235 mS/m in the central segment, and 28 to 270 mS/m in the southern segment. The patterns described above reflect the north to north-easterly flow path followed by the allogenic recharge of mine water in the karst aquifer which is also described in **Figure 27**.

The extent of the mine water impact on the karst aquifer of the Zwartkrans Basin is shown in **Figure 27**, and provides an indication of the sulphate trend at each monitoring station in terms of increasing, stable or decreasing in the recent past, by comparing the sulphate concentration values of July 2016, February 2017, November 2017, March 2018, June 2018, September 2018, December 2018, March 2019, June 2019, September 2019, December 2019, March 2020, October 2020, July 2021, September 2022, December 2022, March 2023, June 2023, September 2023 and March 2024. Consistent with previous reports, the trendlines from September 2023 to March 2024 confirm the spatial variability of sulphate concentrations at individual stations. These trendlines illustrate that sulphate values at the north-eastern end of the study area are typically lower than those at sites closer to the mine locations. Furthermore, there is a general

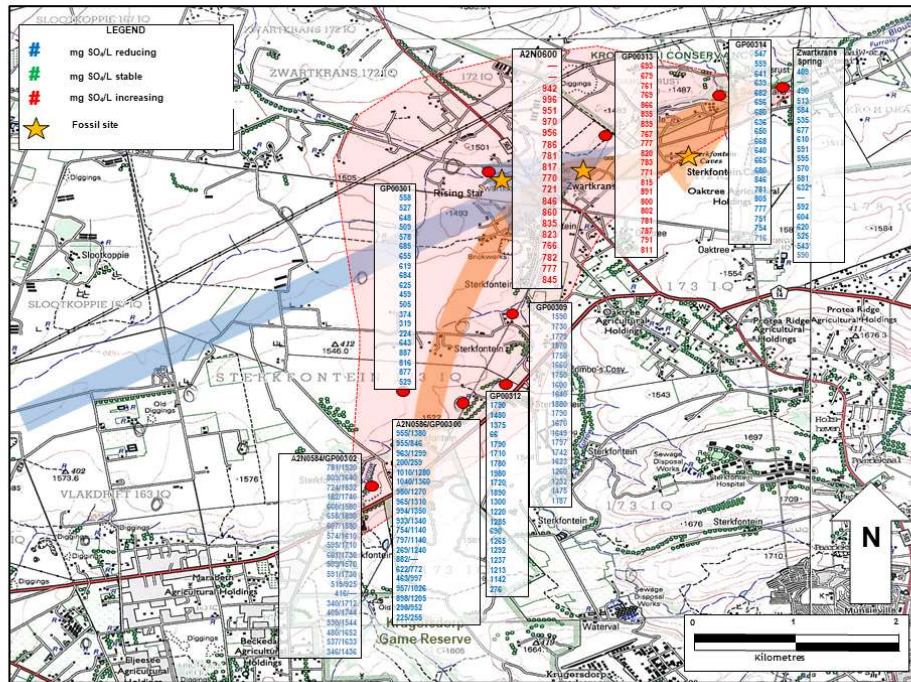
decrease in sulphate levels across the monitoring stations from September 2023 to March 2024, with the exceptions of stations GP00313 and A2N600. The seasonal variation suggests higher sulphate concentrations in water resources during the dry season compared to the wet season.



**Figure 25** Distribution of DWS monitoring boreholes with pH pattern and trend as bar graphs; brown arrow denotes principal direction of impacted groundwater flow, and blue arrow direction of natural karst groundwater flow



**Figure 26** Distribution of DWS monitoring boreholes with SEC pattern and trend as bar graphs; brown arrow denotes principal direction of impacted groundwater flow, and blue arrow direction of natural karst groundwater flow



**Figure 27** Distribution of sulphate levels in groundwater of the Zwartkrans Basin in July 2016 (1<sup>st</sup> value), February 2017 (2<sup>nd</sup> value), November 2017 (3<sup>rd</sup> value), March 2018 (4<sup>th</sup> value), June 2018 (5<sup>th</sup> value), September 2018 (6<sup>th</sup> value), December 2018 (7<sup>th</sup> value), March 2019 (8<sup>th</sup> value), June 2019 (9<sup>th</sup> value), September 2019 (10<sup>th</sup> value), December 2019 (11<sup>th</sup> value), March 2020 (12<sup>th</sup> value), October 2020 (13<sup>th</sup> value), July 2021 (14<sup>th</sup> value), September 2022 (15<sup>th</sup> value), December 2022 (16<sup>th</sup> value), March 2023 (17<sup>th</sup> value), July 2023 (18<sup>th</sup> value), September 2023 (19<sup>th</sup> value) and March 2024 (20<sup>th</sup> value), also showing the principal vectors of allogenic recharge (brown arrow), autogenic recharge (blue arrow), the postulated footprint (shaded area) of a mine water impact in the karst aquifer, and SEC trend as INCREASING (red text), STABLE (green text) or REDUCING (blue text).

### 5.2.3 Sterkfontein Cave

**Table 7** presents the water chemistry results of groundwater samples collected at the Sterkfontein Cave in March 2019, June 2019, September 2019, December 2019, March 2020, September 2020, September 2022, December 2022 and March 2024. Typically, the water quality of the groundwater forming the underground lake in the Sterkfontein Cave is monitored every quarter. However, the Cave was inaccessible since March 2023 due to the collapse of its roof following the heavy rainfall experienced during the early months of the wet season, from November 2022 to February 2023.

The recent results from March 2024 indicate consistency with the historic values reported for this site. None of the chemistry variables reported for, exceed the respective SANS (2015a) health-related limits for potable water. However, the microbiological quality of the water is compromised by the total coliform bacteria which exceeds the standard health-related limits across all sample results and thus reflects the impact of poorer quality surface water on the karst groundwater. The moderately alkaline pH value reflects the continuing neutralising capacity of the carbonate strata. Because the Sterkfontein Cave is situated away from the main groundwater flow vector towards Zwartkrans Spring, mine water has less of an effect on the lake's water chemistry.

**Table 7** Water chemistry results of samples collected at the Sterkfontein Cave between 2019 and 2024.

Variable	Mar 19	Jun 19	Sep 19	Dec 19	Mar 20	Jul 20	Sep 20	Sep 22	Dec 22	Mar 24	SANS (2015a) <sup>(1)</sup>
pH (-log <sub>10</sub> a <sub>H<sup>+</sup></sub> )	8	8.1	8.1	8.2	7.9	4.7	7.9	8	8.2	7.8	5.0–9.7
SEC (mS/m)	92	92	95	94	100	90	100	96	94	88	<170
Ca (mg/L)	90	89	88	89	91	95	88	88	70	86	n.s.
Mg (mg/L)	53	54	54	53	50	58	51	51	51	50	n.s.
Na (mg/L)	35	37	33	34	35	38	33	35	35	33	<200
K (mg/L)	1.5	1.6	1.5	1.5	2	1.5	1.3	1.5	1.5	1.5	n.s.
Cl (mg/L)	37	37	36	37	38	37	37	36	36	35	<300
SO <sub>4</sub> (mg/L)	273	271	259	262	298	286	267	262	270	246	<500
HCO <sub>3</sub> (mg/L)	148	146	148	142	146	160	145	147	147	153	n.s.
NO <sub>3</sub> +NO <sub>2</sub> (mg N/L)	7.1	7	6.6	7	7.4	7.6	7	7.1	7.6	7.2	<11
Si (mg/L)	5.9	7	5.8	6	5.8	5.6	6	6.3	5.9	6.5	n.s.
Fe (mg/L)	0.02	0.31	0.02	0.17	0.04	<0.01	0.03	0.03	<0.02	0.39	<2
Mn (mg/L)	<0.01	0.32	<0.01	0.03	0.02	0.02	0.01	<0.01	<0.03	<0.03	<0.5
Al (mg/L)	0.03	0.14	0.02	0.05	0.03	0.02	0.02	<0.01	<0.02	0.18	<0.3
Total coliform bacteria (MPN/100 ml)	1046.2	125.9	35.5	248.1	547.5	78	32.3	78.9	1299.7	579.4	<10
E. coli (MPN/100 ml)	<9.8	4.1	<1.0	3.1	3.1	<1	<1	14.4	12.2	5.2	n.d.

<sup>(1)</sup> Standard health-related limit for consumption of 2 L/d over 70 years by a 60 kg person

n.s no standard

n.d not detected

#### 5.2.4 Dolomitic Springs

As part of Project GT/WHS/021/2022 the CSIR is responsible for the bi-annual water quality monitoring of the dolomitic springs in the study area (**Figure 28**). Sampling occurred in September 2023 and March 2024, with the corresponding results outlined in **Table 8**. Additionally, **Section 5.2** provides a comparison between historical and recent water quality data concerning the Zwartkrans Spring.

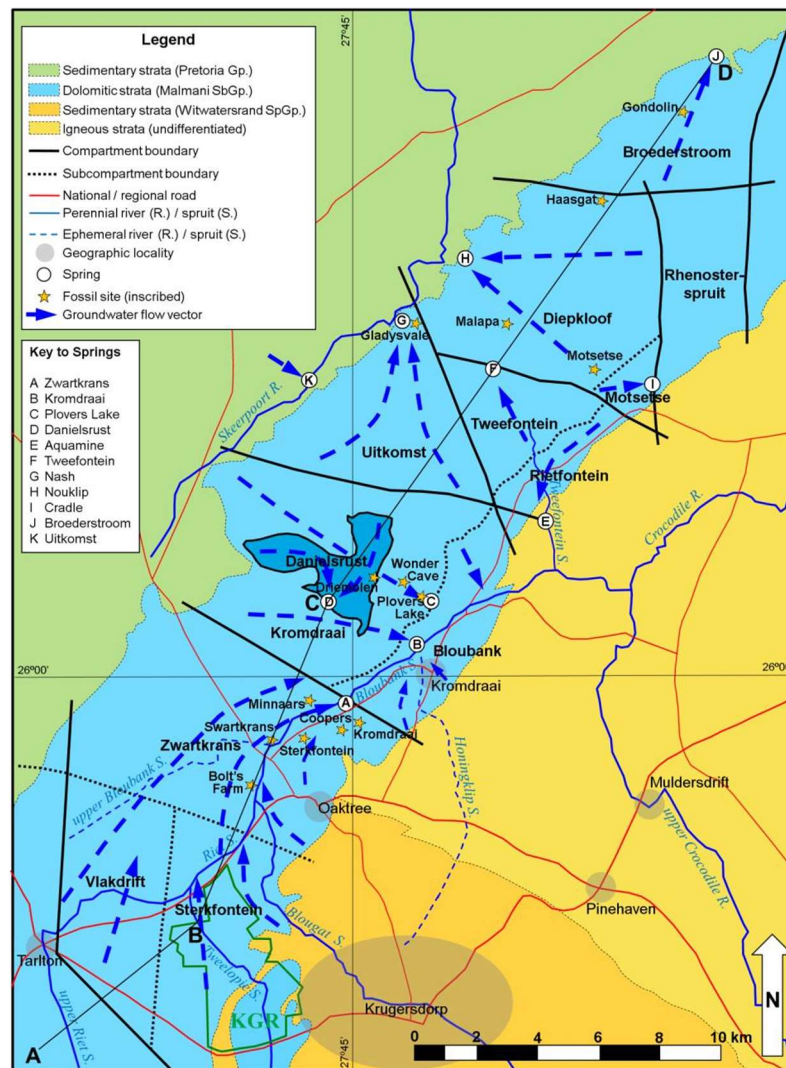
Barring the Zwartkrans and Aquamine springs, the groundwater quality from the main dolomitic springs is of good standard for the chemical parameters indicated in the sample data between September 2023 and March 2024. Consistent with the results from September 2023 (Mvandaba et al., 2023), the chemical analysis of water from the Zwartkrans Spring indicates a probable impact from mine water, as elevated sulphate levels surpass established health-related limits (SANS 241:1, 2015a). The groundwater from the Aquamine Spring continues to exhibit relatively higher concentrations of manganese, iron, and aluminium, as noted in previous reports (Mvandaba et al., 2020b; Mvandaba et al., 2023). These minerals occur naturally in karst sediments, and their fluctuation may be linked to the resuspension of minerals due to sediment movement during periods of heavy rainfall (Diković and Koželj, 2015) and runoff.

Across all the spring sample results, the microbiological quality of the water is compromised by total coliform bacteria, which far exceed standard health-related limits. In water resource management, the counts of total coliform bacteria and E. coli are indicators of the sanitary quality of water and the degree of pollution. Total coliform bacteria are common in natural environments, such as soil, vegetation, and aquatic ecosystems, and

may not necessarily be harmful. However, elevated counts indicate vulnerability to contamination by more harmful microorganisms. *E. coli*, a strain of coliform bacteria found only in the intestines of mammals, including humans, can enter natural environments via poorly treated municipal effluent, stormwater runoff, manure used in agricultural practices, and droppings from domestic animals and wildlife.

The South African Water Quality Guidelines (DWA, 1996) do not specify a target range for coliform in aquatic ecosystems, but the drinking water guidelines (SANS 241:1, 2015) stipulate less than 10 MPN/100 mL for total coliforms and zero detection for *E. coli*. According to the World Health Organization (2011), a count of 1–10 MPN/100 mL is regarded as low risk, 11–100 MPN/100 mL as moderate risk, and greater than 100 MPN/100 mL as high risk. *E. coli* counts greater than 1,000 MPN/100 mL are considered extreme high risk.

The most impacted site, the Kromdraai Spring, exhibited high levels of total coliform bacteria but moderate faecal contamination. Due to its position within the channel of the Bloubankspruit, some intermixing may occur between groundwater emerging from the spring and surface water within the Bloubankspruit, impacting the observed results. The results from the other dolomitic springs showed minimal *E. coli* contamination and can therefore be considered low risk.



**Figure 28** Map showing the regional geology of the study area, dolomitic compartment boundaries, groundwater flow vectors and the major dolomitic springs

**Table 8** Water chemistry and microbiological results of samples collected at the major dolomitic springs during September 2023 and March 2024.

Variable	Zwartkrans Spring		Kromdraai Spring		Plovers Lake Spring		Danielsrust Spring		Aquamine Spring		Tweefontein Spring		Nouklip Spring		Nash Spring		Broederstroom Spring		SANS	
	Sep 23	Mar 24	Sep 23	Mar 24	Sep 23	Mar 24	Sep 23	Mar 24	Sep 23	Mar 24	Sep 23	Mar 24	Sep 23	Mar 24	Sep 23	Mar 24	Sep 23	Mar 24	(2015a)(1)	
pH (– log <sub>10</sub> aH <sup>+</sup> )	7.8	7.8	8.1	8	8.1	8.2	8	8	8	7.9	8.1	8	8.1	8.1	8.1	8.1	7.9	7.9	5.0–9.7	
SEC (mS/m)	140	142	70	70	40	37	30	62	54	56	39	37	38	26	26	37	56	52	<170	
Ca (mg/L)	152	168	66	70	41	47	30	32	61	61	41	43	41	28	27	43	57	59	n.s.	
Mg (mg/L)	76	82	39	41	25	27	19	19	35	35	25	26	25	17	16	26	35	37	n.s.	
Na (mg/L)	55	60	19	1.3	1.8	3	1.5	20	3.3	3.8	1.2	1.2	1.2	1.4	1.3	1.2	1.3	1.6	<200	
K (mg/L)	22	<3	0.9	0.8	<0.6	<0.6	<0.6	<0.6	0.9	0.9	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	n.s.	
Cl (mg/L)	42	42	23	22	22	2.5	3.3	3.2	3.3	5.9	<2.0	3.5	<2.0	<2.0	<2.0	4	<2.0	3.3	<300	
SO <sub>4</sub> (mg/L)	543	590	154	160	6.9	22	6.6	5.2	8.5	12	7.5	6.9	5.6	1.9	1.8	5.6	19	22	<500	
Alkalinity as CaCO <sub>3</sub> (mg/L)	146	149	164	167	197	198	142	141	283	284	198	198	198	135	133	201	267	273	n.s.	
NO <sub>3</sub> +NO <sub>2</sub> (mg N/L)	8.4	8.4	3.9	3.5	0.67	0.73	0.76	0.78	0.21	0.34	0.71	0.73	0.34	0.21	0.18	0.33	0.58	0.58	<11	
Si (mg/L)	6.6	6.4	5.9	5.6	6.2	6	5.5	5.4	7.2	12	5	4.9	5.3	5	5.4	5	5	5	4.8	n.s.
Fe (mg/L)	0.02	0.04	0.02	0.09	0.01	0.03	0.03	0.02	0.89	7.4	0.12	0.04	0.04	0.05	0.04	0.2	0.02	0.09	<2	
Mn (mg/L)	<0.01	<0.01	<0.01	0.01	<0.01	0.02	<0.01	<0.01	0.52	0.68	0.3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.5	
Al (mg/L)	<0.02	<0.01	<0.02	0.03	0.08	<0.02	<0.02	<0.02	0.43	3.3	0.04	<0.02	<0.02	<0.02	0.04	<0.02	<0.02	<0.2	<0.3	
Total coliform bacteria (MPN/100 ml)	48.7	>2419.6	1373.9	>2419.6	50.4	187.2	45.7	387.3	>2419.6	>2419.6	387.3	248.1	50.4	2.0	32.7	29.5	14.6	14.6	<10	
E.coli (MPN/100 ml)	<1	24.1	29.5	260.3	<1	<1	<1	20.1	2	980.4	8.6	10.9	1	<1	4.1	<1	<1	<1	n.d.	

(1)- Standard health-related limit for consumption of 2 L/d over 70 years by a 60 kg person; n.s. – no standard; n.d. – not detected

## 6 RIVER HEALTH ASSESSMENT

In accordance with the SLA governing project GT/WHS/021/2022, the water resources monitoring program includes an assessment of river health on the COHWHS property based on macroinvertebrate biomonitoring and toxicity screening assays. This assessment is typically conducted biannually at the end of the dry and wet seasons, i.e., in September and March. However, the river health assessments scheduled for March 2024 could not be performed due to the unavailability of an accredited SASS 5 practitioner. The assessment will resume in September 2024. Therefore, the analysis included in this section is as presented for the April 2023 to September 2023 reporting (Mvandaba et al., 2023).

Macroinvertebrate assessment is widely recognized as an effective means of determining river health. Benthic macroinvertebrates are valuable for bioassessments due to their visibility, ease of identification, rapid life cycles, and largely sedentary habits (Dickens and Graham, 2002). Bioassessment techniques have been developed over the past three decades with varying complexity. The South African Scoring System 5 (SASS 5), developed by Chutter (1994), has undergone refinement and now forms the backbone of the National River Health Programme. Key changes include tighter definitions of techniques, sampling and analytical methods, and the introduction of quality control procedures.

SASS 5 is suitable for assessing river water quality and health and can be used to:

- Assess the ecological state of aquatic ecosystems;
- Assess spatial and temporal trends in ecological state;
- Identify emerging problems;
- Set objectives for rivers;
- Assess the impact of developments;
- Predict changes in ecosystems due to developments;
- Contribute to the determination of the Ecological Reserve (National Water Act, 1998).

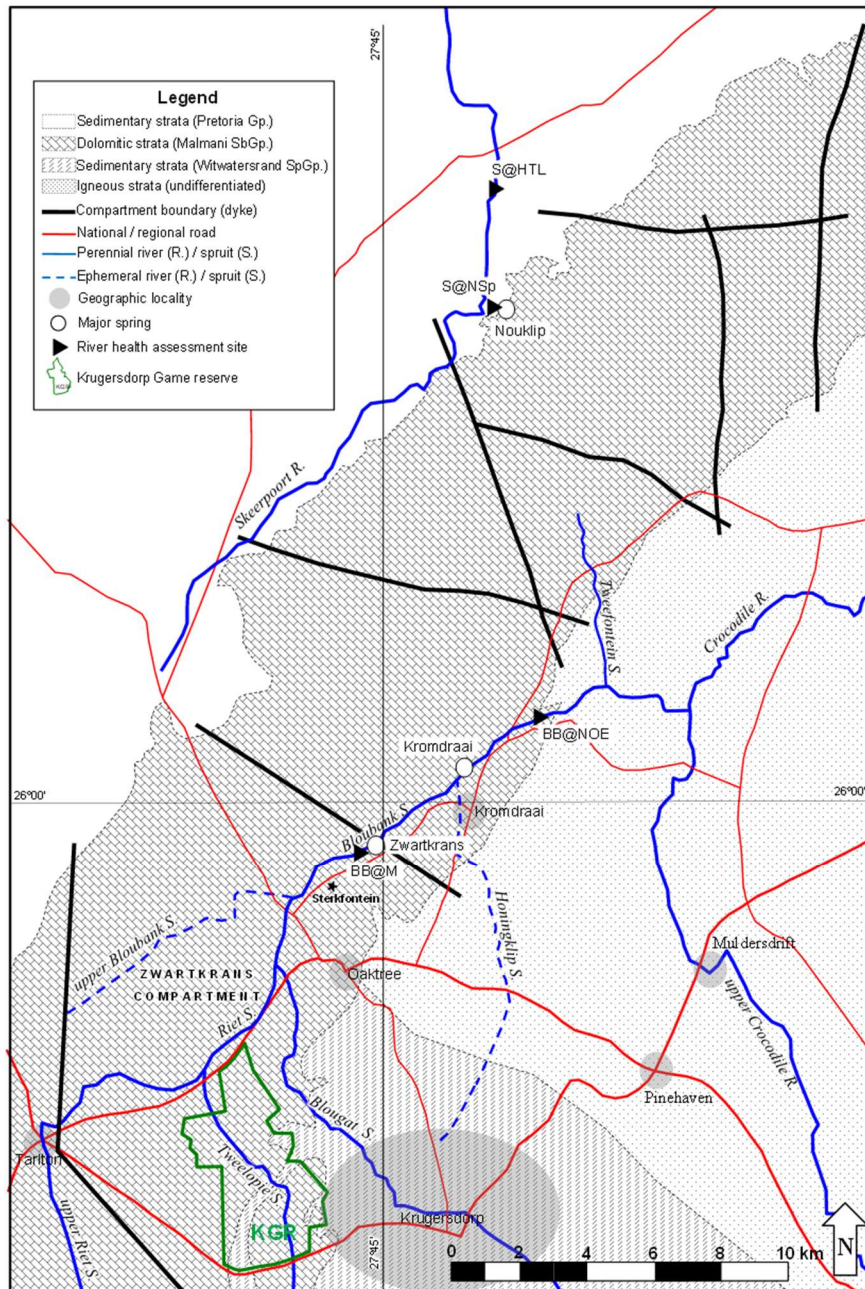
The river health assessment targets two sites on the largely natural Skeerpoort River and two sites on the impacted Bloubankspruit. The sites on the Skeerpoort River are labelled S@NSp and S@HTL, while the sites on the Bloubankspruit are respectively labelled BB@M and BB@NOE (**Figure 29**).

In the case of the springwater-driven Skeerpoort River, the 'upper' site is located on a small (short) perennial tributary at a distance of ~125 m downstream from a major karst spring, and the 'lower' site at a position ~4000 m further downstream where the river has left the dolomitic substrate and traverses sedimentary strata (mainly shale). In the case of the Bloubankspruit, both the 'upstream' and 'downstream' sites experience the combined impact of mine water and municipal wastewater discharges. The difference is that the upper site is located before the first substantial springwater (Zwartkrans Spring) input, and the lower site after the last substantial springwater (Kromdraai Spring) input.

The sites on the Bloubankspruit replicate two of those surveyed earlier as part of a CSIR Strategic Research Project (SRP) assessment of the biotic response in streams of the Western Basin that receive neutralised acid mine drainage. The outcome of this project is reported in Hill *et al.* (2014).

The Skeerpoort River sites ostensibly represent largely undisturbed, natural conditions for reference purposes, although the lower site is located ~120 m downstream of a weir and adjacent to a trout farm that discharges into the river. The lower site has been surveyed on numerous occasions in the past (Fourie *et*

al., 2014 and references therein). No published material is available for the aquatic ecosystem status of the upper site, and it is not known whether this drainage was surveyed prior the establishment of the COHWHS water resources monitoring programme . In any event, this site represents as natural a condition of a spring water-driven headwater stream in a karst landscape as can be found in the COHWHS.



**Figure 29** Map showing sites of relevance to the river health assessment

## 6.1 Assessment & Data Analysis

The assessment entailed the application of the integrated habitat assessment system IHAS (McMillan, 1998) and SASS5 (Dickens and Graham, 2002) procedures to evaluate respectively the instream habitat quality and associated benthic macroinvertebrate integrity at each site. Together, the procedures have proven in countless studies nationally their efficacy in assessing aquatic macroinvertebrate diversity as a function of instream habitat and water quality. Impairment of either habitat or water quality reduces

biodiversity and, therefore, results in a 'poorer' river condition (generically referred to as river health) when compared to the natural (or reference) condition. River health is classified according to the criteria set out in **Table 9** below. A description of the survey sites is given in **Annexure A**.

**Table 9** River Health Classification (Dallas, 2007).

Biological Band / Ecological Category	SASS5 Score	ASPT Score	Band / Category Name	Description
<b>A</b>	>104-180	>5.8-8	Natural	Unmodified natural
<b>B</b>	>71-104	>4.8-5.8	Good	Largely natural with few modifications
<b>C</b>	>53-71	>4.2-4.5	Fair	Moderately modified
<b>D</b>	>42-53	0-4.2	Poor	Largely modified
<b>E/F</b>	0-42		Seriously modified	Seriously modified

## 6.2 Macroinvertebrate Biomonitoring Results

### 6.2.1 Current Assessment Outcome

A synthesis of the current results is presented as a synoptic assessment in **Table 10**, and the results discussed in **Section 6.2.2**. The current macroinvertebrate biomonitoring results have indicated an improvement in the general river health and ecological category at both sites on the Skeerpoort River, i.e. S@NSp and S@HTL, despite the noticeable disturbance at the S@HTL site due to cutting away of the natural vegetation and disturbance of the instream habitat as evidenced by the presence of stones, gravel and as a result of fast-flowing water due to flooding. Upstream on the Bloubankspruit, while there was a clear decline in the quality of water and instream habitat at the BB@M site, the ecological category remained the same. As a result of the recent flood events in the study area, the streamflow at the BB@NOE site was found to be too turbid and deep to allow safe assessment of the river health. However, water samples for the assessment of the toxicity, chemistry and microbiological quality were collected.

**Table 10** Synoptic river health assessment outcome for September 2023.

SITE	Date	Ecological category	Condition	Description
S@NSp	14/09/2023	<b>A</b>	Natural	Unmodified natural
S@HTL	14/09/2023	<b>A</b>	Natural	Unmodified natural
BB@M	13/09/2023	<b>E/F</b>	Seriously modified	Seriously modified
BB@NOE	13/09/2023	Macroinvertebrate biomonitoring could not be conducted		

## 6.2.2 Comparison with Historical Results and General Observations

### 6.2.2.1 Skeerpoort River

#### S@NSP

In **Table 11**, seasonal sampling (spring and summer) is compared for site S@NSp. The inclusion of this site in the river health assessment aims to analyse the suitability of site S@HTL as a reference site for the COHWHS property even though it is located downstream of the karst area.

The surrounding area is largely undisturbed, natural vegetation. A few hundred metres upstream there was evidence of heavy erosion from flooding and cutting of the natural vegetation which could easily affect the invertebrate sampling scores. In the current monitoring period (March 2023), the number of taxa was the least in all sampling period due to lack of good habitat for macroinvertebrates sampling.

The River Health Category rating for this site in March 2023 was B (good) with an ASPT score of 5.8 – 5.9. It is expected that this particular site will continue to oscillate between a Category B and C.

S@NSp is a headwater stream, and macroinvertebrate diversity in headwaters do tend to differ, but are generally lower than in the middle reaches of a river network (widely reported in numerous sources, and reviewed in Clarke *et al.*, 2008). This would also account for higher scores in the downstream Skeerpoort River site (even though it is downstream of a trout farm).

**Table 11** Comparison of present biomonitoring results for site S@NSp with previous results.

Site: S@NSp		River: Grootspruit (tributary of the Skeerpoort River)							
Date	Ecological category	SASS associated scores						Dominance *	
		SASS 5	Taxa	ASPT	IHAS (%)	Highest Sensitivity	Total Invertebrates	Dominant Feeding Group	Dominant Taxa
27/02/2018	C	105	18	5.8	71	12	130	Predators	<i>Baetidae</i>
26/09/2018	B	95	15	6.3	73	10	238	Predators	<i>Corixidae</i>
26/03/2019	C	99	17	5.8	75	12	160	Predators	<i>Gomphidae</i>
16/09/2019	C	70	12	5.8	70	12	169	Predator	<i>Pleidae</i>
02/03/2020	B	138	21	6.6	66	14	338	Predator	<i>Palaemonidae</i>
14/09/2020	C	53	14	4.5	59	12	229	Predator	<i>Simuliidae</i>
19/09/2022	<i>Sampling could not be conducted</i>								
14/03/2023	B	31	6	5.17	40	8	NA	Predator	<i>Baetidae</i>
14/09/2023	A	102	15	6.8	40	8	N/A	Predator	<i>Batidae</i>

## S@HTL

In **Table 12** seasonal sampling for site S@HTL is compared. The River Health Category generally borders between A (Natural), and B (Good) while it was categorized as D only in one occasion.

The sampling area has a pool of slow-moving/still water, leading to a narrower region of faster-flowing riffles. Most habitats were well represented. The Skeerpoort River at this site is more in the middle reaches of the river system, and as such is expected to have a larger diversity of macroinvertebrates. The current ASPT score was the highest of all sampling runs.

**Table 12** Comparison of present biomonitoring results for site S@HTL with those of the Fourie et al. (2014) "site B" results.

Site: S@HTL		River: Skeerpoort River							
Date	Ecological category	SASS associated scores						Dominance *	
		SASS5	Taxa	ASPT	IHAS	Highest Sensitivity	Total Invertebrates	Dominant Feeding Group	Dominant Taxa
##/01/2014 <sup>1</sup>	A	~200 <sup>2</sup>	~34 <sup>2</sup>	~6.0 <sup>2</sup>	-	-	-	-	-
13/02/2018	A	185	27	6.9	72%	13	653	Collector-Gatherers	<i>Tricorythidae</i>
17/10/2018	B	170	29	5.9	73%	12	524	Collector-Gatherers	<i>Baetidae</i>
25/03/2019	B	170	28	6.1	76%	13	437	Predators	<i>Baetidae</i>
17/09/2019	B	99	16	6.2	70	12	354	Predators	<i>Baetidae and Pleidae</i>
02/03/2020	A	90	13	6.9	77%	15	399	Predators	<i>Pleidae</i>
15/09/2020	D	70	16	4.3	69%	12	328	Predators	<i>Baetidae</i>
19/09/2022	Sampling could not be conducted								
14/03/2023	A	103	14	7.36	82%	12	NA	NA	<i>Caenidae</i>
14/09/2023	A	99	14	7.07	82%	12	NA	NA	<i>Caenidae</i>

<sup>1</sup> From Fourie et al. (2014) <sup>2</sup> Approximate value interpolated from bar graph in Fourie et al. (2014)

### 6.2.2.2 Bloubankspruit

## BB@M

The study by Hill et al. (2014) provides a similarly quite recent assessment against which to gauge the present river condition. This is provided in **Table 13** (site BB@M) and **Table 14** (site BB@NOE)

Site BB@M typically has in the past showed a variety of good habitat with large areas of sand, stones in current, and leafy vegetation. However, as shown in the Table 12, there is a clear trend in the deterioration of aquatic habitats (biotopes)..The River Health Category remains similar to the previous sampling campaign as it scored E/F. Furthermore, it was noticeable that the ASTP score was the worst compared to the previous sampling campaigns while the habitat quality was very poor of all the sampling trips due to

site destruction owing to recent flooding.

**Table 13** Comparison of present biomonitoring results for site BB@M with those of the Hill et al. (2014) study.

Date	Ecological category	SASS associated scores						Dominance	
		SASS 5	Taxa	ASPT	IHAS (%)	Highest Sensitivity	Total Invertebrates	Dominant feeding group	Dominant Taxa
23/02/12	E/F	32	9	3.6	57				
16/05/12	E/F	53	14	3.8	81	13	653	Collector-Gathers	<i>Tricorythidae</i>
24/10/12	E/F	35	10	3.5	72	12	524	Collector-Gathers	<i>Baetidae</i>
06/03/13	E/F	52	13	4	74	13	437	Predator	<i>Baetidae</i>
15/08/13	E/F	34	9	3.8	65	12	277	Predators	<i>Baetidae</i> and <i>Pleidae</i>
12/12/13	E/F	38	10	3.8	61				
13/02/18	E/F	27	8	3.4	67	13	653	Collector-Gathers	<i>Tricorythidae</i>
25/09/18	E/F	48	11	4.4	71	12	524	Collector-Gathers	<i>Baetidae</i>
28/03/19	E/F	46	12	3.8	66	13	437	Predator	<i>Baetidae</i>
16/09/19	E/F	36	10	3.6	65	12	277	Predators	<i>Baetidae</i> and <i>Pleidae</i>
04/03/20	E/F	27	8	3.9	56%	6	735	Predators	<i>Chironomidae</i> , <i>Ceratopogonidae</i> and <i>Culicidae</i>
16/09/20	E/F	13	4	3.2	75	5	382	Predators	<i>Chironomidae</i>
19/09/22		Sampling could not be conducted							
06/12/22	E/F	35	12	2.9	49	6	861	Predators	<i>Chironomidae</i>
16/03/23	E/F	15	6	2.5	55%	5	861	Predators	<i>Chironomidae</i>
13/09/2023	E/F	16	6	2.6	35%	5	861	Predators	<i>Chironomidae</i>

## BB@NOE

As illustrated in **Table 14**, the ecological state of the Bloubankspruit at the Nedbank Olwazini has been observed to be variable over the various monitoring periods. Less diversified than the upstream site, the instream habitat at this location is largely made up of heavy stones and rocks that pose a safety threat to the river health assessor. There is a little sand, and the overhanging vegetation is often limited. The SASS 5 and ASPT scores continue to be low, as seen in Table 13 below.

**Table 14** Comparison of present biomonitoring results for site BB@NOE with those of the Hill et al. (2014) study.

Site: BB@NOE		River: Bloubankspruit							
Date	Ecological category	SASS associated scores						Dominance *	
		SASS5	Taxa	ASPT	IHAS (%)	Highest Sensitivity	Total Invertebrates	Dominant Feeding Group	Dominant Taxa
23/02/12	E/F	52	12	4.3	52	6	206	Collector-Filterers	<i>Hydropsychidae</i>
16/05/12	E/F	41	10	4.1	59	6	269	Collector-Gatherers	<i>Baetidae</i>
24/10/12	C	59	11	5.4	61	12	230	Collector-Gatherers	<i>Baetidae</i>
06/03/13	B	60	10	6.0	55	12	225	Collector-Gatherers	<i>Baetidae</i>
12/12/13	D	32	6	5.3	53	12	329	Collector-Gatherers	<i>Baetidae</i>
13/02/18	D	57	12	4.8	58	12	234	Collector-Gatherers	<i>Baetidae</i>
25/09/18	E/F	30	8	3.8	55	6	275	Predators	<i>Corixidae</i>
25/03/19	E/F	33	8	4.1	54	6	178	Predators	<i>Simuliidae</i>
17/09/19	E/F	64	14	4.6	58	8	781	Predators	<i>Ceratopogenidae</i> and <i>Chironomidae</i>
04/03/20		Sampling could not be conducted							
16/09/20	E/F	38	11	3.4	50	6	320	Predators	<i>Chironomidae</i>
19/09/22		Sampling could not be conducted							
06/12/22		Sampling could not be conducted							
04/03/23		Sampling could not be conducted							
13/09/2023		Sampling could not be conducted							

Previous results indicate the deterioration in the system as the ecological category is consistently in the E/F category. IN the last three monitoring runs of 2022/23, the river health assessment at this

site could not be conducted due to highly turbulent and unsafe flow that posed a danger to the operator. According to (Dickens and Grahams, 2002), it is the prerogative of the operator to desist from sample collection if she/he considers the conditions in the river or the surroundings to be unsafe.

### 6.3 Toxicity Testing

Laboratory toxicity tests are used worldwide to manage environmental resources such as water quality and are considered to be the first step in a tiered approach in establishing guidelines for setting up maximum acceptable concentrations of specific pollutants (Muller and Palmer, 2004). The use of bioassays incorporating complex mixtures has proven to be a relevant and complementary tool in evaluating the adverse toxic effects of effluents and receiving waters (Rand et al., 1995; Grothe et al., 1996). The techniques are site specific, and account for both point and non-point sources of aquatic contamination and uncharacteristic sources of toxicity and can detect their potentially unknown interactions (Smolders et al., 2003; Martins et al., 2007). However, no single toxicity test has proven to be suitable to assess all adverse ecological effects because individual organisms differ in susceptibility and sensitivity to different chemicals (Rand et al., 1995; DWAF, 2003). Therefore, several different bioassays at different levels of biological complexity and trophic levels need to be used simultaneously to adequately assess if a potential hazard posed (Jergentz et al., 2004).

The approaches used internationally in water quality monitoring programmes include the whole effluent toxicity (WET), used in the USA, the direct toxicity assessment (DTA), used in the UK and the 'totale effluent milieu' hygiene (TEM) or 'whole effluent environmental risk' approach, used in the Netherlands (DWAF 2003). These approaches are based on similar fundamental concepts by using an array of acute or chronic toxicity test endpoints and in certain instances such as TEM, and other indirect hazard parameters such as oxygen depletion potential, bioaccumulation, and mutagenicity to ascertain ecological effects of pollutants.

In South Africa, the Department of Water Affairs and Forestry (DWAF) presently known as the Department of Water and Sanitation (DWS) has adopted a multifaceted approach referred to as the 'direct estimation of ecological effect potential' (DEEEP) which includes these international approaches (using TEM as a foundation) and has become an integral first-tier tool used in the ecological hazard assessment of complex waste discharges (DWAF, 2003). The DEEEP approach uses representative organisms from different trophic levels of the food chain (fish, invertebrates, microalgae, and bacteria) and endpoints (both lethal and sublethal) to reflect the overall impact of toxicants in the water samples, with provision to define acceptable ecological hazards to protect aquatic ecosystems (Jooste and Herbst 2004). The same suite of hazard assessment methods was selected for inclusion in the National Toxicity Monitoring Programme (NTMO) for surface water (DWAF, 2005). The main objectives of the NTMO are to monitor South African water resources in terms of (a) the toxic effects on selected organisms and (b) selected potentially toxic substances.

The two rapid and relatively inexpensive internationally standardized toxicity bioassays that form part of the DEEEP and NTMP were adapted and incorporated into the present study. These were (1) the water flea (*Daphnia magna*) mortality test, and (2) the microalgal (*Raphidocelis subcapitata*

(previously: *Selenastrum capricornutum*) growth inhibition test. Differential responses of organisms such as these represent diverse physiological capabilities and reach in aquatic systems that can help focus field studies where non-target effects due to off-site movement, for example acid mine drainage and wastewater effluents, are suspected. Water fleas (*Daphnia* spp.) are routinely employed to detect chemical pollution in river waters based on their toxicity (Rand et al., 1995). *Daphnia* sp. has been used for many years as 'standard aquatic test species'. Unicellular microalgae form the basis of the energy flow in the aquatic trophic chain and show great advantage for use in estimating the toxicity of effluents and receiving waters (Sbrilli et al., 2005). They are relatively sensitive to a variety of pollutants and are easy to handle and have a short life cycle (Rioboo et al. 2001).

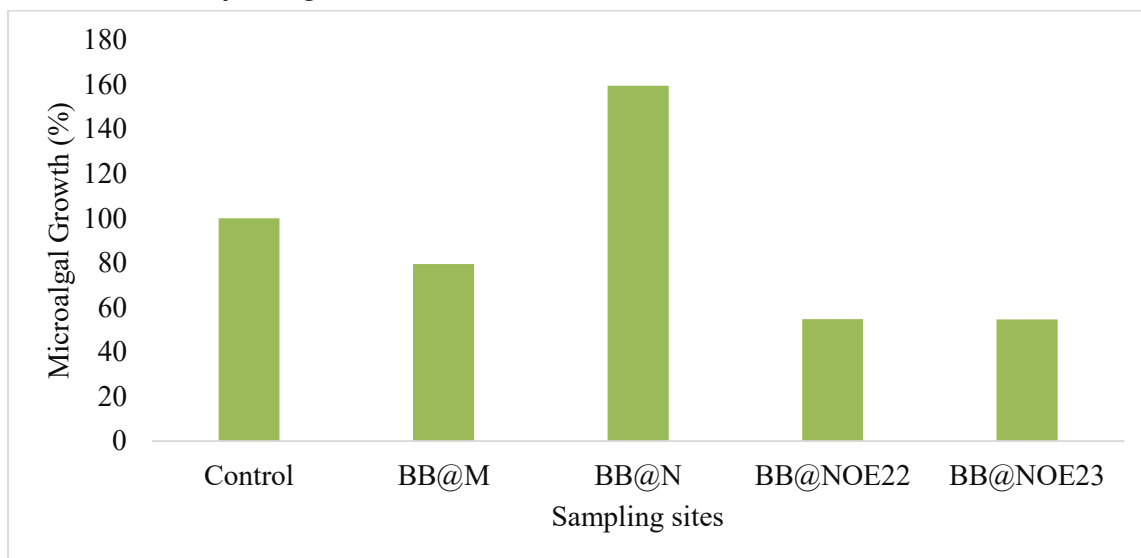
### Toxicity testing results

The results are reported per bioassay for toxicity screening tests with and microalgae (*Raphidocelis subcapitata* (previously: *Selenastrum capricornutum*), and water fleas (*Daphnia magna*). The bioassays were performed on surface water samples collected from two sites at the Bloubankspruit (BB@M and BB@NOE), and one site upstream of BG@N14 on the Blougatspruit. For this study, acute 48-hour *D. magna* mortality and 72-hours *R. subcapitata* tests were conducted under static conditions to assess the short-term toxicity potential of water samples from the selected sites using organisms on two different trophic levels of the aquatic ecosystem.

Physicochemical parameters were measured at the start and end of the tests with a hand-held Hach HQ 40D multi-parameter (temperature, pH, electrical conductivity and dissolved oxygen) meter. The results for the three tests are summarised below.

### December 2023

#### 72-hour *R. subcapitata* growth inhibition/stimulation test



**Figure 30** Growth inhibition results of *R. subcapitata* after 72-hour exposure to water samples from different sites at the COHWHS sampling area

The water samples induced effects to varying degrees after the exposure period, when compared to the control. All growth was  $\geq 20\% \leq 50\%$  for all samples except BB@N which showed a 40% growth stimulation when compared to the control (**Figure 30**). The growth inhibition in all other samples showed moderately high pollution in the water samples which inhibited the growth of microalgae. This evidenced perturbation in the system resulting from the presence of harmful chemicals in the assessed water system (Rodrigues *et al.*, 2021). The overstimulation in BB@N14 (BB@N) may be a result of nutrient overload which overfeeds the algae; the result of such overstimulation may be uncontrolled algal bloom which will compromise the integrity of the aquatic ecosystem.

#### **48-hour *D. magna* lethality test**

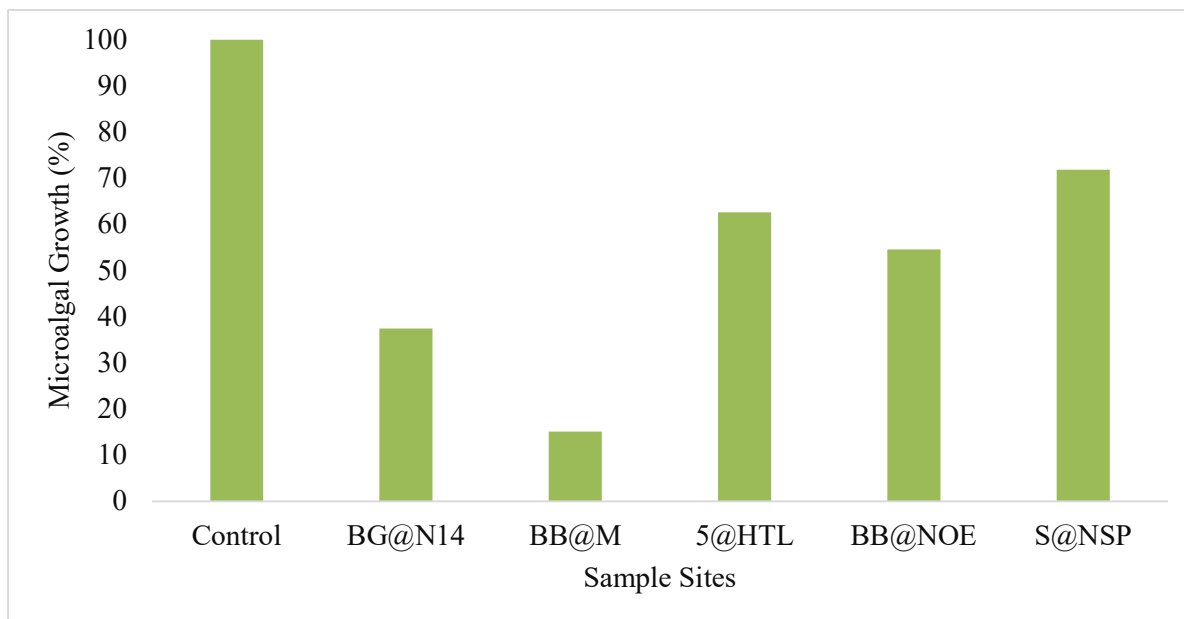
**Table 15** Results of the *D. Magna* screening assays expressed as percentage mortality after 24 and 48 hours.

Sample	Time (hrs)	Mortality (No. of organisms)	Mortality (%)	Hazard Level
BB@M	24	2	10	Acute hazard
	48	11	55	
BG@N14	24	3	15	Slight acute hazard
	48	7	35	
BB@NOE2022	24	0	0	Slight acute hazard
	48	7	35	
BB@NOE2023	24	1	5	Slight acute hazard
	48	4	20	

The water induced mortality effects in the *D. magna* to varying degrees after the exposure period. Although the mortality was recorded for all samples, the effects were generally low across all sites; the highest mortality was 55% (BB@M), with the noted effects of  $\leq 35\%$  across all the other samples. Given the sensitivity of *D. magna* to environmental pollution, the current data shows that the levels of pollution in the assessed water samples were only moderate.

March 2024

**R. subcapitata growth inhibition/stimulation test**



**Figure 31** The growth inhibition effects of water samples from different sites at the COHWHS sampling area on *R. subcapitata*.

The *R. subcapitata* growth inhibition was induced to varying levels after exposure, with the highest effects reaching >80% in BB@M. The lowest growth inhibition was induced in the S@NSP at ±30%. The growth inhibition effects in other sample exposures ranged between ≥37% and ≤63%. The relatively high inhibition reflects the moderately high potency of the assessed water samples as a result of relatively high pollutant levels in the water.

**48-hour *D. magna* lethality test**

**Table 16** The mortality of *D. magna* after 48-hour exposure to water samples from the COHWHS sampling area

Sample	Time	Mortality (No. of organisms)	Mortality (%)	Hazard level
BB@NOE	24	4	20	
	48	8	40	Slight acute hazard
BB@M	24	0	0	
	48	1	5	Slight acute hazard
BB@N14	24	5	25	
	48	9	45	Slight acute hazard
S@NSP	24	6	30	
	48	7	35	Slight acute hazard
S@HTL	24	2	20	
	48	8	40	Slight acute hazard

**Table 17** Physicochemical properties of the assessed water samples.

Date	Site	pH	Temp (°C)	DO (mg/L)
Dec'23	Control	7.79	22.3	4.55
	BB@M	7.56	18.5	4.91
	BG@N14 (BB@N)	7.27	17.4	3.78
	BB@NOE22	7.94	18	1.29
	BB@NOE23	7.07	18.9	2.76
Mar'24	Control	6.61	16	8.01
	BG@N14	6.66	12.5	1.36
	BB@M	6.14	14.2	3.09
	S@HTL	6.95	13.2	9.08
	BB@NOE	6.23	15.5	4.97
	S@NSP	6.84	14	8.85

### 6.2.3 Hazard classification for natural waters

A risk/hazard class was determined by application of the DEEEP (Direct Estimation of Ecological Effect Potential) (DWAF, 2003; Slabbert, 2004) recommended protocols and the hazard classification system for natural waters (Persoone *et al.*, 2003). This hazard class equates to the level of acute or chronic risk posed by the water sample tested. A percentage effect (PE)<sup>1</sup> is determined for each of the tests in the battery of screening bioassays by measuring either immobility/mortality or inhibition/stimulation, depending on the type of test. The sample is then ranked into one of five classes (**Table 15**), based on either screening or definitive testing protocols.

**Table 18** Hazard classification system for screening tests (Persoone *et al.*, 2003).

Class	Description
CLASS I	No acute hazard – none of the tests shows toxic effect.
CLASS II	Slight acute hazard – a statistically significant percentage effect is reached in at least one test, but the effect level is below 50%.
CLASS III	Acute hazard – the 50 % effect level is reached or exceeded in at least one test, but the effect level is below 100%.
CLASS IV	High acute hazard – the 100% effect is reached in at least one test.
CLASS V	Very high acute hazard – the 100%percentage effect is reached in all the tests.

<sup>1</sup> Percentage effect: 10% effect = slight toxicity for daphnia and fish; 20% effect = slight toxicity for algae and bacteria; 50% and > effect = toxicity for all test organisms (bacteria, algae, daphnia, fish).

### 6.2.4 Discussion on River Health and Toxicity Assessment

The macroinvertebrates assessment indicates that the anthropogenic activities occurring in Bloubankspruit affect the ecosystem health leading to a decrease in taxa and total SASS5 scores. This is best evidenced by the E/F rating of past biomonitoring results. Effluent pollution has clearly led to the deterioration of the health of both sites on the Bloubankspruit with ASTP being at lowest

compared to all sampling campaigns. The river water at Bloubankspruit River has been turbid in the past field trips which confirms the impact of recent flooding and probable effluent discharge into the system. However, there has been an improvement in ecological category of S@HTL and S@NSP compared to the previous results.

## Toxicity Assessment

The DEEEP methods, as developed for South Africa and applied herein, are specifically aimed at assessing the ecological risks of complex surface water media (Slabbert *et al.*, 2004). Accordingly, the battery of tests used herein was aimed at indicating how the current pollution levels affect the aquatic ecosystem. This is critical to the decision-making process for intervention; the ecotoxicity data help to detect early perturbations in the aquatic ecosystem (Rodrigues *et al.*, 2021). In the current study, the effects were noted to varying degrees in both the microalgae (*R. subcapitata*) and the water fleas (*D. magna*), indicating the deteriorating water quality in the assessed river systems. Starting with *R. subcapitata*, the effects were moderate to high between December and March, respectively. The December samples seemed to induce only moderate effects, even causing overstimulation in one sample (BB@N). However, the samples from the March campaign only reduced the growth of microalgae, albeit moderately. The high inhibition of >80% was noted in BB@M. The noted growth inhibition indicates the deteriorating water quality in the sampled system. This means increased pollution or the presence of chemicals in this system which have led to the destruction of the microalgal cellular structures and therefore hindering the growth of the microalgae (Wang and Liu, 2023). This disrupts the functions of the microalgae in the environment. Specifically, primary functions such as the primary production for the freshwater ecosystem, carbon sequestration, and reduction of pollutants are disrupted (Caroppo and Pagliara, 2022). On the other hand, the overstimulation observed in the samples from the December campaign indicated the stress experienced by the microalgae; the presence of excess nutrients such as phosphates and phosphorus has been shown to put stress on the microalgae thereby stimulating their growth (Wang *et al.*, 2022).

The *D. magna* results corroborated the effects observed in the microalgae test. The effects were moderate, with the highest mortality of 55% observed in the samples from the December 2023 sampling period. The mortality generally ranges below 45% across all samples from both the sampling campaigns. Although the effects were moderate, the slight acute effects still represent potential hazard to the aquatic ecosystem if the pollution increases beyond the current levels. In particular, the current moderate results show that further disruption to current water quality could affect the functionality of *D. magna* in this system; *D. magna* are planktonic freshwater organisms that are filter feeders in the aquatic ecosystem (Ecology, 2005). *D. magna* feeds on excess algae and suspended solids in the aquatic ecosystem thereby reducing the amount of debris and maintaining the integrity of the aquatic ecosystem (Ecology, 2005).

Overall, the March 2024 ecotoxicity results show only slight acute hazards posed by the assessed water samples. However, the current results should not be interpreted as representative of pristine conditions in the current system. Rather, further monitoring should be considered to ensure that the pollution does not slip beyond the current levels as it will compromise the integrity of the assessed freshwater systems. Based on the current data, one specific system needs consistent monitoring and attention, i.e. BB@M, to ensure that the pollution levels are under control. Although the effects

are still moderate and there is only a potential hazard, the consistently increasing effects are a cause for concern.

These toxicity tests of the current monitoring and macroinvertebrates assessment clearly indicates that the Bloubankspruit sites are heavily affected by anthropogenic impacts occurring on the catchment. Proper rehabilitation of the current wastewater treatment activities is also essential to avoid or minimise effluent related impacts in the future. The results of this assessment study clearly indicates that improved management and maintenance of WWTWs is still crucial. Furthermore, the results of this assessment further indicates the importance of macroinvertebrates monitoring and toxicity tests river health status, toxicity potential and also potential pollution hotspots.

## **7 DISCUSSION & CONCLUSIONS**

The outcome of monitoring activities as documented in this report has revealed that the 2024 wet season's notably low rainfall has resulted in decreased discharge volumes compared to 2023, with the overall trend indicating reduced flow rates and potential impacts on water quality. The data from the 16-year rainfall record indicates that the 2024 wet season rainfall at station HDS was one of the lowest recorded, ranking at the 7<sup>th</sup> percentile, with station SC recorded moderately lower rainfall, ranking at the 42<sup>nd</sup> percentile. Despite data limitations, the rainwater chemistry suggests consistent low salinity and acidity, with variations across different stations.

In the context of river flow and groundwater dynamics, the historical total annual discharge data shows that the 2023 hydrological year experienced the highest discharge on record, aligned with flash floods between November 2022 and March 2023. Conversely, the 2024 hydrological year displayed significantly lower discharge volumes. Groundwater levels in the northern and central segments of the COHWHS property have shown a slight decline, while the Sterkfontein Cave lake water level has decreased notably since its peak in March 2023. These variations highlight the impact of seasonal and environmental changes on hydrological conditions within the region.

The recent hydrochemical and microbiological analyses of water samples reveal ongoing challenges with water quality. The Tweelopiespruit and Rietspruit show low pH values and relatively elevated concentrations of sulphate and manganese, while the Blougatspruit and Bloubankspruit exhibit alkaline conditions with significant bacteriological contamination from municipal wastewater effluent. The springs within the COHWHS property demonstrate variability in discharge and water quality, influenced by seasonal changes and direct extraction. Despite improvements from mine water control measures, the presence of coliform bacteria in groundwater remains a concern, underscoring the need for ongoing monitoring and effective management strategies to mitigate the impact on regional water resources.

The study underscores the complex interplay of hydrological and environmental factors affecting water resources within the COHWHS property. The variability in river discharge and groundwater levels further illustrates the impact of seasonal changes and environmental events, such as the flash floods of 2023. The hydrochemical and microbiological data highlight persistent water quality issues, particularly with bacteriological contamination from municipal wastewater and the ongoing influence of mine water. The reduced quantity and deteriorating quality of surface and groundwater resources,

exacerbated by low rainfall, acid mine drainage, and faecal contamination, pose significant environmental and public health risks. The increased salinity, acidity, and elevated contaminant levels, including manganese, iron, and sulphates, can lead to adverse effects on aquatic ecosystems, including habitat degradation and reduced biodiversity. For public health, the high levels of *E. coli* and total coliform bacteria in surface water raise concerns about waterborne diseases and potential health hazards for local communities. Additionally, the compromised microbiological quality of groundwater and surface waters can affect drinking water safety. The environmental impact extends to the fossil sites within the COHWHS, as altered water chemistry and contamination can threaten the preservation of these important paleontological resources, potentially leading to accelerated degradation and loss of critical fossil evidence. These findings emphasize the necessity for continuous monitoring, improved data collection, and robust management strategies to address the challenges posed by both natural variability and anthropogenic impacts on water resources in the region.

It can be concluded that the water resources monitoring results documented in this report confirm the continued suitability of the monitoring programme and conceptual hydro(geo)logical model developed for the COHWHS by Hobbs (2011). However, the integrity and impact of the monitoring programme is compromised by the multiple gaps of missing data that perforate the various data records that are used to achieve a holistic understanding of the COHWHS hydrological system. It is therefore imperative that improved efforts are made to ensure continuous monitoring and up-to-date maintenance of the respective institutional databases.

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# ANNEXURE A

## DESCRIPTION OF THE AQUATIC BIOMONITORING SURVEY SITES

### A.1 GENERAL

All four sites are located Western Bankenveld ecoregion. Site S@HTL, however, is considered a transitional location as it is located close to the boundary with the Bushveld Basin ecoregion to the north.

### A.2 Site S@HTL

This is located on the Skeerpoort River approximately 50 metres downstream of a small trout farm. Although much of the surrounding area is natural and undisturbed, several riparian trees were observed to be chopped and cut down. The sampling area comprises a pool of slow-moving/still water, leading to a narrower region of faster-flowing riffles. Most habitats are well represented, although there was a lack of sandy areas.



Figure A.1: Downstream site S@HTL on the Skeerpoort River

The macroinvertebrate family diversity for this site is summarised in **Table A.1** below.

**Table A.1:** Macroinvertebrate families present at site S@HTL

Macroinvertebrate families	13/02/2018	17/10/2018	25/03/2019	16/09/2019	02/03/2020	15/09/2020	15/03/2023
Turbellaria	40	3	1				
Oligochaeta		1	2				
Potamonautidae	3	3	12	11	3	7	
Hydracarina	40	1	4				
Baetidae	80	240	80	109	88	120	
Caenidae	30	20	6	3		11	
Prosopistomatidae					8		
Teloganodidae	2		2	12			
Heptageniidae	10	40	30				
Leptophlebiidae	240	100	60				
Tricorythidae	1		4				
Chlorocyphidae	20	20	15				
Coenagrionidae		2		4	4	4	
Lestidae	3	1	6				
Aeshnidae	2	15	20		1	5	
Gomphidae	1	2	2				
Libellulidae	1		1	3		1	
Pyralidae			1				
Belostomatidae	30	2	60				
Corixidae	40	4	2			1	
Gerriidae	2				10		
Naucoridae			1				
Nepidae	2	6					
Notonectidae			30		9	120	
Pleidae	20	15	30	101	180		
Veliidae	40	1	2				
Hydropsychidae	6	2	1	80	10		
Philopotamidae		2	30				
Dytiscidae		3				6	
Psychomyiidae					7		
Elmidae	20	8	10	4			
Gyrinidae	2	1					
Libellulidae	40	20	10	4			
Ceratopogonidae		2		3	7	4	
Athericidae	5	6					
Chironomidae			3	20	44	4	
Dixidae	2	2					
Simuliidae	1	1	12		28	4	
Tabanidae		1				4	
Tipulidae						20	
Ancylidae							
Planorbinae*							

### A.3 Site S@NSp

This is located on a spring-fed tributary (the Groot Spruit) of the Skeerpoort River some 4000 m upstream from site S@HTL. The survey site itself is located ~120 m downstream of the Nouklip Spring, and would therefore count as a headwater site. The habitat is varied and adequate, and large areas of the riverbed are sandy. The surrounding area is largely undisturbed, natural vegetation. The macroinvertebrate family diversity for this site is summarised in **Table A.2**.



Figure A.2: Upstream site S@NSp on the Skeerpoort River

**Table A.2:** Macroinvertebrate families present at site S@NSp

Macroinvertebrate families	27/02/2018	26/09/2018	26/03/2019	16/09/2019	02/03/2020	14/09/2020	13/03/2023
Oligochaeta	1		1		45		
Potamonautidae	8		8		5	1	1
Atyidae					8		
Palaemonidae					120		
Hydracarina	1	4		3			
Plecoptera					1		
Baetidae	40	30	6	5	10	29	36
Notonemouridae					1		
Caenidae	6	4	30	3		20	
Teloganodidae				5	1		
Leptophlebiidae		10					
Tricorythidae		6					
Chlorocyphidae		1	1				
Calopterygidae					1		
Coenagrionidae	12		3		1		
Aeshnidae	1			12	12		
Gomphidae	16	20	50			14	
Pyralidae	1		1				
Libellulidae					1		
Corixidae	5	100	20			1	
Gerridae	5			4	1	1	8
Hydrometridae					1	14	
Naucoridae			12				1
Notonectidae				3	1		
Pleidae	1	2	6	107			
Veliidae	10	15	10	6	88	11	
Ecnomidae					7		
Hydropsychidae		30	3		9		
Psychomyiidae				3		3	
Gyrinidae	12						
Psephenidae	6	1	2				
Ceratopogonidae			2				
Chironomidae	3			6	8	4	6
Dixidae					8	9	
Simuliidae		12		12	9		
Tabanidae	1		3			9	
Tipulidae		2				110	
Ancylidae	1		2				
Planorbinae*		1					
Hydraenidae							5

#### A.4 Site BB@M

The upstream site on the Bloubankspruit was originally immediately downstream of the causeway over the stream at Makiti. Although there is excellent habitat abundance and diversity, concern exists for the impact of the causeway and culverts on the morphology of the site. The site has therefore been moved ~350 m downstream where more natural conditions prevail, but maintains its position upstream of the Zwartkrans Spring, i.e. located upstream of the first major groundwater discharge into the Bloubankspruit. A significant difference between the 'old' and the 'new' sites is the abundance of a sandy substrate and absence of riffles at the 'old' site, compared to the abundance of riffles and moderate sandy substrate at the 'new' site. The macroinvertebrate family diversity for this site is summarised in **Table A.3** below.



Figure A.3: Site BB@M on the Bloubankspruit

**Table A.3:** Macroinvertebrate families present at site BB@M

BB@M	13/02/2018	25/09/2018	25/03/2019	17/09/2019	02/03/2020	16/09/2020	06/12/2022	15/03/2023
Turbellaria		1	8	4	15	44	8	2
Leeches	1		1				1	
Oligochaeta		1		6	2		5	1
Crustacea	3	1	2	6				
Potamonautidae	40	40		8				
Baetidae		40	10	12	19		10	
Coenagrionidae		10		3			11	8
Aeshnidae		8	3					
Gomphidae		1	6					
Corixidae	30							
Gerridae	4	3	12					
Pleidae			1	7				
Ecnomidae	40	100	20	18				
Veliidae	1							
Hydropsychidae	100	200	300	55	38			
Psychomyiidae			3	15				
Gyrinidae			1					
Ceratopogonidae				161	120	1	1	
Chironomidae				33	219	330	450	460
Culicidae					180			
Simuliidae				29	5		370	280
Physidae					7	7	10	
Ancylidae				4				
Coelenterata								
Hirunidae								
Libellulidae							1	
Belostomatidae							1	
Dytiscidae							1	

#### A.5 Site BB@NOE

Site BB@NOE is located on the Bloubankspruit ~7400 m downstream of site BB@M. This position places it downstream of the last major groundwater discharge (that from the Kromdraai and Plover's Lake springs) into the Bloubankspruit. This site appears to be turbid, even when the upstream site BB@M is clear. There is a trout farm upstream of the site. The habitat diversity at the site is far less compared to the upstream site and is mostly bedrock and large stones. There is little sand, and the overhanging vegetation is often limited. The macroinvertebrate family diversity for this site is summarised in **Table A.4**.



Figure A.4: Site BB@NOE on the Bloubankspruit

**Table A.4:** Macroinvertebrate families present at site BB@NOE

<b>BB@NOE</b>	13/02/2018	25/09/2018	25/03/2019	17/09/2019	16/09/2020
Turbellaria		1		4	33
Annelida					4
Leeches		1			
Oligochaeta	2			6	6
Crustacea	2	1	3	6	
Potamonautidae	80	40	4	8	3
Baetidae	10	40	30	12	24
Coenagrionidae		10		3	
Aeshnidae		8	25		
Gomphidae		1			
Corixidae	2				
Gerriade	2				
Pleidae			15	7	
Ecnomidae	40		1	18	
Veliidae	3	3			
Hydropsychidae	2			55	6
Psychomiidae	40	100	40	15	
Gyrinidae	1				6
Ceratopogonidae	50	200	60	161	3
Chironomidae				33	220
Culicidae					
Simuliidae				29	
Syrphidae					17
Ancylidae				4	1

# ANNEXURE B

## MATERIALS AND METHODS

### Toxicity assays

A battery of four screening toxicity assays was conducted on each sample according to standard procedures, under laboratory conditions (**Table B.1, B.2, B.3 and B.4**):

- 15-minute *Vibrio fischeri* (bacterium)
- 72-hour *Selenastrum capricornutum* (algae)
- 48-hour *Daphnia magna* (water flea)

The assays were performed to assess the toxicity potential of the test samples by assessing the response of the exposed test organisms. The test organisms were applied directly to the test samples (screening assays), except in the case of the algal assay and bacterium assay where samples were filtered through a 45 µm filter prior to testing. When a 50% or more toxicity effect was detected, definitive tests were conducted.

### 15-minute *Vibrio fischeri* screening assay

**Table B.1** Summary of test conditions and test acceptability criteria for the bacterium *Vibrio fischeri* growth tests (ISO, 1998).

Parameter	Condition maintained during test
Test type	Static non-renewal
Volume of test sample	0.5 mℓ
Exposure period	15 and /or 30 minutes
Number of replicate chambers	2
Measurement equipment	Luminoskan Sirius Luminometer
Adjustment	20% salinity adjustment
Effects measured and interpretation	Screening test - % Luminescence inhibition (-) / stimulation (+) relative to the Control; Inhibition / Stimulation of ≥20% over control indicates toxic activity; Definitive test - EC20 and EC50 –values

## 72 / 96-hour *Selenastrum capricornutum* screening assay

**Table B.2:** Summary of test conditions and test acceptability criteria for the *Selenastrum capricornutum* growth inhibition tests – microplate assay for effluents and receiving waters (Slabbert, 2004).

Parameter	Condition maintained during test
Test type	Static non-renewal
Temperature	24± 2° C
Light quality	“Cool white” fluorescent lighting
Light intensity	4306 lux
Photoperiod	Continues
Volume of test sample	180 µl (plus 20 µl algal inoculum and medium)
Age of algal culture	4 to 6 days
Inoculum size	200 000 cells/ml
Number of replicate chambers	5
Shaking rate:	100 cpm continuous
Aeration	None
Dilution water	Algal stock culture media
Test duration	72 to 96 hours
Effects measured	Percentage inhibition or stimulation of growth compared to Control
Interpretation	Inhibition of ≥20% over Control indicates toxic activity, while growth of ≥20% over controls indicates stimulation

## 48-hour *Daphnia magna* screening assay

**Table B.3** Summary of test conditions and test acceptability criteria for *Daphnia magna* acute toxicity tests with effluents and receiving waters (Slabbert, 2004).

<b>Summary of toxicity test</b>	
Test system	<i>Daphnia</i> test
Test species	<i>Daphnia magna</i>
Age of test organisms	Less than 48h old
Trophic level	Grazer
Toxicity level	Acute toxicity
Test procedure	USEPA, 2002
<b>Summary of test conditions for the <i>Daphnia magna</i> acute toxicity test</b>	
Test type	Static-renewal
Water temperature	20 °C ± 1 °C; or 25 °C ± 1 °C
Light quality	Ambient laboratory illumination
Photoperiod	8 hours dark: 16 hours light
Feeding regime	Feed algae and commercial fish flakes while in holding prior to test
Aeration	None
Size of test chamber	50 ml
Volume of test sample	25 ml
Number of test organisms per chamber	5
Number of replicate chambers	4
Total number of test organisms per sample	20
Control and dilution water	Moderately hard, reconstituted water
Test duration	48 hours
Effect measured	Percentage lethality (no movement on gentle prodding), calculated in relation to control
Test acceptability	90% or greater survival in control
Interpretation	Lethality >10% indicates toxicity, provided that control lethality is ≤10%

### **Phytotoxicity test**

The toxicity classification of the sediment and pore water samples is generally performed using direct-contact tests Phytotoxkit (sediment) and Phytotestkit (pore water). In all tests the pore water samples were tested at one concentration without dilution. Sediment samples were not used in this round of analysis due to insufficient sampling of sediment as sediment tox training took place after the sampling trip, therefore phytotestkit (pore water samples) was conducted instead of Phytotoxkit (sediment samples).

Phytotoxkit and phytotestkit are generally applied for the sediment and pore water toxicity classification using three test plant seeds: *Sorghum saccharatum*, *Lepidium sativum* and *Sinapis alba*. The measures parameters were inhibition of seed germination and root length inhibition in the tested sediment/pore water in comparison with the control sediment/redistilled water. The test was conducted in accordance with the procedure recommended by the manufacturer (Phytotoxkit, 2004). The studied sediment samples (90 cm<sup>3</sup>) were placed on test plates (21 15.5 0.8 cm). In the Phytotestkit assay the lower compartment of the test plate is not filled with soil, but with a foam pad and thick filter paper which is subsequently soaked with the chemical compound under analysis. This alternative test procedure allows you to determine any significant visible effect of chemical compounds on plants, without adding the chemical(s) to a soil substrate. 20 cm<sup>3</sup> of the pore water was slowly spread over the entire surface of the white filter paper in order to hydrate the filter paper completely. The bottom part of the test plates with sediment/pore water samples were covered by a black filter paper, and ten test seeds were placed in a row at 1 cm below the middle ridge of the plate. The plates prepared in this way were incubated in a horizontal position at 25 °C in darkness for 72 h. A photo of the test plate was then taken with a digital camera and transferred to a computer. Root lengths were subsequently measured using the "Image Tools" image analysis program. The test was replicated three times for each test plant. The percent inhibition of seed germination (IG) and inhibition of root growth (IR) for the plant were calculated.

### **Ostracod test exposure**

Ostracod exposure tests were conducted as 6 replicates per site sample and 1 control per sample by using reference sediment (consisting of washed marine sediment less than 2 mm in size) according to the standard operating procedures of the test kit (Ostracodtoxkit F, 2001) and the ISO (2012) method. Ostracod neonates were exposed to test sediments (6 days in darkness at 25°C ± 2°C). Upon completion of the test period, all surviving ostracods were collected and photographs were taken with a compound microscope (with a built-in digital camera) using a micrometre cover slip as a reference point. Using the UTHSCSA ImageTool (Version 3) software, growth inhibition of surviving ostracods exposed to test sediments was determined in relation to the control exposure. Mortality data were also noted. Data from the two sediment bioassays were analysed using Microsoft Excel.

### **Hazard classification for natural waters**

A risk/hazard class was determined by application of the DEEEP (Direct Estimation of Ecological Effect Potential) (DWAF, 2003; Slabbert, 2004) recommended protocols and the hazard classification system for natural waters (Persoone *et al.*, 2003). This hazard class equates to the level of acute or chronic risk posed by the water sample tested. A percentage effect (PE)<sup>1</sup> is determined for each of the tests in the battery of screening bioassays by measuring either immobility/mortality or inhibition/stimulation, depending on the type of test. The sample is then ranked into one of five classes, based on either screening or definitive testing protocols.

**Table B.4** Hazard classification system for screening tests (Persoone *et al.*, 2003)

Class	Description
CLASS I	No acute hazard – none of the tests shows toxic effect.
CLASS II	Slight acute hazard – a statistically significant percentage effect is reached in at least one test, but the effect level is below 50%.
CLASS III	Acute hazard – the 50 % effect level is reached or exceeded in at least one test, but the effect level is below 100%.
CLASS IV	High acute hazard – the 100% effect is reached in at least one test.
CLASS V	Very high acute hazard – the 100%percentage effect is reached in all the tests.

<sup>1</sup> Percentage effect: 10% effect = slight toxicity for daphnia and fish; 20% effect = slight toxicity for algae and bacteria; 50% and > effect = toxicity for all test organisms (bacteria, algae, daphnia, fish).

**Table B.5** Physicochemical parameters per sample measured at the start and end of the tests (March 2023).

Sites	Time	Temperature	pH	EC	Oxygen
Control	0	21	7.99	301	7.66
	48	20.9	7.61	298	6.47
S@HTL	0	21.4	7.44	409	7.82
	48	21.4	7.18	425	7.88
S@HTC	0	20.9	7.42	352	7.8
	48	21.4	7.48	347	7.9
BB@M	0	21.8	6.99	1489	7.45
	48	20.7	7.1	1477	7.19
BG@N14	0	21.5	6.54	1457	7.69
	48	21.9	6.98	1449	7.18
BB@NOE	0	21.1	7.31	1266	7.45
	48	20.9	7.39	1301	7.22

## PREVIOUS TOXICITY TESTING RESULTS

### 15-minute *Vibrio fischeri* test (September 2022)

Physical parameters measured at the start and end of each test with a hand-held Hach HQ 40D multi parameter meter, are summarised in **ANNEXURE B**. 15-minutes for the *V. fischeri* luminescence inhibition (-) / stimulation (+) test after a 15-minute exposure period were as follows: S@HTL test sample: 3.58%; S@NSP test sample: 5.95%; BB@M test sample: -14.29% and B@NOE test sample: -7.72%. Inhibition of > 20% indicates toxicity and stimulation of > 20% indicates potential eutrophication. In all the sites, there was no indication of toxicity or potential eutrophication; However there was contrasting results on both rivers. There was inhibition at Bloubank Spruit with the highest inhibition occurring in site BB@M while at Skeerpoort River sites there was minimal stimulation. Tests were accepted as the % CV was > 3%.

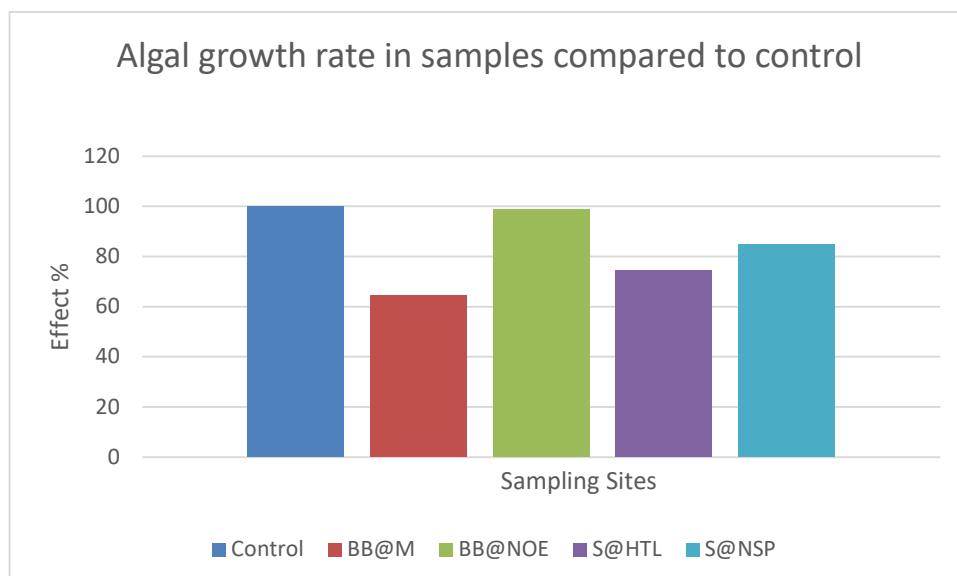
### 48-hour *Daphnia magna* test (September 2022)

**Error! Reference source not found.** summarises the results of *D. magna* exposed to the undiluted test samples S@HTL, S@NSP, BB@M and B@NOE. The tests were accepted as no mortality ( $\leq 10\%$ ) was observed in the Control. At the end of the exposure period (48 hours), the highest mortality was found in sample BB@M and BB@NOE of which the mortality percentage was 30% and 20% respectively. The mortality percentage remained low at Skeerpoort Spruit samples.

Sample	Time (Hrs)	Mortality (No. of organisms)	Mortality (%)
Control	24	0	0
	48	0	0
S@NSP	24	1	5
	48	1	5
S@HTL	24	0	0
	48	1	5
BB@NOE	24	1	5
	48	4	20
BB@M	24	2	10
	48	6	30

### 72-hour *Selenastrum capricornutum* test (September 2022)

The percentage algal growth rate in the undiluted test samples was compared to the algal growth rate in the Control (**Figure 32**). Samples with a growth rate less than 80% (> 20% growth inhibition) compared to the Control, are regarded as samples with a toxicity potential. According to the results, samples at BB@M and S@HTL were potentially considered toxic as growth rate of less than 80% was detected. This was significant in BB@M as the growth rate was 64.37% compared to Control.



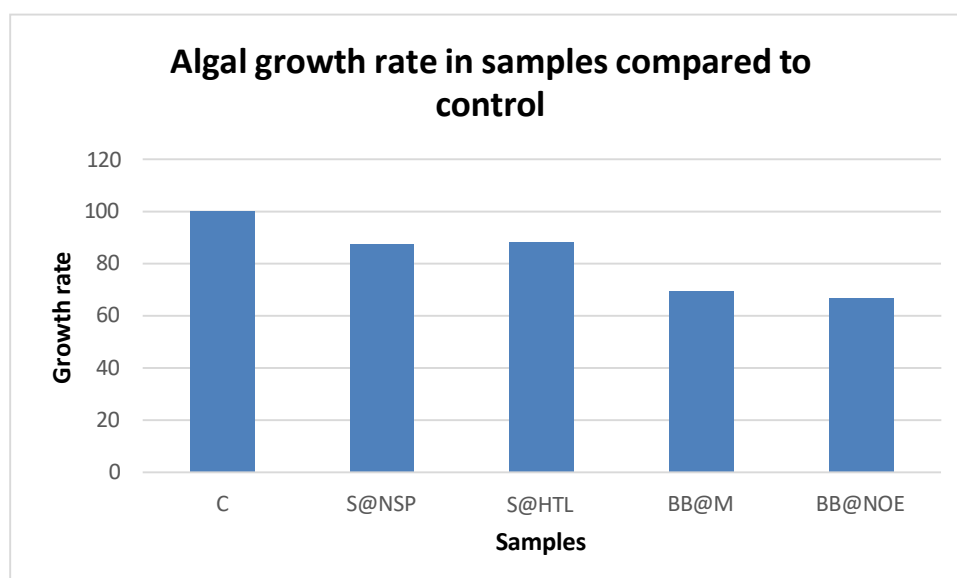
### V. fischeri test (September 2020)

Physical parameters measured at the start and end of each test with a hand-held Hach HQ 40D multi parameter meter, are summarised in **ANNEXURE B**. 15-minutes for the *V. fischeri* luminescence inhibition (-) / stimulation (+) test after a 15-minute exposure period were as follows: S@HTL test sample: 6.45%; S@NSP test sample: 1.98%; BB@M test sample: 29.06% and B@NOE test sample: 21.44%. Inhibition of >20% indicates toxicity and stimulation of > 20% indicates potential eutrophication. In all the sites, there was an indication of potential eutrophication in two sites namely; BB@M and B@NOE as stimulation exceeded 20%. Tests were accepted as the % CV was > 3%.

### V. fischeri test (March 2020)

15-minutes for the *V. fischeri* luminescence inhibition (-) / stimulation (+) test after a 15-minute exposure period were as follows: S@HTL test sample: 4.36%; S@NSP test sample: 0.98%; BB@M test sample: 24.06% and B@NOE test sample: 20.18%. Inhibition of > 20% indicates toxicity and stimulation of > 20% indicates potential eutrophication. In all the sites, there was an indication of potential eutrophication in two sites namely; BB@M and B@NOE as stimulation exceeded 20%. Tests were accepted as the % CV was > 3%.

### S. Capricornatum test (March 2020)



**D. magna test**

**Table 16 B.2a** Results of the *D. Magna* screening assays expressed as percentage mortality after 24 and 48 hours (September 2019).

<b>Sample</b>	<b>Time (hrs)</b>	<b>Mortality (No. of organisms)</b>	<b>Mortality (%)</b>
CONTROL	24	0	0
	48	0	0
S@NSp	24	0	0
	48	5	25
S@HTL	24	0	0
	48	0	0
BB@NOE	24	0	0
	48	0	0
BB@M	24	0	0
	48	0	0

**Table B.2b** Results of the *D. magna* screening assays expressed as per cent mortality after 24 and 48 hours (March 2019).

Sample	Time (hrs)	Mortality (No. of organisms)	Mortality (%)
CONTROL	24	0	0
	48	0	0
S@NSp	24	0	0
	48	0	0
S@HTL	24	0	0
	48	0	0
BB@NOE	24	0	0
	48	0	0
BB@M	24	0	0
	48	0	0

**Table B.2c** Results of the *D. magna* screening assays expressed as per cent mortality after 24 and 48 hours (September 2018).

Sample	Time (hrs)	Mortality (No. of organisms)	Mortality (%)
CONTROL	24	0	0
	48	1	5
N@NSp	24	0	0
	48	0	0
HTC (S@HTL)	24	1	0
	48	1	5
BB@NOE	24	1	5
	48	3	15
BB@M	24	1	5
	48	1	5

**Table B.2d** Results of the *D. magna* screening assays expressed as per cent mortality after 24 and 48 hours (February 2018)

Sample	Time (hrs)	Mortality (No. of organisms)	Mortality (%)
CONTROL	24	0	0
	48	0	0
HTC (S@HTL)	24	0	0
	48	0	0
BB@NOE	24	1	5
	48	3	15
BB@M	24	0	0
	48	1	5

**Table 17 B.3b.** Physicochemical parameters per sample measured at the start and end of the tests (September 2019).

Sample	Time (hrs)	Temperature (°C)	pH	SEC (mS/m)	Dissolved oxygen (mg/L)
CONTROL	0	19.3	7.04	254	7.71
	48	20.0	7.48	349	7.37
S@NSP	0	19.6	7.48	405	7.79
	48	20.0	7.78	438	7.28
S@HTL	0	19.5	7.40	340	8.30
	48	20.1	7.64	426	7.12
BB@NOE	0	19.6	7.10	1204	7.31
	48	20.2	7.40	1464	6.94
BB@M	0	19.7	6.93	1795	6.87
	48	20.1	7.01	2092	6.73

**Table B.3a** Physicochemical parameters per sample measured at the start and end of the tests (March 2019).

Sample	Time (hrs)	Temperature (°C)	pH	SEC (mS/m)	Dissolved oxygen (mg/L)
CONTROL	0	19.3	7.04	254	7.71
	48	20.0	7.48	349	7.37
S@NSP	0	19.6	7.48	405	7.79
	48	20.0	7.78	438	7.28
S@HTL	0	19.5	7.40	340	8.30
	48	20.1	7.64	426	7.12
BB@NOE	0	19.6	7.10	1204	7.31
	48	20.2	7.40	1464	6.94
BB@M	0	19.7	6.93	1795	6.87
	48	20.1	7.01	2092	6.73

**Table B.3b.** Physicochemical parameters per sample measured at the start and end of the tests (September 2018).

Sample	Time (hrs)	Temperature (°C)	pH	SEC (mS/m)	Dissolved oxygen (mg/L)
CONTROL	0	20.0	8.21	25.5	7.52
	48	20.0	7.78	36.7.7	7.47
N@NSP	0	20.0	7.99	36.4	8.31
	48	20.1	8.41	45.4	7.43
HTC (S@HTL)	0	20.0	7.63	35.0	7.62
	48	20.0	8.08	33.6	7.31
BB@NOE	0	20.0	7.93	136.4	8.73
	48	20.0	7.96	148.2	7.45
BB@M	0	20.0	6.67	184.4	7.61
	48	20.0	6.96	209.4	7.33

**Table C.3c.** Physicochemical parameters per sample measured at the start and end of the tests (February 2018).

Sample	Time (hrs)	Temperature (°C)	pH	SEC (mS/m)	Dissolved oxygen (mg/L)
CONTROL	0	20.0	8.10	21.3	7.24
	48	20.1	8.01	23.7	7.04
HTC (S@HTL)	0	20.1	8.31	30.8	7.59
	48	20.0	7.73	43.8	6.05
BB@NOE	0	20.2	7.96	124.7	6.73
	48	20.0	8.22	138.7	6.35
BB@M	0	20.2	7.72	187.2	6.68
	48	20.1	7.76	201.9	6.66