



# Groundwater Complete

## ANGLO PLATINUM MINES RUSTENBURG SECTION

### ANNUAL REPORT ON GROUNDWATER MONITORING RESULTS FOR 2018/2019

**SEPTEMBER 2019**

**Contact Details:**

Phone: 0844091429

Fax: 0866950191

P.O. Box 448

Riversdale

6670

[gcomplete@outlook.com](mailto:gcomplete@outlook.com)

Compiled by: Gerdes Steenekamp (B.Sc. Hons. Hydrology/Geohydrology)

Reviewed by: Gerhard Steenekamp (M.Sc. Geohydrology Pr.Sci.Nat.400385/04)

## TABLE OF CONTENTS

1	Introduction .....	4
2	Interpretation of Monitoring Data.....	6
2.1	Waterval Smelter, Concentrator, and Acid Plant.....	11
2.2	The Rustenburg Base Metal Refinery (RBMR) .....	19
2.3	The Precious Metal Refinery (PMR) and Central Deeps Shaft.....	29
2.4	The Waterval Tailings Complex .....	37
3	Conclusions and Recommendations.....	41

## LIST OF FIGURES

Figure 1:	Map of the RPM area with distribution of groundwater monitoring points during 2018/2019 .....	5
Figure 2:	Layout of the Expanded Durov diagram.....	7
Figure 3:	Positions of monitoring boreholes in the Waterval Processing area.....	12
Figure 4:	Time-series plot of indicator chemical parameters in the Waterval Processing area – TDS and SO <sub>4</sub> .....	15
Figure 5:	Time-series plot of indicator chemical parameters in the Waterval Processing area – NO <sub>3</sub> , Cl and Na.....	16
Figure 6:	EDD of groundwater chemistry in the Waterval Processing area.....	17
Figure 7:	Stiff diagrams of groundwater chemistry in the Waterval Processing area.....	17
Figure 8:	Time series plot of water levels for in the Waterval Processing area .....	18
Figure 9:	Positions of monitoring boreholes in the RBMR area.....	20
Figure 10:	Time-series plot of indicator chemical parameters in the RBMR area – TDS and SO <sub>4</sub> .....	24
Figure 11:	Time-series plot of indicator chemical parameters in the RBMR area – Na, NO <sub>3</sub> and Cl.....	25
Figure 12:	EDD of groundwater chemistry in the RBMR area.....	26
Figure 13:	Stiff diagrams of groundwater chemistry in the RBMR area.....	27
Figure 14:	Time series plot of water levels for in the RBMR area .....	28
Figure 15:	Positions of monitoring boreholes in the PMR and Central Deeps Shaft areas .....	30
Figure 16:	Time-series plot of indicator chemical parameters in the PMR and Central Deeps Shaft areas – TDS and SO <sub>4</sub> .....	33
Figure 17:	Time-series plot of indicator chemical parameters in the PMR and Central Deeps Shaft areas – Na, NO <sub>3</sub> and Cl.....	34
Figure 18:	EDD of groundwater chemistry in the PMR area.....	35
Figure 19:	Stiff diagrams of groundwater chemistry in the PMR area .....	35
Figure 20:	Time series plot of water levels for in the PMR area .....	36
Figure 21:	Positions of monitoring boreholes in the Waterval Tailings Complex area .....	38
Figure 22:	EDD of groundwater chemistry in the Waterval Tailings Complex area ..	40
Figure 23:	Stiff diagrams of groundwater chemistry in the Waterval Tailings Complex area.....	40

**LIST OF TABLES**

Table 1: Guideline concentrations according to RPM Water Use Licence .....	8
Table 2: South African National Standards for drinking water (SANS 241:2011) .....	8
Table 3: Average concentrations of indicator parameters for the 2018/2019 monitoring year .....	10

## ANGLO PLATINUM MINES, RUSTENBURG SECTION: ANNUAL REPORT ON GROUNDWATER MONITORING RESULTS FOR 2018/2019, AUGUST 2019

### 1 INTRODUCTION

Groundwater Complete was contracted by Aquatico Scientific to evaluate the groundwater quality and water level monitoring results for Anglo Platinum's Rustenburg Section (herein after referred to as RPM) for the 2018/2019 monitoring year. This evaluation therefore focuses on the monitoring data from September 2018 to September 2019, but also correlates with earlier data where necessary.

The distribution of all the groundwater monitoring points that were actively sampled during the past year is presented on a regional map of the RPM operations area in **Figure 1. More detailed site maps on a larger scale are provided in each section with the discussion of the water quality properties in each area and are orientated with north at the top of all maps and figures.** The regional groundwater seepage directions (flow lines) are also indicated as blue arrows on each of the maps in the source areas.



Figure 1: Map of the RPM area with distribution of groundwater monitoring points during 2018/2019

## 2 INTERPRETATION OF MONITORING DATA

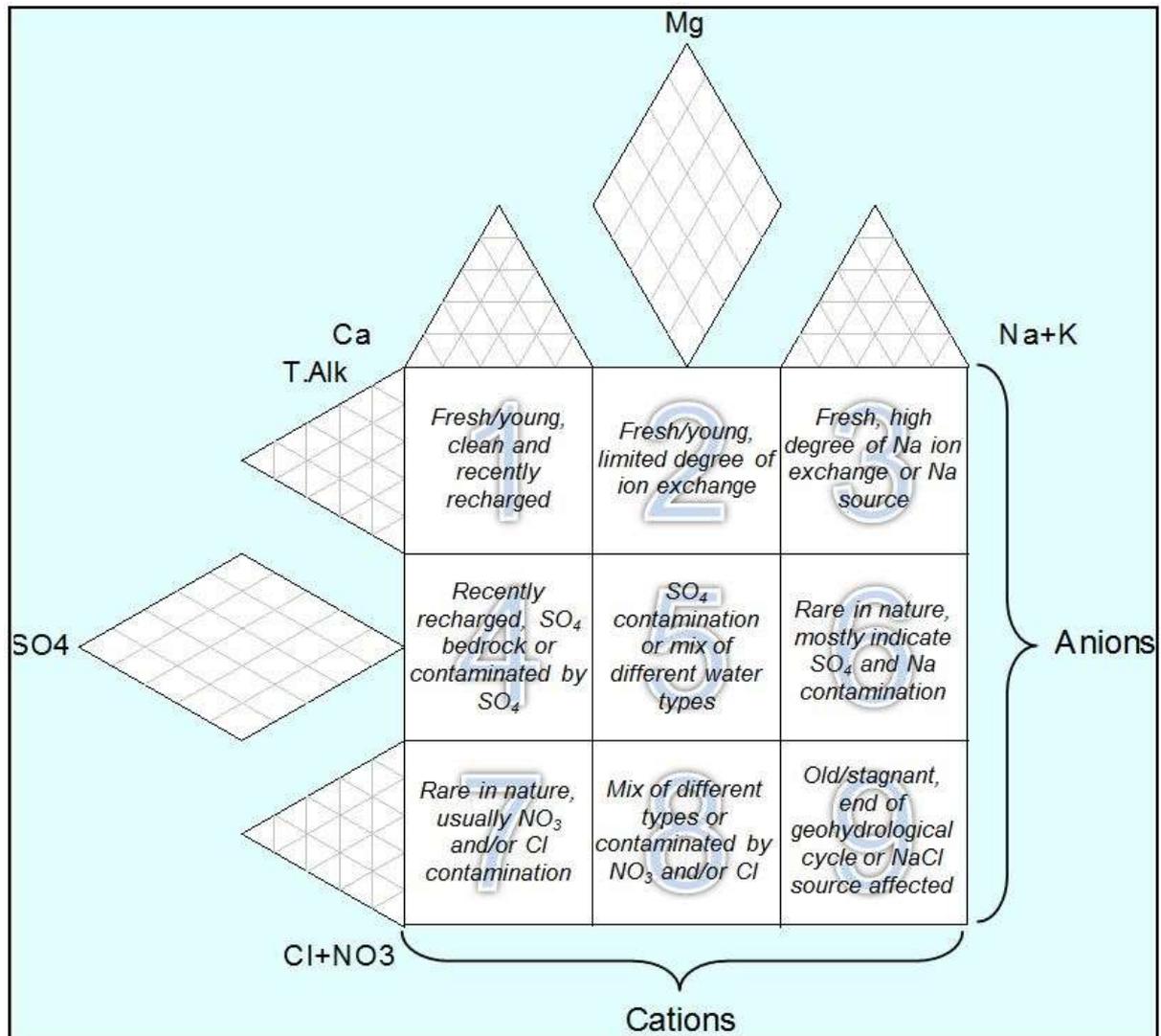
Five chemical parameters, namely Total Dissolved Solids (TDS), nitrate (NO<sub>3</sub>), sulphate (SO<sub>4</sub>), chloride (Cl) and sodium (Na) were chosen from the full list of analytes as indicators of the specific type of contamination commonly occurring at RPM:

- The **TDS** value provides a holistic measurement of the total inorganic ion content of the water.
- **Nitrate** content often increases in the vicinity of shafts, discard dumps and tailings facilities as a result of traces of nitrate-based explosives used in the mining process. As will be discussed frequently in this report, nitrate contamination is the most direct and prominent parameter that is influenced by the mining activities at RPM. Nitrate is affected because of remnants of explosives attached to run-of-mine rocks, including ore, waste and discard – wet or dry. These nitrates are very soluble in water and any contact with water of blasted rock results into dissolution in water or leachate formation when rainwater percolates through rock dumps, stockpiles or discard facilities. One of the areas where the most pronounced impacts of the nitrate contamination occur is discard rock dumps and settling dams at the shaft areas. Nitrate concentrations are also elevated in the tailings dam water but dilution with make-up water imported from outside source aid significantly in reducing the concentrations.
- **Sulphate** is a prominent and widespread contaminant in the base metal processing areas such as the concentrators, smelters and refineries. Most ore and gangue minerals occur in the form of metal sulphides. When liberated, crushed and washed in the mining process oxidation of these materials occurs and a reactions chain forms commonly referred to as acid-mine-drainage. Sulphuric acid forms in this process and sulphate levels increase significantly. Sulphate is therefore a common indicator of pollution resulting from the processing facilities and waste products.
- **Sodium** and **chloride** are usually present in high concentrations in connate water within the crystal structure or matrix of rocks. When blasted, crushed, smelted or processed in some other way, sodium and chloride are liberated and serve as a conservative indicator of the impact of mining and processing activities on the environment.

These parameters will be plotted for all the different areas and all boreholes where data exists. Although only the five parameters will be plotted in each case, all inorganic parameters will be assessed and anomalies will be discussed. Groundwater quality conditions are compared to the water quality objectives set by the RPM Water Use Licence (*WUL*) as well as the South African National Standards for drinking water (*SANS 241:2015*). The respective standards are provided in **Tables 1** and **2**.

The purpose with the time-series plots is not to show exact concentrations for each monitoring point and each parameter, but rather to present an overall impression of the trends over the past year.

One of the most appropriate ways to interpret the type of water at a sampling point is to assess the plot position of the water quality on different analytical diagrams like a Piper, Expanded Durov and Stiff diagrams. Of these three types, the Expanded Durov diagram probably gives the most holistic water quality signature. The characteristics of the different fields of the Expanded Durov diagram (EDD) are discussed briefly in **Figure 2**.



**Figure 2: Layout of the Expanded Durov diagram**

Another way of presenting the signature or water type distribution in an area is by means of Stiff diagrams. These diagrams plot the equivalent concentrations of the major cations and anions on a horizontal scale on opposite sides of a vertical axis. The plot point on each parameter is linked to the adjacent one resulting in a polygon around the cation and anion axes. The result is a small figure/diagram of which the geometry typifies the groundwater composition at the point. Ambient groundwater qualities in the same aquifer type and water polluted by the same source will for example display similar geometries.

**Table 1: Guideline concentrations according to RPM Water Use Licence**

<b>Chemical Parameter</b>	<b>Unit</b>	<b>RPM WUL Guideline Concentration</b>
Calcium	<i>mg/l</i>	34
Chloride	<i>mg/l</i>	14
EC	<i>mS/m</i>	45
Fluoride	<i>mg/l</i>	0.4
Magnesium	<i>mg/l</i>	16
Nitrate	<i>mg/l</i>	0.2
pH	<i>N/A</i>	6 - 9.5
Sodium	<i>mg/l</i>	22
Sulphate	<i>mg/l</i>	20

**Table 2: South African National Standards for drinking water (SANS 241:2011)**

<b>Determinant</b>	<b>Risk</b>	<b>Unit</b>	<b>Standard limits</b>
<b>Physical and aesthetic determinants</b>			
Free chlorine	Chronic health	<i>mg/L</i>	≤ 5
Monochloramine	Chronic health	<i>mg/L</i>	≤ 3
Conductivity at 25 °C	Aesthetic	<i>mS/m</i>	≤ 170
Total dissolved solids	Aesthetic	<i>mg/L</i>	≤ 1 200
Turbidity	Operational	<i>NTU</i>	≤ 1
	Aesthetic	<i>NTU</i>	≤ 5
pH at 25 C	Operational	<i>pH units</i>	≥ 5 to ≤ 9.7
<b>Chemical determinants - macro-determinants</b>			
Nitrate as N	Acute health – 1	<i>mg/L</i>	≤ 11
Nitrite as N	Acute health – 1	<i>mg/L</i>	≤ 0.9
Sulphate as SO <sub>4</sub> <sup>2-</sup>	Acute health – 1	<i>mg/L</i>	≤ 500
	Aesthetic	<i>mg/L</i>	≤ 250
Fluoride as F <sup>-</sup>	Chronic health	<i>mg/L</i>	≤ 1.5
Ammonia as N	Aesthetic	<i>mg/L</i>	≤ 1.5
Chloride as Cl <sup>-</sup>	Aesthetic	<i>mg/L</i>	≤ 300
Sodium as Na	Aesthetic	<i>mg/L</i>	≤ 200
Zinc as Zn	Aesthetic	<i>mg/L</i>	≤ 5
<b>Chemical determinants - micro-determinants</b>			
Aluminium as Al	Operational	<i>mg/L</i>	≤ 0.3
Antimony as Sb	Chronic health	<i>mg/L</i>	≤ 0.02
Arsenic as As	Chronic health	<i>mg/L</i>	≤ 0.01
Barium Ba	Chronic health	<i>mg/L</i>	≤ 0.7
Boron B	Chronic health	<i>mg/L</i>	≤ 2.4
Cadmium as Cd	Chronic health	<i>mg/L</i>	≤ 0.003
Total chromium as Cr	Chronic health	<i>mg/L</i>	≤ 0.05
Cobalt as Co	Chronic health	<i>mg/L</i>	≤ 0.5

<b>Determinant</b>	<b>Risk</b>	<b>Unit</b>	<b>Standard limits</b>
Copper as Cu	Chronic health	<i>mg/L</i>	≤ 2
Cyanide (recoverable) as CN <sup>-</sup>	Acute health – 1	<i>mg/L</i>	≤ 0.07
Iron as Fe	Chronic health	<i>mg/L</i>	≤ 2
	Aesthetic	<i>mg/L</i>	≤ 0.3
Lead as Pb	Chronic health	<i>mg/L</i>	≤ 0.01
Manganese as Mn	Chronic health	<i>mg/L</i>	≤ 0.4
	Aesthetic	<i>mg/L</i>	≤ 0.1
Mercury as Hg	Chronic health	<i>mg/L</i>	≤ 0.006
Nickel as Ni	Chronic health	<i>mg/L</i>	≤ 0.07
Selenium as Se	Chronic health	<i>mg/L</i>	≤ 0.04
Uranium as U	Chronic health	<i>mg/L</i>	≤ 0.015
Vanadium as V	Chronic health	<i>mg/L</i>	≤ 0.2
<b>Organic determinants</b>			
Total organic carbon	Acute health – 1	<i>mg/L</i>	≤ 10

**Table 3: Average concentrations of indicator parameters for the 2018/2019 monitoring year**

Site Name	pH	TDS	Ca	Mg	Na	K	Cl	SO <sup>4</sup>	NO <sup>3</sup>	Fe	Mn
		mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
BMRWWTW	8.4	951	99	60	135	2	23	460	3.016	-0.009	0.081
EM11	7.5	2179	264	236	223	12	886	331	0.692	0.263	0.888
EM16	8.1	3437	402	266	410	4	1083	1018	0.781	-0.009	3.525
NB01	-	-	-	-	-	-	-	-	-	-	-
NB03	8.3	1104	91	110	109	18	64	533	-0.459	-0.009	0.196
NB04	8.0	451	30	27	43	12	18	1	0.085	-0.009	0.166
NB48	-	-	-	-	-	-	-	-	-	-	-
NB52	7.9	1797	104	41	87	57	58	119	54.429	0.830	0.567
NB56	8.4	681	17	47	133	18	68	84	0.596	-0.009	0.080
NB57	8.4	775	13	50	138	18	61	114	0.152	-0.009	0.061
NBH07	7.4	25630	3332	824	4674	121	15538	1	0.097	0.505	53.760
S011	7.4	6664	565	203	1218	5	78	4074	10.480	0.017	4.873
S051	8.0	1406	117	133	158	7	104	594	2.249	-0.009	0.058
S102	8.2	6448	115	265	1822	15	1209	2567	-0.212	-0.002	0.091
S104	8.2	1123	93	63	197	4	43	466	1.222	0.038	0.243
S120	9.3	20319	29	185	6708	43	596	11796	-0.034	0.159	0.328
S160	8.2	2223	163	98	463	4	530	675	0.746	-0.009	0.024
S230	8.4	6175	142	74	1785	81	352	2395	-0.459	0.019	0.496
S373	8.1	2982	334	264	359	10	1198	380	0.696	0.000	0.050
S374	7.0	6781	778	512	915	47	3816	363	47.432	0.007	1.351
S386	7.8	4448	759	136	610	7	723	1927	-0.459	-0.009	0.138
S388	7.2	3333	480	174	365	26	56	1678	-0.459	1.930	1.920
S389	8.0	15305	422	430	4360	18	2235	7148	-0.459	0.126	0.331
S400	7.5	3055	281	208	461	2	299	1483	2.770	-0.009	0.188
S403	7.8	1471	119	60	298	8	33	744	1.688	-0.002	0.023
S405	-	-	-	-	-	-	-	-	-	-	-
S407	8.2	2756	213	184	479	4	287	1252	2.493	-0.009	0.057
S409	8.5	2749	146	151	481	18	99	1209	2.599	0.013	0.045
S410	8.3	2271	160	182	313	9	136	1025	3.370	-0.009	0.076
S418	7.2	56992	480	136	18210	66	481	36978	-0.264	18.306	27.906

**\*RED – Exceeds SANS maximum limit for drinking water**

## 2.1 WATERVAL SMELTER, CONCENTRATOR, AND ACID PLANT

Three boreholes were in use to monitor groundwater impacts at the Waterval Processing area during the 2006/07 monitoring year. Thirteen monitoring boreholes were added in order to sufficiently cover the area. Ten boreholes were monitored during the 2015/2016 monitoring year. In the 2018/2019 monitoring year, 7 boreholes remained as part of the monitoring plan of which 5 were sampled and their positions are indicated in **Figure 3**

This processing complex consists of a number of processing modules including the Waterval Concentrator, Waterval Smelter, UG2 Concentrator and the Acid Plant. The larger part of the surface area underlying the Waterval Processing area is lined by concrete surfaces and effluent dams like storm water control, settling and return water dams are lined with synthetic or clay liners. Seepage and leachate formation however still emanates from the Waterval Processing area.

The Waterval Processing area is situated to the south of an east-west trending surface water divide and groundwater flow will be in the same direction as surface flow, namely southwards. Groundwater seepage and mass transport will thus also occur southwards and then west in the direction of flow of the Klipfontein Spruit.

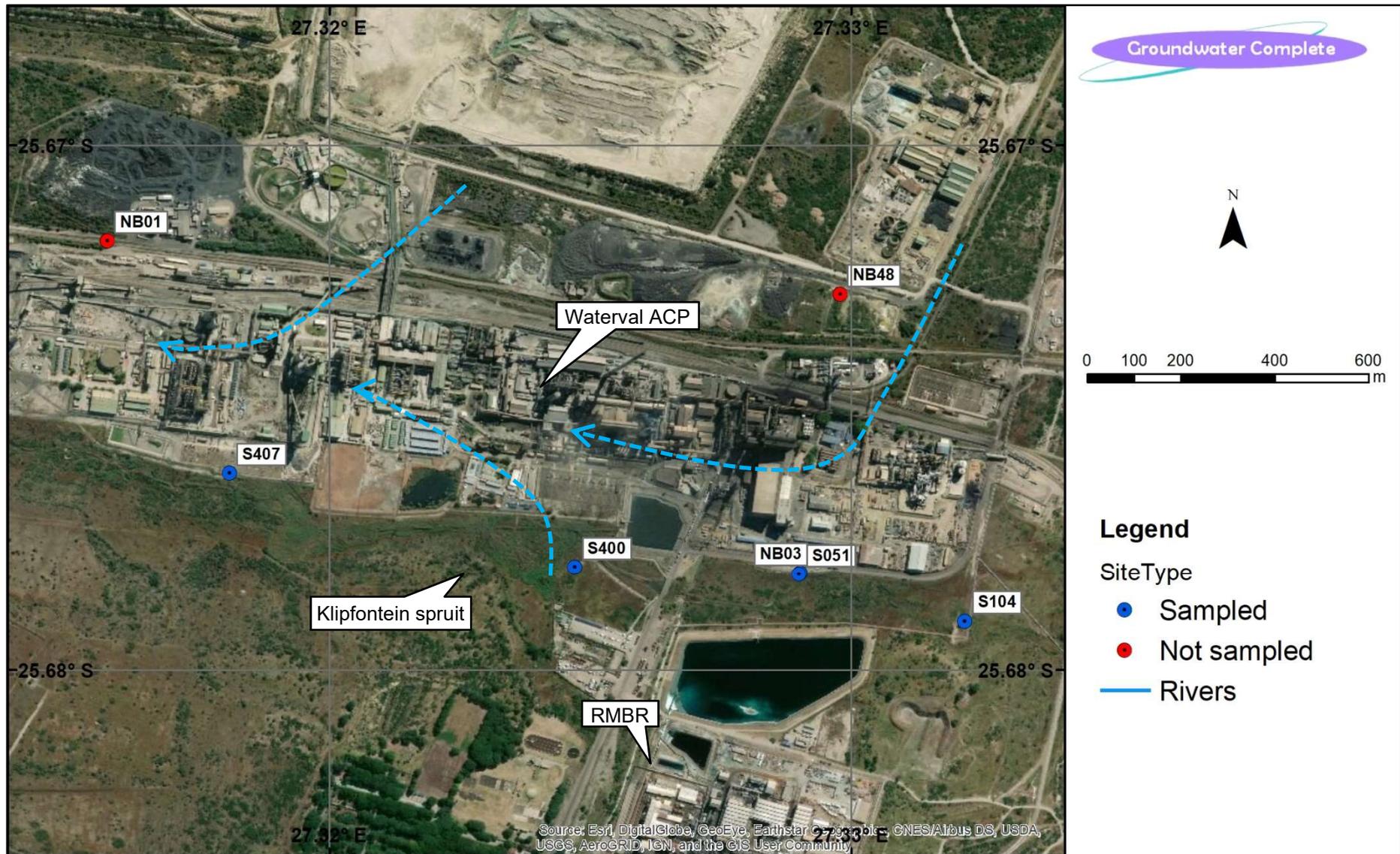


Figure 3: Positions of monitoring boreholes in the Waterval Processing area

Time-series plots of indicator chemical parameters for the Waterval Processing area are presented in **Figures 4 and 5**.

Average groundwater **TDS** concentrations for the 2018/2019 monitoring year in S104 and NB03 varied between  $\pm 1100$  mg/l and  $1\,125$  mg/l, which are just below the permissible SANS value of  $1\,200$  mg/l, however, they did on numerous sampling runs exceed the permissible levels. Groundwater salinity measured in monitoring boreholes S051, S400 and S407 exceeded the maximum concentration allowed in drinking water and displayed averages of between  $\pm 1\,400$  mg/l and  $3\,060$  mg/l. The highest concentration was measured in S407 and time-series graphs provided in **Figure 4** display an increasing trend for this borehole. **No guideline TDS value is specified by the Water Use License for RPM.**

The **sulphate** content measured in S051, S400, S407 and NB03 exceeded the SANS guideline value of  $500$  mg/l and displayed averages of between  $\pm 530$  mg/l and  $1\,480$  mg/l. Again, the highest concentrations were measured in monitoring borehole 407, which also displayed a general increase in the groundwater sulphate content (**Figure 4**). An average of  $466$  mg/l was measured in borehole S104, which just falls short of the maximum concentration allowed in drinking water. **The groundwater sulphate content measured in all monitoring boreholes far exceeded the RPM WUL guideline concentration of  $20$  mg/l throughout the entire evaluation period.**

Groundwater **chloride** concentrations measured in the majority of monitoring boreholes remained below the permissible SANS value of  $300$  mg/l during the 2018/2019 monitoring period (**Figure 5**). The highest concentrations were once again measured in monitoring boreholes S400 and S407, which displayed an average  $\pm 300$  and  $290$  mg/l respectively. The downgradient monitoring borehole S407 also displayed an increasing concentration trend for chloride, not exceeding the maximum permissible limits for drinking water ( $300$  mg/l). **The groundwater chloride content within the immediate vicinity of the Waterval Processing area exceeded the RPM WUL guideline concentration of  $14$  mg/l.**

Groundwater **sodium** concentrations measured in monitoring boreholes S400 and S407 exceeded the permissible SANS value of  $200$  mg/l during the past monitoring year and displayed averages of  $\pm 460$  mg/l and  $480$  mg/l respectively. Averages of between  $\pm 110$  mg/l and  $200$  mg/l were measured in the remainder of groundwater monitoring boreholes, which are within the maximum concentration allowed in drinking water. The groundwater sodium content in S140 seems to have decreased during the past monitoring year, while the concentrations in S407 and S102 increased (**Figure 5**). **The WUL guideline concentration of  $22$  mg/l was exceeded in all boreholes during the 2018/2019 monitoring year.**

Groundwater **nitrate** concentrations remained below the permissible SANS value of  $11$  mg/l throughout the entire monitoring period. Monitoring boreholes displayed averages from less than  $1$  mg/l to  $2.8$  mg/l. Higher than ambient nitrate concentrations were observed in S051, S400 and S407. Increasing nitrate concentration trends were also observed in both these boreholes over the monitoring period. **The nitrate content of groundwater within the immediate vicinity of the Waterval Processing area exceeded the RPM WUL guideline concentration of  $0.2$  mg/l in all boreholes, except for NB03.**

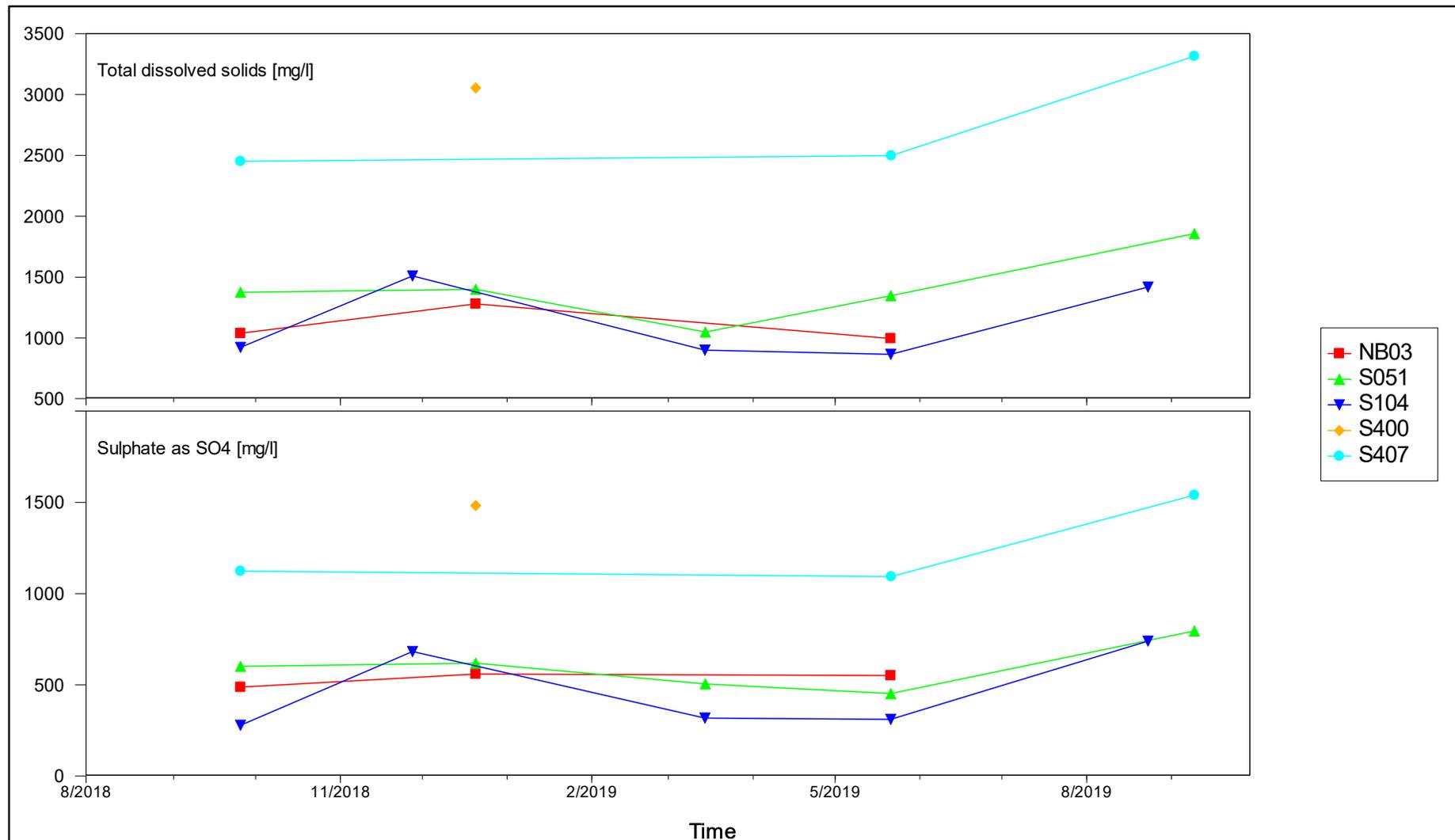
According to **Figures 6** and **7** the Waterval Processing area is mainly dominated by groundwater that is usually a mix of different types – either clean water from fields 1 and 2 of the Expanded Durov diagram (EDD) that has undergone sulphate and sodium chloride mixing/contamination or old stagnant sodium chloride dominated water that has mixed with clean water – groundwater is therefore dominated by **magnesium/sodium** cations, while **sulphate/chloride** dominates the anion content. This indicates that the groundwater has definitely experienced adverse effects from the Waterval processing area activities.

**The dominant plot position in field 5 of the EDD confirms definite impacts of the Waterval Processing area on the natural groundwater environment.**

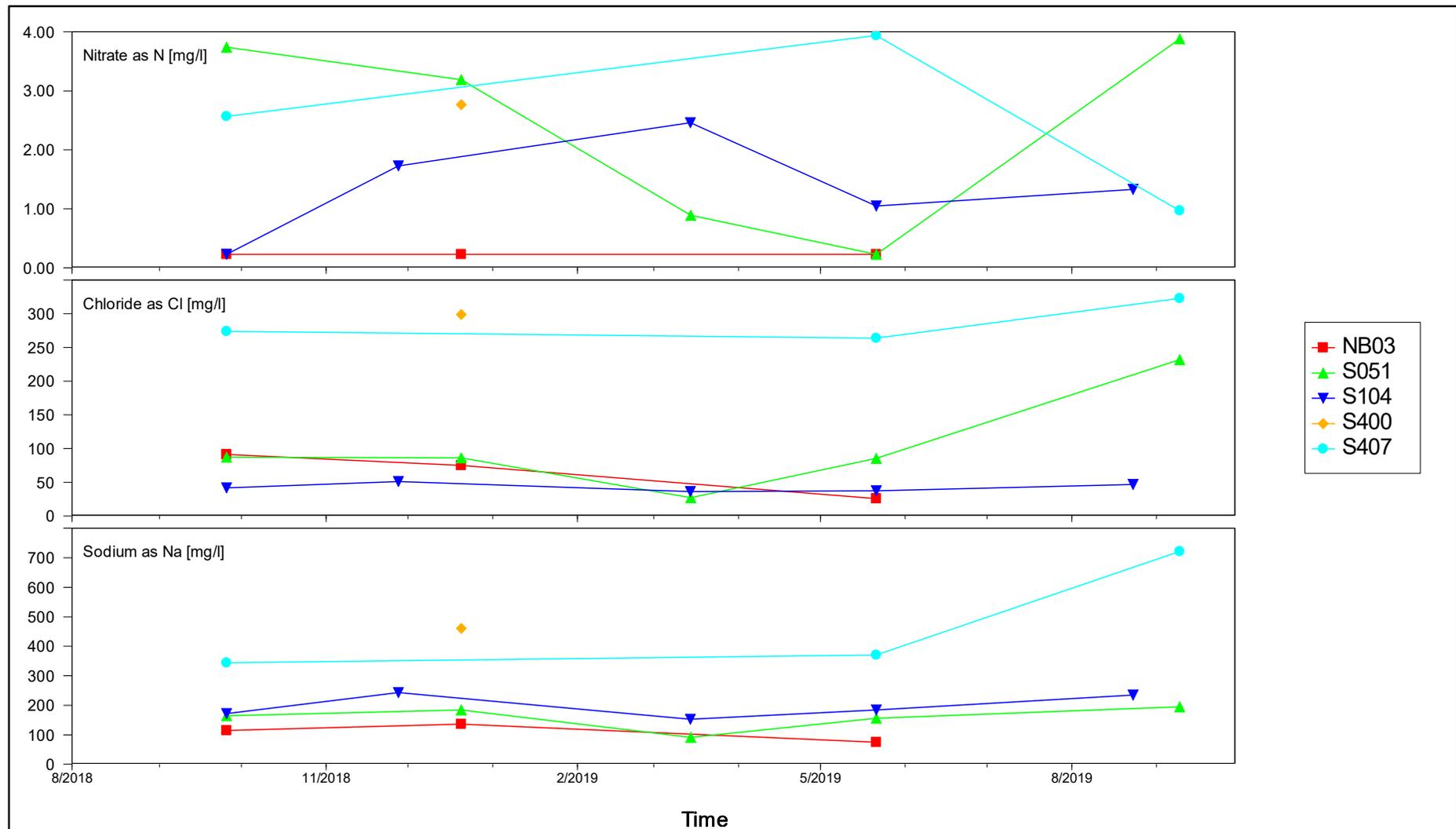
Average **water levels** for the Smelter and ACP monitoring boreholes varied between 2 and 4 meters below surface (mbs). NB03 displayed a decreasing water level trend (**Figure 8**).

**Summary:**

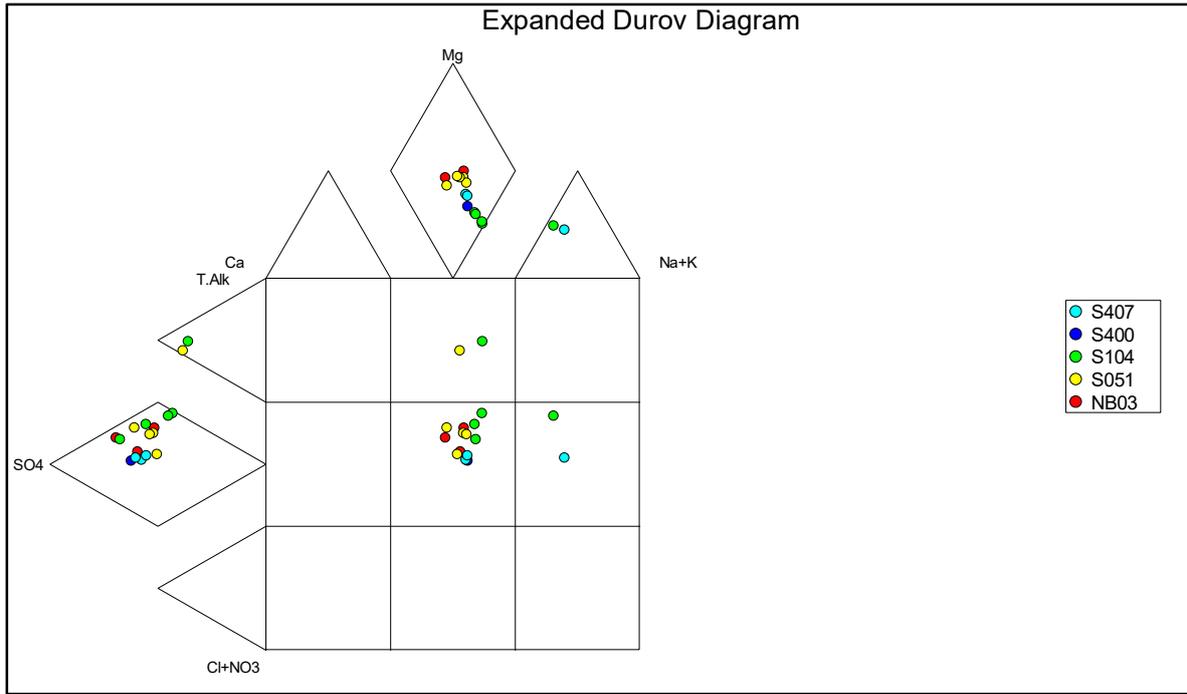
- Definite impacts from the Waterval Processing area occur on the down gradient groundwater environment.
- Sulphate is especially of concern, as the majority of groundwater monitoring boreholes indicated elevated concentrations.
- Up gradient groundwater monitoring borehole S407 displayed the highest levels of pollution throughout the 2018/2019 monitoring year, however the pollution is unlikely to originate from the Waterval Processing area.
- S400 also had overall high borehole concentration levels, however it only had a single sample taken and its level of pollution can't be determined definitively.
- Increasing parameter concentrations in the downgradient monitoring borehole, S407 are often observed over the monitoring period.
- **Concentrations of indicator chemical parameters do not comply with the water quality objectives stated in the RPM Water Use License.**
- Water levels vary between 2 and 4 mbs.



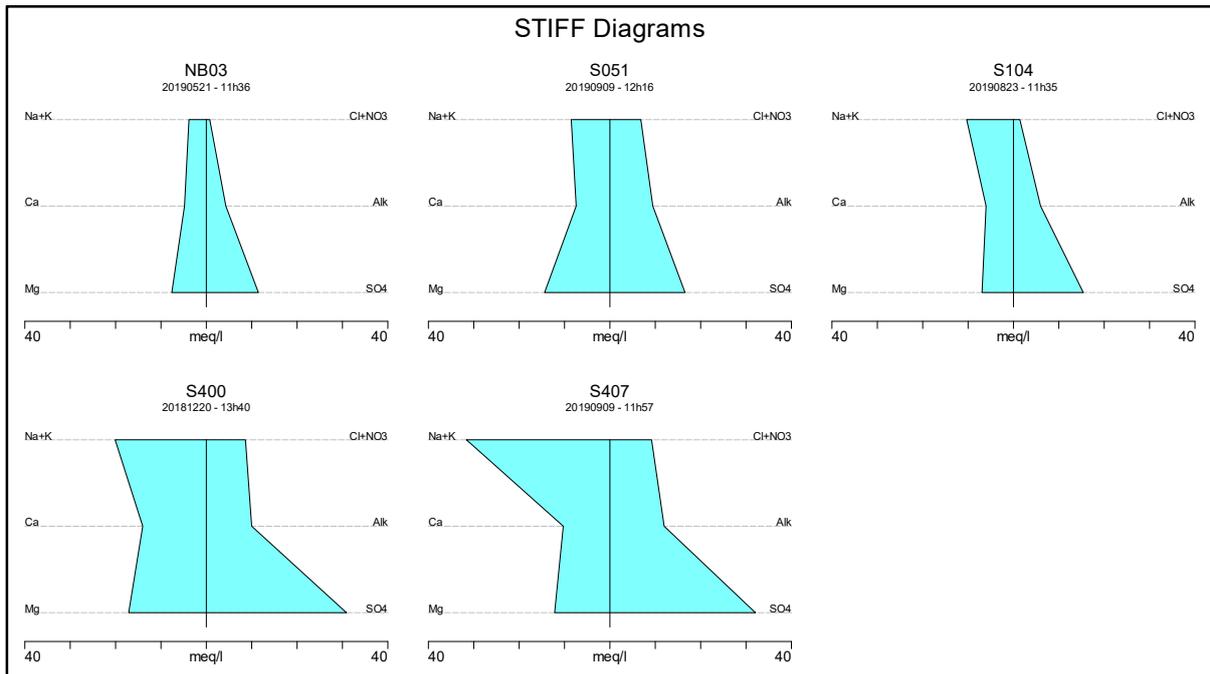
**Figure 4: Time-series plot of indicator chemical parameters in the Waterval Processing area – TDS and SO<sub>4</sub>**



**Figure 5: Time-series plot of indicator chemical parameters in the Waterval Processing area – NO<sub>3</sub>, Cl and Na**



**Figure 6: EDD of groundwater chemistry in the Waterval Processing area**



**Figure 7: Stiff diagrams of groundwater chemistry in the Waterval Processing area**

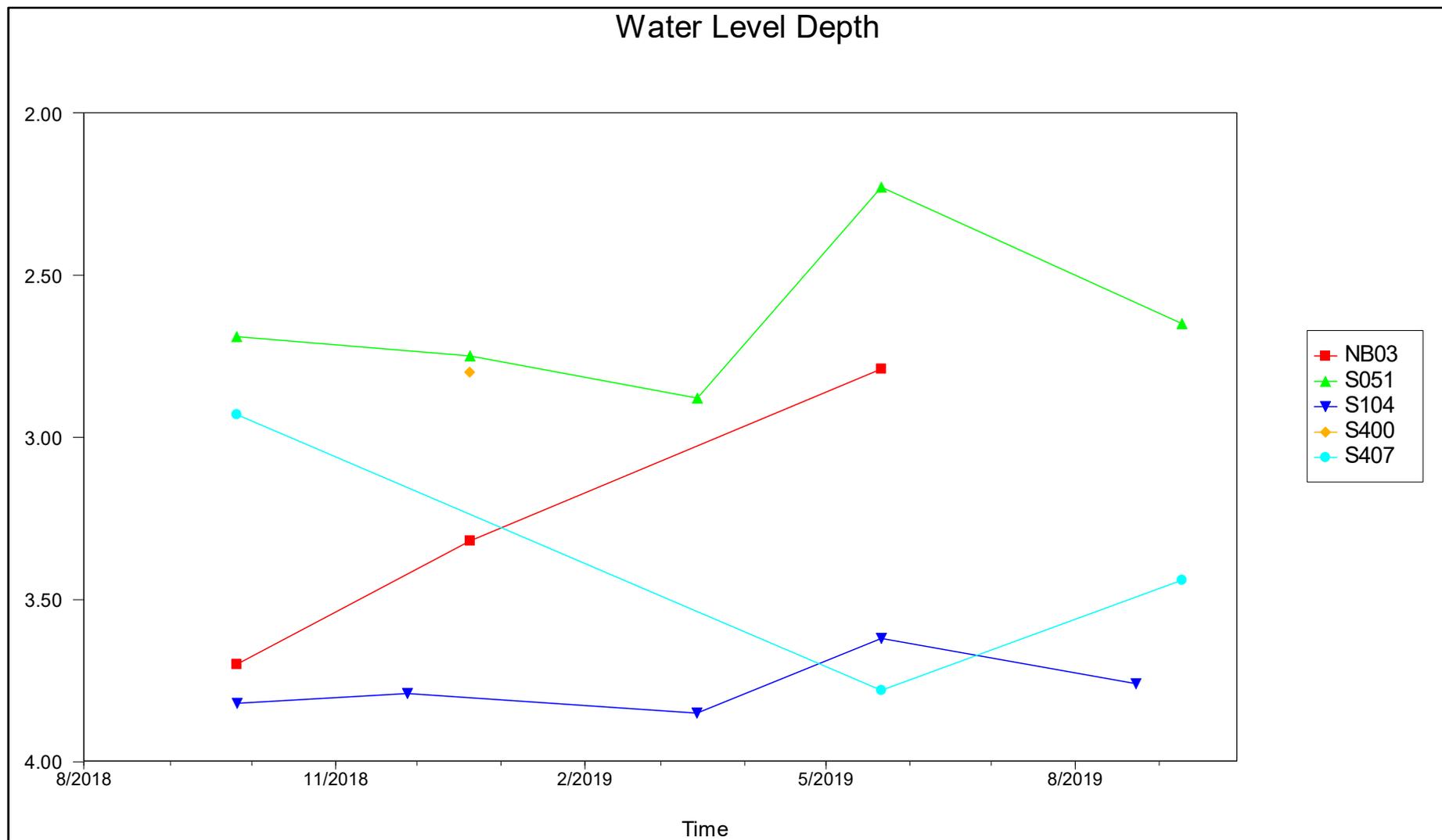


Figure 8: Time series plot of water levels for in the Waterval Processing area

## 2.2 THE RUSTENBURG BASE METAL REFINERY (RBMR)

Seven boreholes were historically used to monitor groundwater impacts at the Rustenburg Base Metal Refinery (RBMR). The distribution and number of monitoring boreholes were insufficient during previous monitoring years, after which boreholes were drilled and existing ones were added to the more extensive monitoring programme.

A total of 15 boreholes were monitored in the Rustenburg Base Metal Refinery area during the 2018/2019 monitoring period and their positions are indicated in **Figure 9**.

This processing complex consists of a large base metal refinery area with associated effluent dams for storage of process water. The most notable of these are the sodium sulphate solution area to the south-east of the refinery where highly concentrated sodium sulphate solution by-product is treated and dried. The groundwater pollution in this area is by far the dominant impact of the RBMR area as a result of leachate formation as well as seepage from effluent dams where historical liners were not fully impervious.

The larger part of the surface area underlying the actual refinery is lined by concrete surfaces, but historical leaks and dumping caused the formation of a large diffuse source area for contamination. Seepage and leachate formation thus still emanates from the RBMR area and remediation plans target the RBMR as the first priority area. The RBMR is situated on the southern banks of the Klipfontein Spruit directly opposite the Waterval Processing area. Groundwater flow and mass transport from the site is northwards in the direction of the Klipfontein Spruit (**Figure 9**).

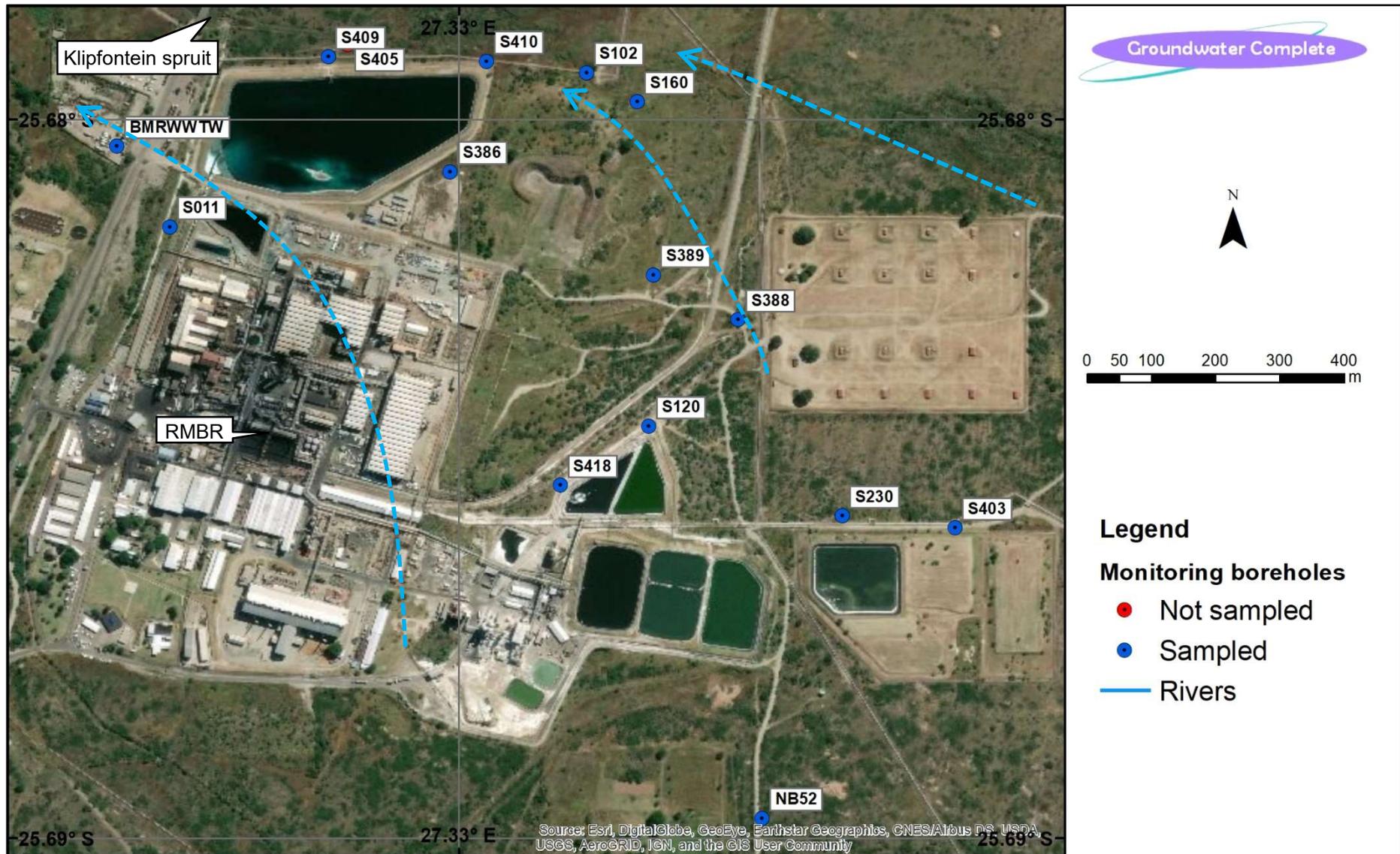


Figure 9: Positions of monitoring boreholes in the RBMR area

Time-series plots of the indicator chemical parameters for the RBMR area are presented in **Figures 10 and 11**.

The groundwater **TDS** concentrations for all the RBMR boreholes, except BMRWWTW, far exceeded the permissible SANS value of 1 200 mg/l during the 2018/2019 monitoring year. Averages in the down gradient groundwater flow direction varied between  $\pm$  1 470 mg/l and 57 000 mg/l. The highest concentrations by far were measured down gradient from the sodium sulphate solution area in monitoring borehole S418. The TDS concentration in S418 increased further over the monitoring period with the highest concentration measured in May 2019 at 86 750 mg/l. The TDS concentrations in the remainder of the boreholes remained relatively constant over the monitoring period (**Figure 10**). Exceptions did occur and some boreholes displayed much lower salinities throughout the year. Variations within such short distances might indicate the presence of groundwater compartments created by low transmissivity dykes. **No guideline TDS value is specified by the Water Use License for RPM.**

Sulphate and sodium (**Figures 12 and 13**) make up most of the inorganic salinity in the groundwater. Average **sulphate** concentrations measured in the majority of groundwater monitoring boreholes varied between  $\pm$  740 mg/l and 36 980 mg/l, which far exceed the permissible SANS value of 500 mg/l. The groundwater sulphate content measured in boreholes BMRWWTW and NB52 did however remain below the SANS acute health guideline value for drinking water throughout the evaluation period, with averages of 460 and 120 mg/l respectively. Borehole S418 is located directly down gradient from the sodium sulphate solution area and indicated the most profound sulphate pollution. Similar to groundwater salinity, the sulphate content in S418 have increased during the past monitoring year (**Figure 10**). A concentration exceeding 55 000 mg/l was measured in May 2019 in this borehole. **The RPM WUL guideline concentration of 20 mg/l was exceeded by all monitoring boreholes.**

Groundwater **sodium** and sulphate concentrations displayed much the same distribution and trends with the highest levels of pollution being measured down gradient from the sodium sulphate solution area in monitoring borehole S418. The groundwater sodium content measured in the majority of the monitoring boreholes far exceeded the permissible SANS concentration of 200 mg/l and displayed averages of between  $\pm$  300 mg/l and 18 200 mg/l. Once again similar to groundwater salinity and sulphate, the sodium content in S418 has increased during the past monitoring year (**Figure 10**). **The groundwater sodium content measured in all monitoring boreholes exceeded the RPM WUL guideline concentration of 22 mg/l throughout most of the past monitoring year.**

Average **nitrate** concentrations measured in groundwater both up and down gradient from the RBMR area are below the permissible SANS value of 11 mg/l. Monitoring borehole S409 displayed an average concentration of 10 mg/l, which is higher than the surrounding monitoring boreholes. The remainder of the boreholes had average nitrate concentrations from below detection limit to 3.5 mg/l. Some fluctuation in concentrations were measured in

most boreholes throughout the past monitoring year (**Figure 11**). The nitrate concentration in NB52 increased significantly over the past monitoring year. The reason for the increase is unknown, but definitely not caused by the RBMR, as it is in the up-gradient direction of groundwater flow. **The RPM WUL guideline concentration of 0.2 mg/l was exceeded in all monitoring boreholes, except S386, S389, S388 and S230, of which the concentrations were all below detection level.**

Groundwater monitoring boreholes S102, S120, S160, S230, S386, S389 and S418 displayed average groundwater **chloride** concentrations of between  $\pm$  350 mg/l and 2 230 mg/l, which exceed the permissible SANS value of 300 mg/l. Averages measured in the remainder of boreholes are within the maximum concentration allowed in drinking water. The highest concentrations were measured in S389, which, however, displayed a decreasing trend over the monitoring period (**Figure 11**). The concentration in S102 also decreased significantly over the monitoring period. **The chloride content of groundwater within the immediate vicinity of the RBMR operations exceeded the RPM WUL guideline concentration of 14 mg/l.**

The groundwater **iron** content measured in the majority of monitoring boreholes remained below the detection limit of 0.0045 mg/l throughout the past monitoring year. Monitoring borehole S418 was however the exception and displayed an average of  $\pm$  18 mg/l, which far exceeds the permissible SANS concentration of 2 mg/l. Significant fluctuations in the groundwater iron content have been measured in S418 throughout the past 10 years, which is only possible under unstable groundwater pH conditions. **No guideline concentration is specified for iron in the Water Use License for RPM.**

According to **Figures 12 and 13** the following types of groundwater are predominant within the immediate vicinity of the RBMR area:

- Fresh, clean, relatively young groundwater – groundwater is therefore dominated by **calcium/magnesium/sodium** cations, while the anion content is dominated by **bicarbonate alkalinity**.
- Groundwater that is usually a mix of different types – either clean water from fields 1 and 2 of the EDD that has undergone sulphate and sodium chloride mixing/contamination or old stagnant sodium chloride dominated water that has mixed with clean water – groundwater is therefore dominated by **magnesium** cations and **sulphate** anions.
- Groundwater that has been in contact with a source rich in sodium or old stagnant sodium chloride dominated water that resides in sodium rich host rock/material – groundwater is therefore dominated by **sodium/potassium** cations, while **sulphate** dominates the anion content.

**The plot positions in fields 5 and 6 of the EDD confirm definite impacts of the processing facilities on the natural groundwater environment.**

From the stiff diagrams in **Figure 13** it is clear that S120, S389 and S418 are the most significantly impacted on by the processing facilities with sulphate and sodium being the dominant ions.

The average **water levels** of the RBMR area are between 3 and 15 mbs. No significant increasing or decreasing water level trends (**Figure 14**) occur.

**Summary:**

- Significant pollution impacts from the RBMR occur on the groundwater environment.
- Impacts are by far the most significant in the sodium sulphate solution area to the south-east of the refinery.
- Groundwater iron concentrations measured in monitoring borehole S418 fluctuated significantly throughout the year, which may be the result of varying groundwater pH conditions.
- The most significant impacts from the refinery were measured in groundwater from boreholes S120, S389 and S418.
- The main contaminants of concern are sodium and sulphate.
- The extent of impact (plume) is however limited to within a few meters of the sources due to poor aquifer hydraulic properties.
- **The indicator chemical parameters do not comply with the water quality objectives stated in the RPM Water Use License.**

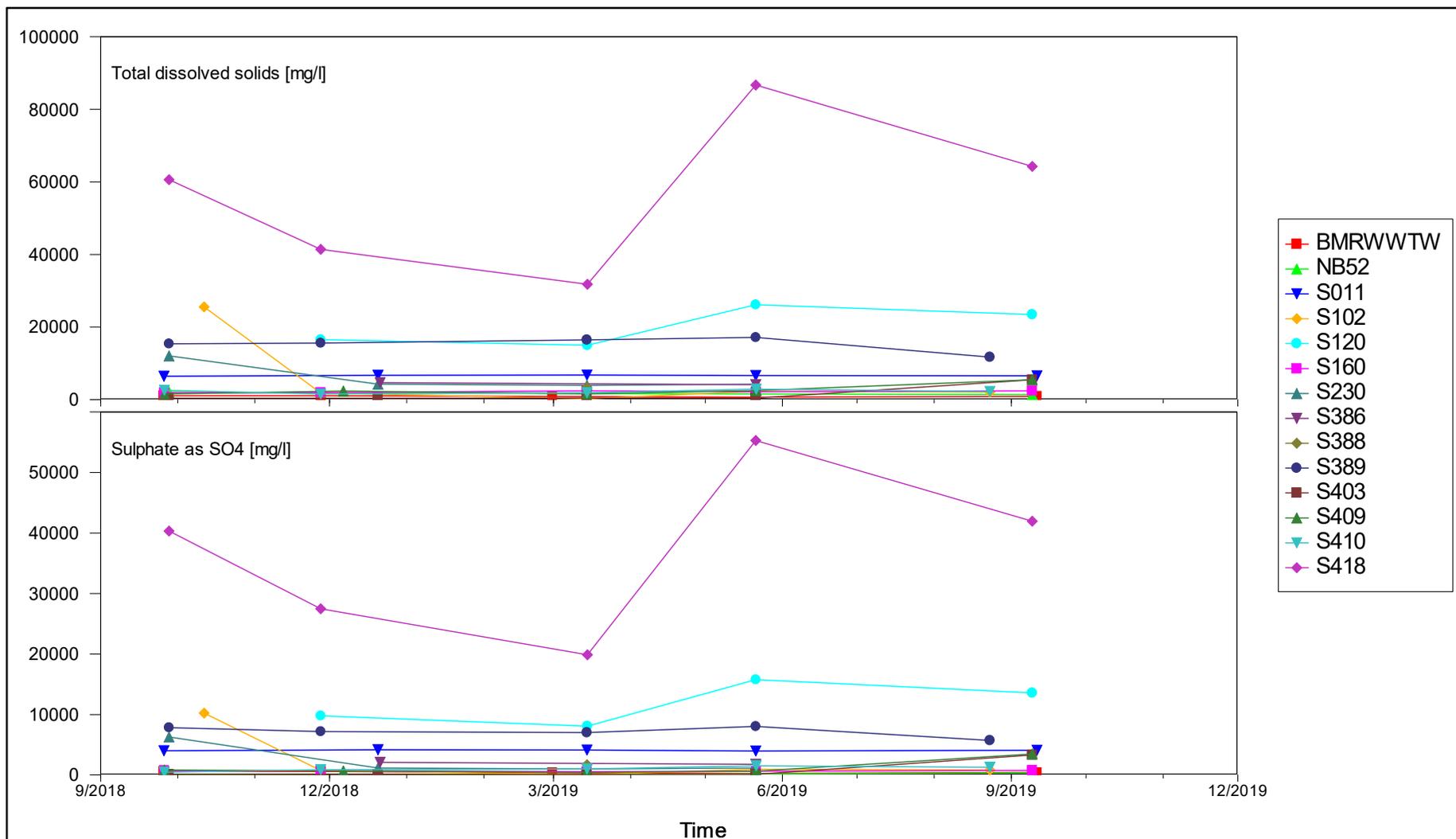


Figure 10: Time-series plot of indicator chemical parameters in the RBMR area – TDS and SO<sub>4</sub>

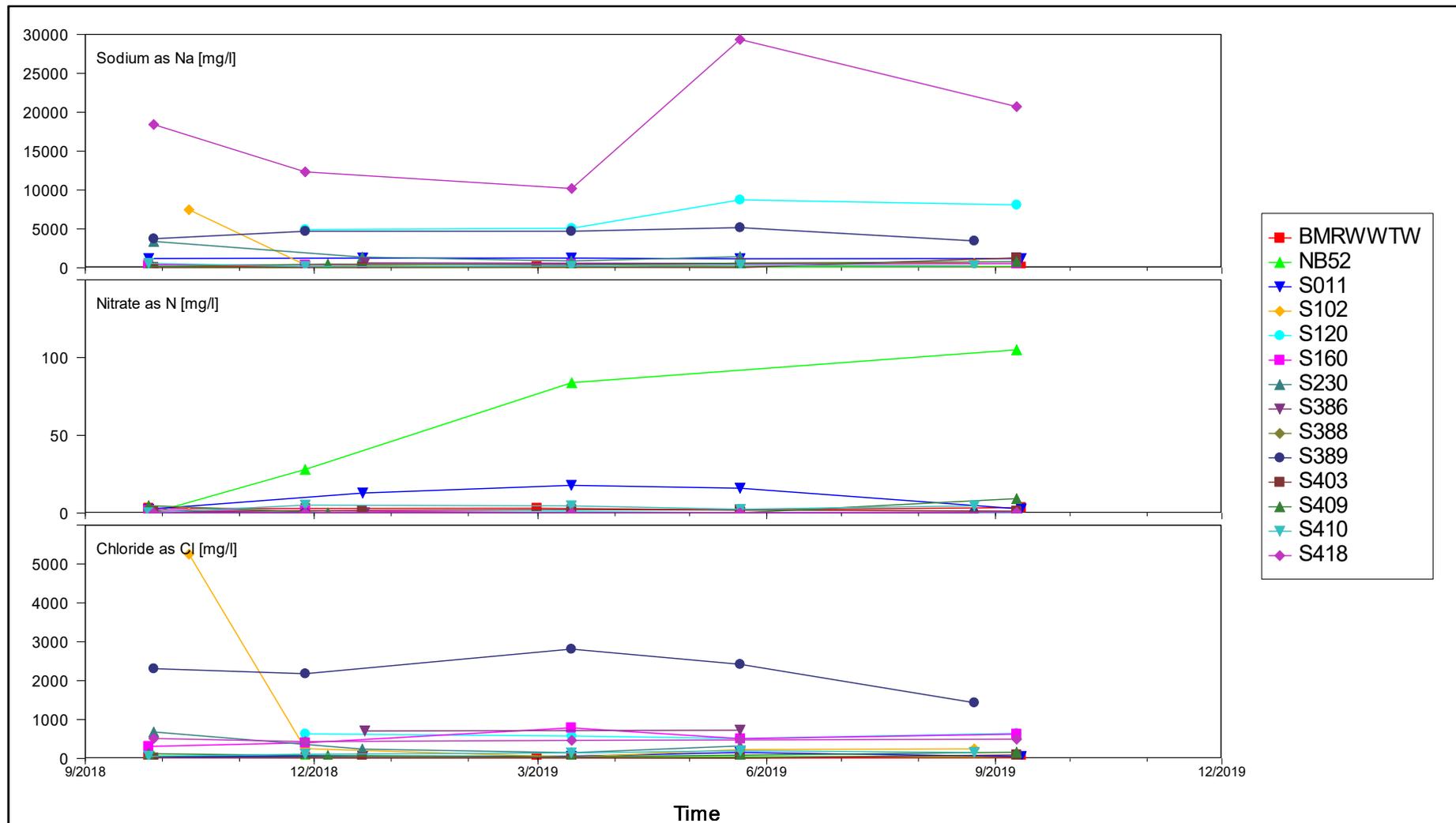
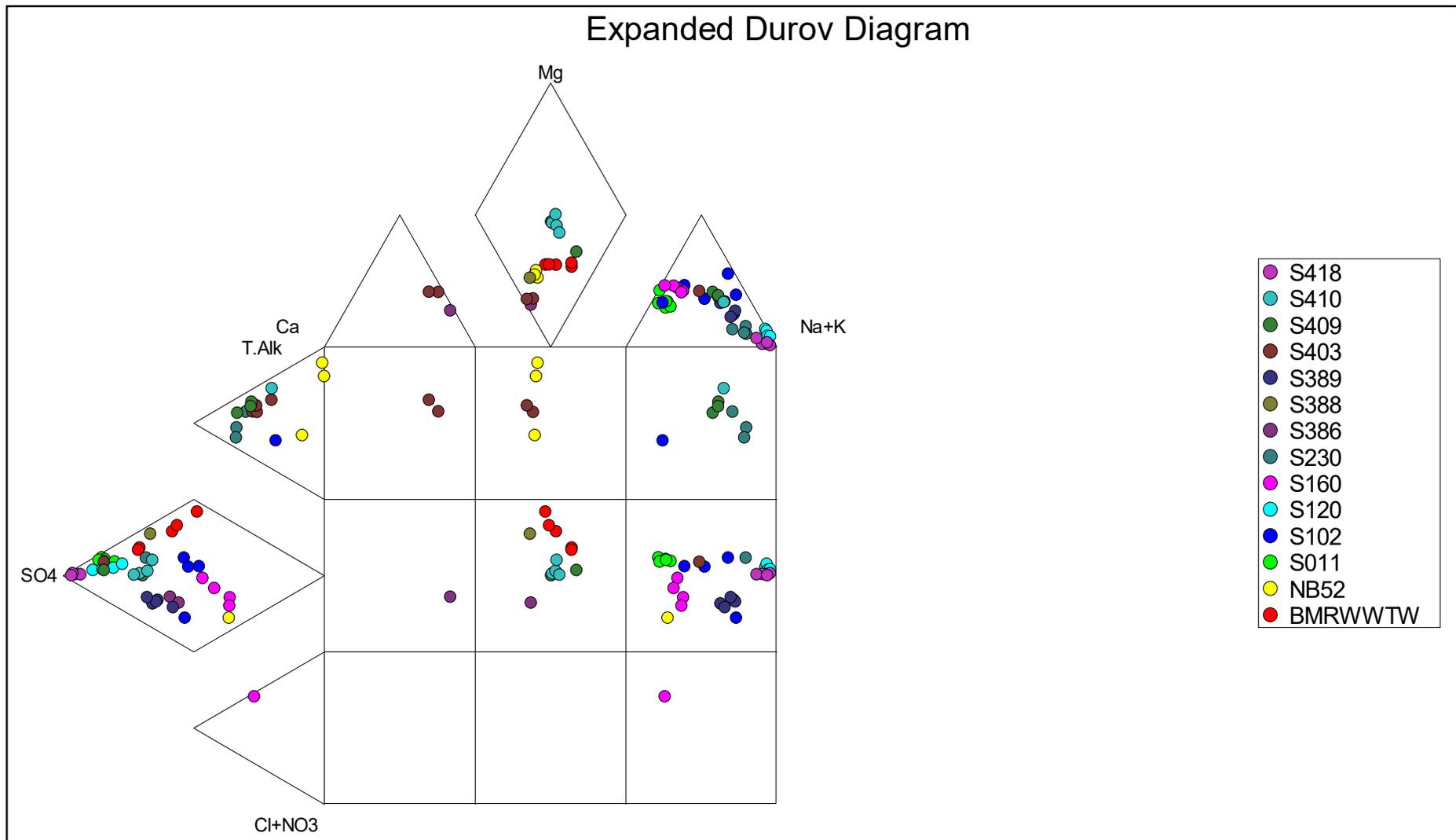


Figure 11: Time-series plot of indicator chemical parameters in the RBMR area – Na, NO<sub>3</sub> and Cl



**Figure 12: EDD of groundwater chemistry in the RBMR area**

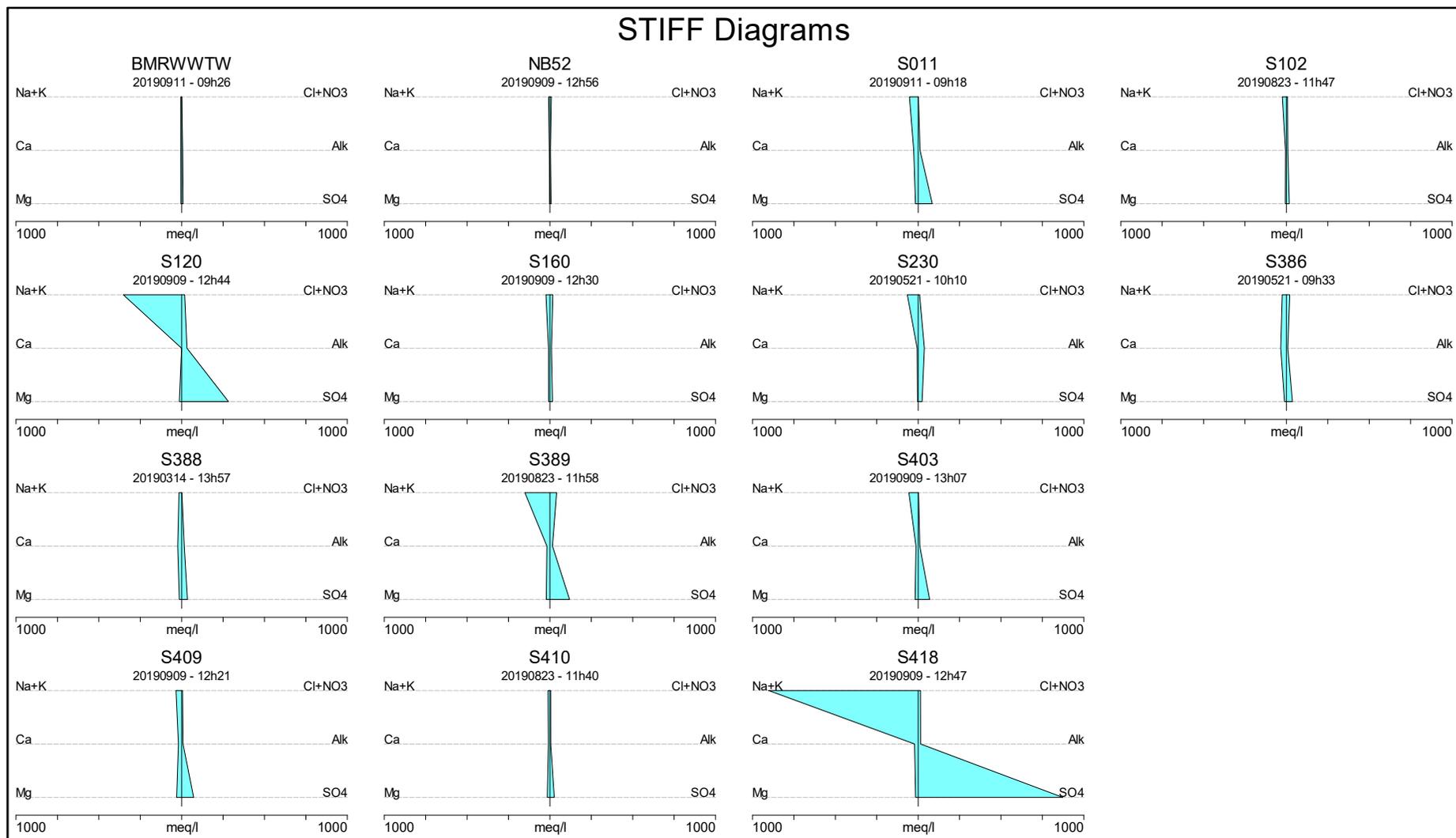


Figure 13: Stiff diagrams of groundwater chemistry in the RBMR area

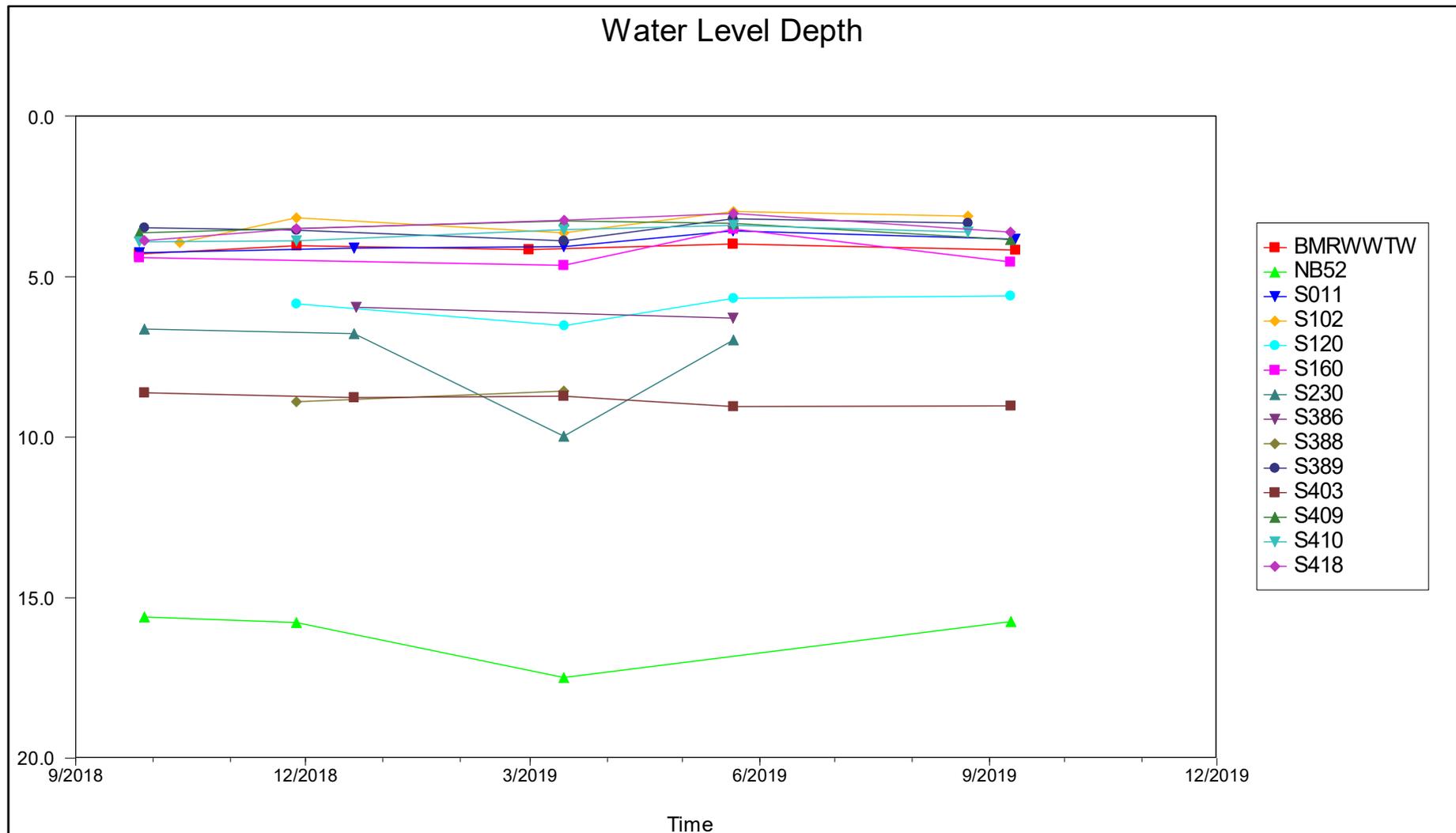


Figure 14: Time series plot of water levels for in the RBMR area

### 2.3 THE PRECIOUS METAL REFINERY (PMR) AND CENTRAL DEEPS SHAFT

Three boreholes were in use to monitor groundwater impacts at the Precious Metal Refinery (PMR) in 2006/2007. In an effort to increase the accuracy and efficiency of monitoring five existing boreholes were added to the monitoring program. Over the years boreholes became blocked and/or demolished and an additional source monitoring borehole, namely NBH07 was drilled approximately 60 meters down gradient from the refinery in 2013. The PMR consists of a refinery with a number of effluent dams for process water storage, settling and re-use. The precious platinum group metals are extracted at the PMR. The Central Deeps Shaft, which is located approximately 900 meters north of the PMR, is also included in this evaluation. A total of seven boreholes were monitored during the 2018/2019 monitoring year and their positions are indicated in **Figure 15**.

The PMR is a relatively new facility compared to other infrastructure at RPM and pollution control measures have been constructed according to more advanced pollution prevention technology. Leaking dam liners have however resulted in some groundwater contamination occurring in the area. The PMR is situated on the southern banks of the Klipfontein Spruit approximately 1.6 km east and up gradient from the RBMR. Groundwater flow and mass transport from the site is northwards in the direction of the Klipfontein Spruit (**Figure 15**).

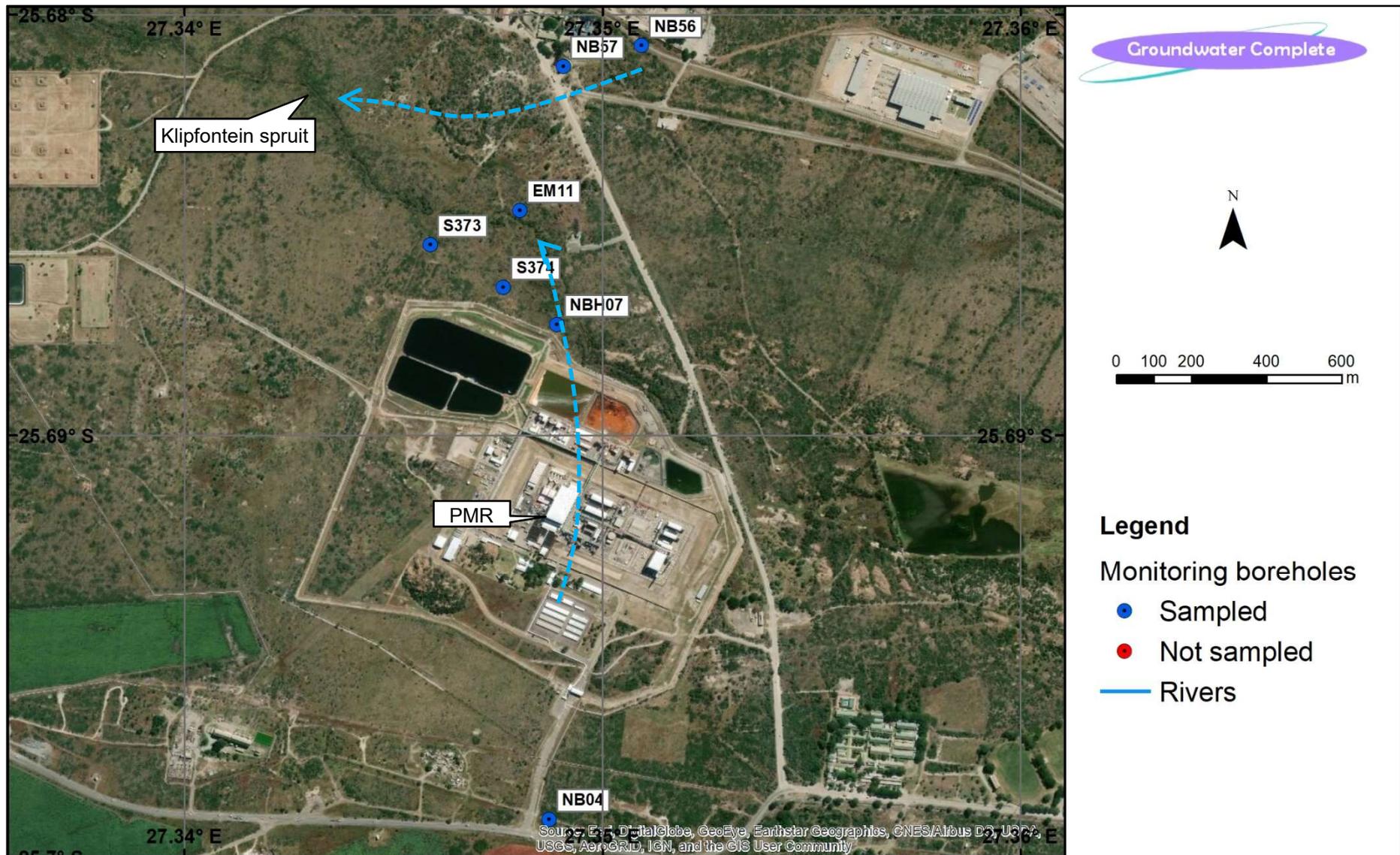


Figure 15: Positions of monitoring boreholes in the PMR and Central Deeps Shaft areas

Time-series plots of the indicator chemical parameters for the PMR and Central Deeps Shaft areas are presented in **Figures 16 and 17**.

Groundwater **TDS** concentrations directly down gradient from the refinery exceeded the permissible SANS value of 1 200 mg/l and displayed averages of between  $\pm$  2180 mg/l and 25 630 mg/l. This indicates severe adverse impacts associated with polluted seepage from the refinery. Concentrations measured up gradient from the refinery and down gradient from the Central Deeps Shaft area remained below the SANS guideline value for drinking water purposes (**Figure 16**). **No guideline TDS value is specified by the Water Use License for RPM.**

The **sulphate** content of groundwater within the immediate vicinity of the PMR and Central Deeps Shaft remained below the permissible SANS concentration of 500 mg/l and displayed averages of between  $\pm$  1 mg/l and 380 mg/l. The relatively low sulphate content measured in NBH07 suggests that sulphate is not the dominant anion in the polluted seepage from the refinery.

Elevated sulphate concentrations were however measured in monitoring boreholes EM11, S373, S374, NB56 and NB57 during the monitoring year, but the concentration decreased significantly over the period from exceeding maximum permissible limits to within acute health and aesthetic limits (**Figure 16**). **The RPM WUL guideline concentration of 20 mg/l was exceeded in all monitoring boreholes except NB04 and NBH07.**

Groundwater **sodium** concentrations measured down gradient from the PMR exceeded the permissible SANS value of 200 mg/l with average concentrations varying between  $\pm$  220 mg/l and 4 670 mg/l. The sodium content in NBH07 has increased over the monitoring period (**Figure 17**). The sodium content of groundwater up gradient from the refinery and down gradient from the Central Deeps Shaft area remained well below the SANS guideline value for drinking water. **The RPM WUL guideline concentration of 22 mg/l was exceeded in all groundwater monitoring boreholes.**

Average groundwater **chloride** concentrations measured in boreholes EM11, S373 and S374 exceeded the permissible SANS value of 300 mg/l and displayed averages of between 890 mg/l and 3 800 mg/l. A much higher average concentration of approximately 15 540 mg/l was measured in monitoring borehole NBH07. Average concentrations measured in the remainder of boreholes were within the maximum concentration allowed for drinking water. Once again, the chloride concentration in NBH07 increased over the monitoring period (**Figure 17**). **The groundwater chloride content within the immediate vicinity of the PMR and Central Deeps Shaft areas exceeded the RPM WUL guideline concentration of 14 mg/l.**

**The exceptionally high levels of sodium and chloride pollution that were measured in NBH07 are clear indications that both parameters are dominant ions in pollution emanating from the PMR.**

**Nitrate** concentrations in most boreholes in the PMR and Central Deeps Shaft areas are not of concern and remained below the permissible SANS value of 11 mg/l/ during the past monitoring year. Monitoring borehole S374 was however the exception and displayed an average concentration of  $\pm 47$  mg/l. **The RPM WUL guideline concentration of 0.2 mg/l was exceeded in all groundwater monitoring boreholes.**

According to **Figures 18** and **19** the following types of groundwater are predominant within the immediate vicinity of the PMR and Central Deeps Shaft areas:

- Fresh, clean, relatively young groundwater that has started to undergo magnesium and sodium ion exchange – groundwater is therefore dominated by **magnesium/sodium** cations and **bicarbonate alkalinity**.
- Relatively old, stagnant groundwater that has undergone a significant degree of ion exchange reactions – groundwater is therefore dominated by **magnesium** cations and **chloride** anions.

**The plot positions in fields 8 of the EDD confirm definite impacts of the processing facilities on the natural groundwater environment, especially with regards to sodium and chloride pollution.**

**Water levels** for the PMR area range from 2 mbs to 20 mbs, of which none of the boreholes display increasing or decreasing concentration trends (**Figure 20**).

**Summary:**

- Monitoring borehole EM11 is affected by groundwater contamination, but the PMR is not considered to be the source.
- The Central Deeps Shaft and its discard area north of the Klipfontein Spruit are also not considered to be significant sources of groundwater contamination.
- Exceptionally high levels of sodium and chloride pollution were measured in all boreholes directly down gradient from the PMR, indicating clear impacts from PMR.
- **The majority of indicator chemical parameters do not comply with the water quality objectives stated in the RPM Water Use License.**

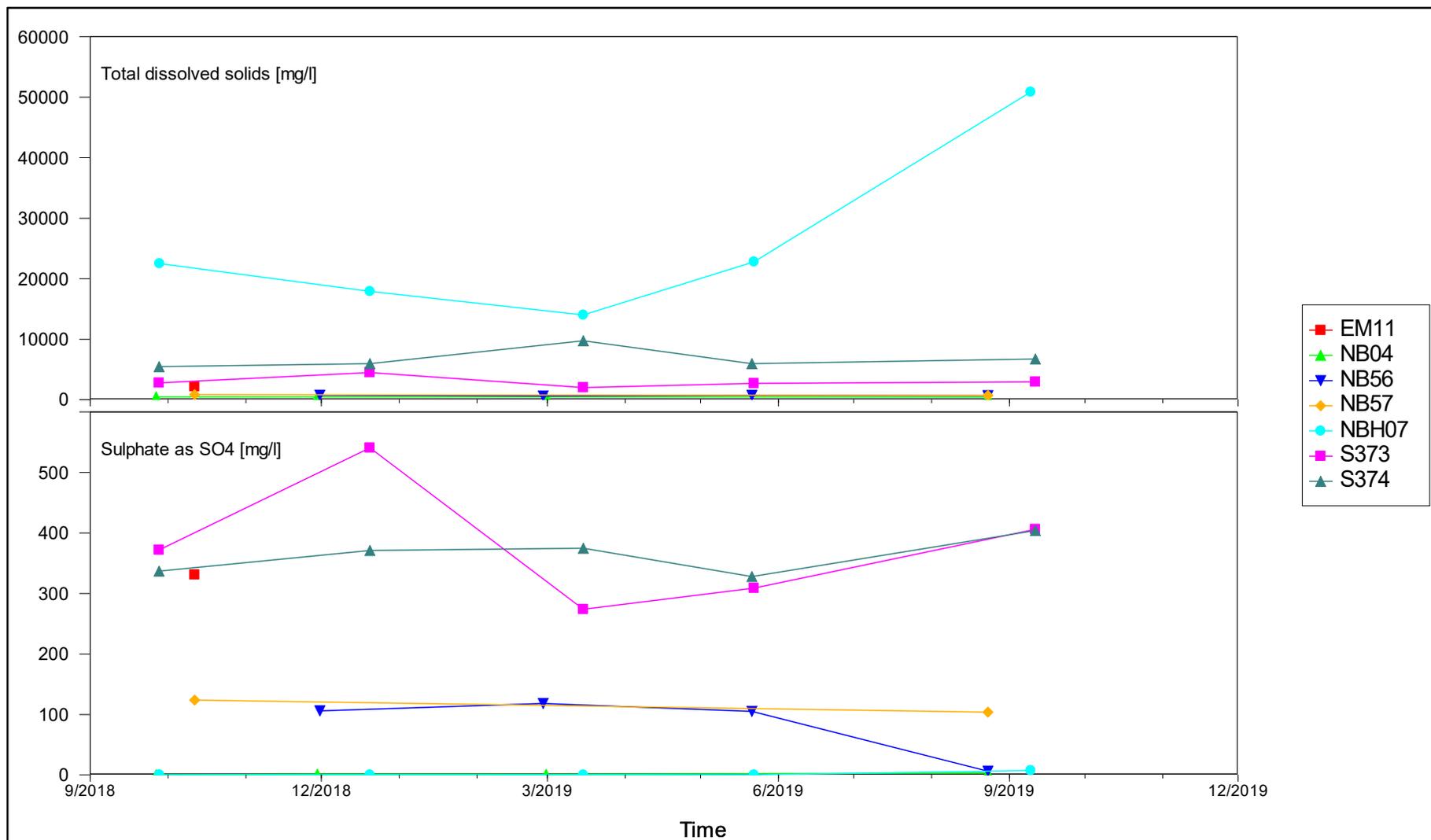


Figure 16: Time-series plot of indicator chemical parameters in the PMR and Central Deeps Shaft areas – TDS and SO<sub>4</sub>

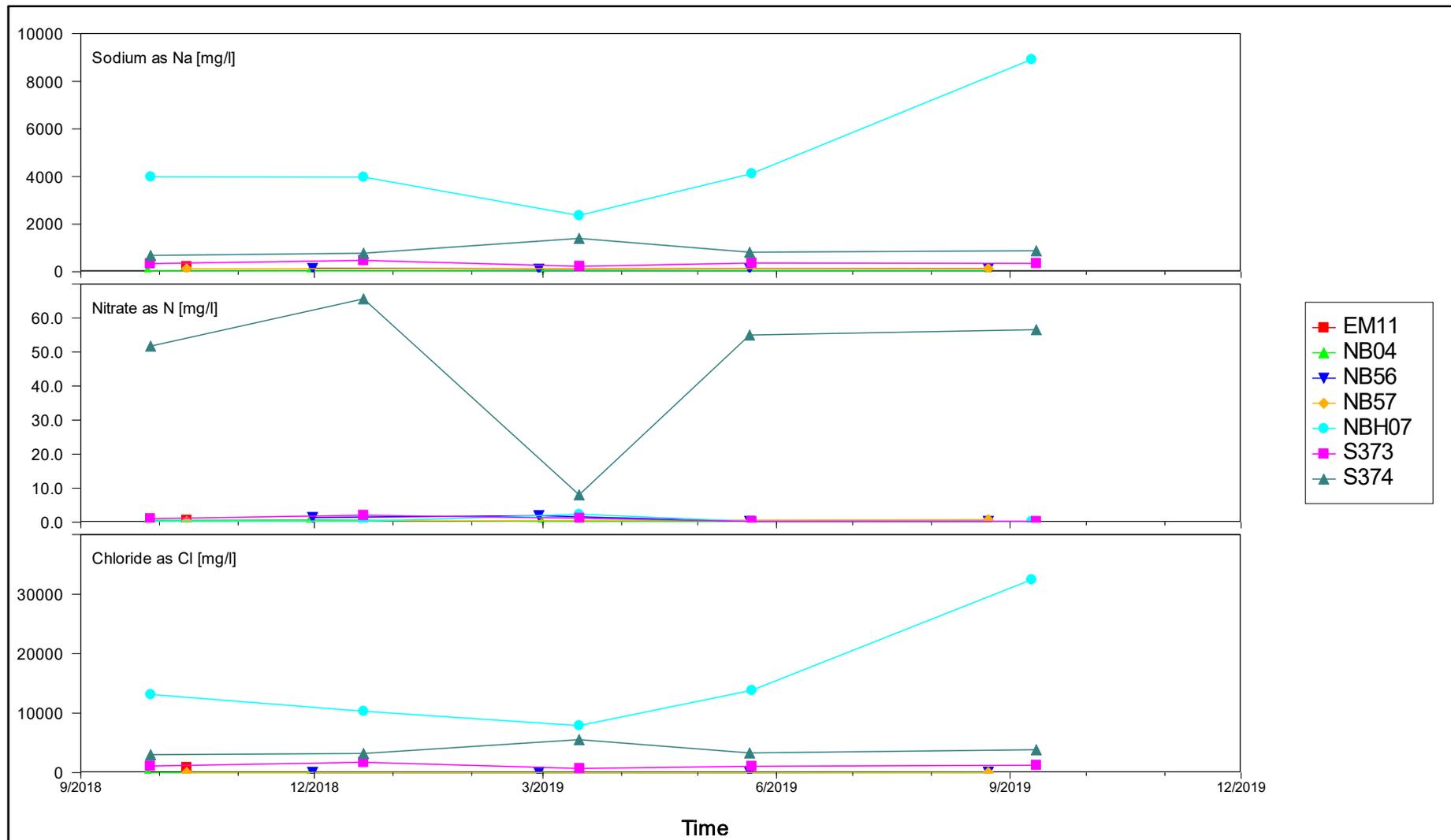


Figure 17: Time-series plot of indicator chemical parameters in the PMR and Central Deeps Shaft areas – Na, NO<sub>3</sub> and Cl

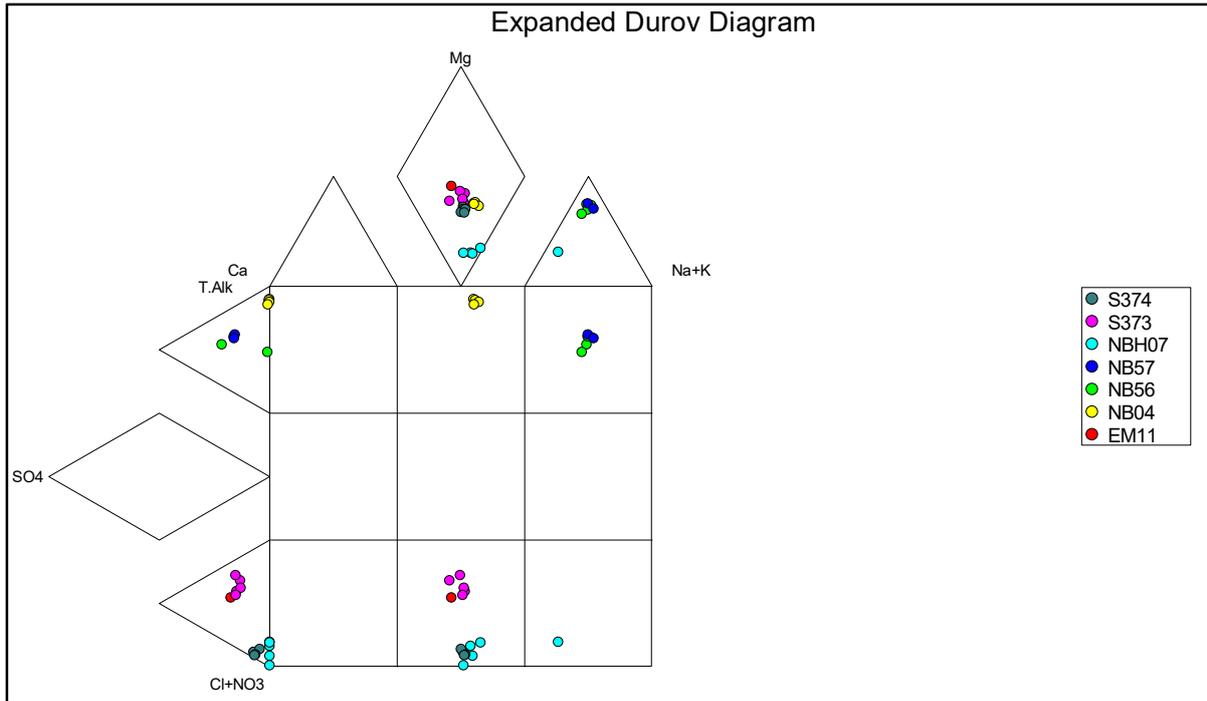


Figure 18: EDD of groundwater chemistry in the PMR area

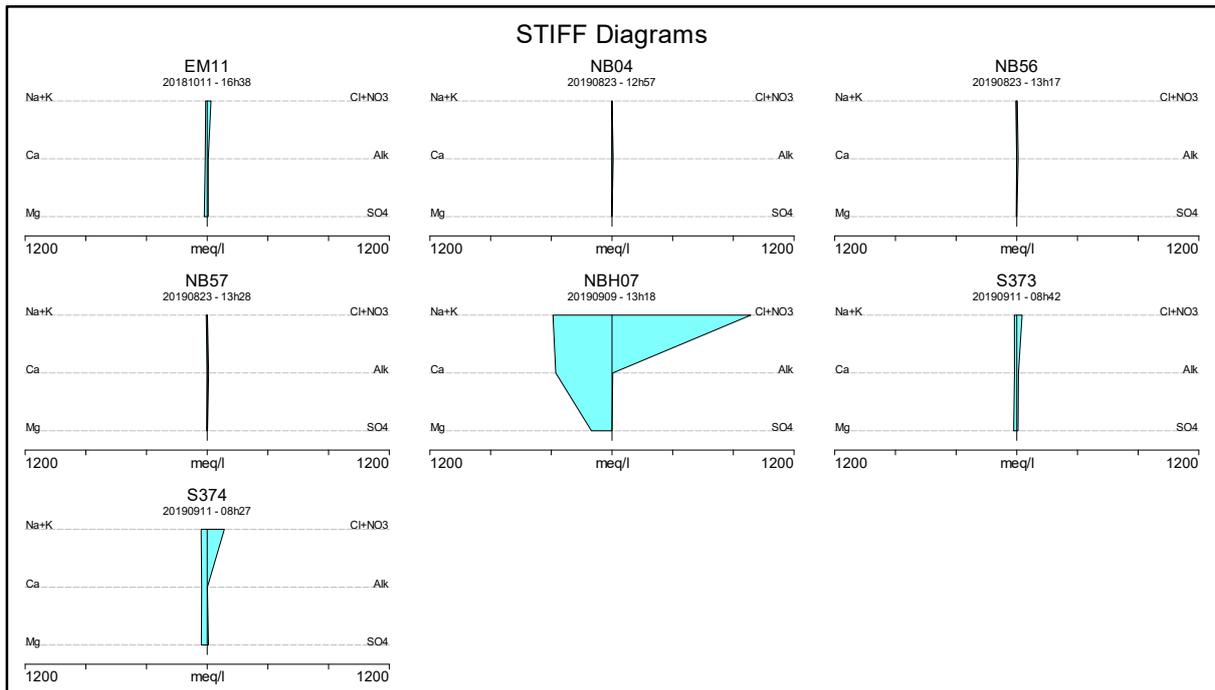


Figure 19: Stiff diagrams of groundwater chemistry in the PMR area

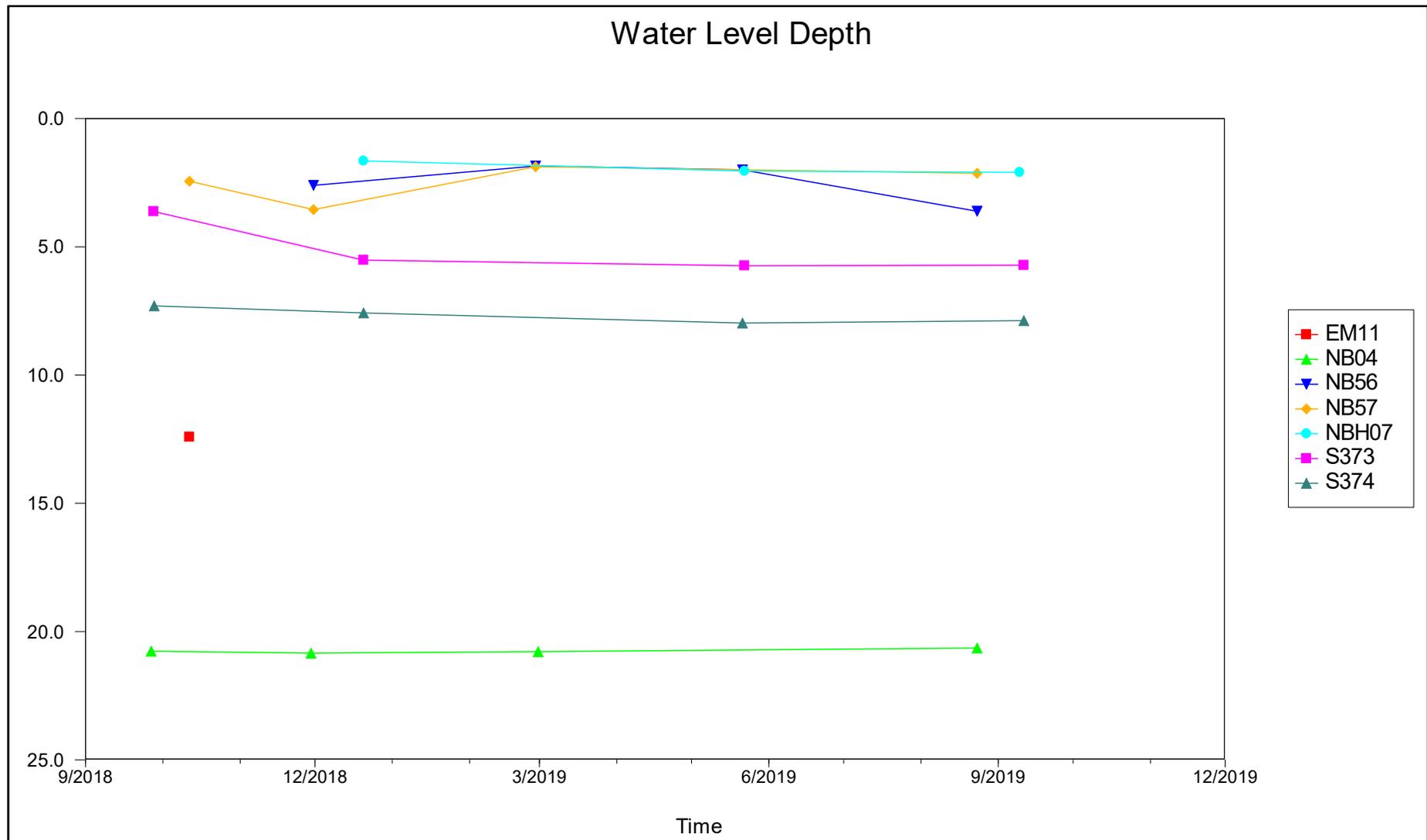


Figure 20: Time series plot of water levels for in the PMR area

## 2.4 THE WATERVAL TAILINGS COMPLEX

The Waterval Tailings Complex straddles a north-west trending groundwater divide and seepage from the tailings is towards the west/south-west and north-west in the direction of the Klipfontein Spruit and Klipgat Spruit respectively. Only one monitoring borehole was sampled during the 2018/2019 monitoring year and its position is indicated in **Figure 21**.

Due to the fact that only a single borehole was monitored for the Tailings complex and it was only sampled twice in the 2018/2019 monitoring year, no meaningful time series information can be gained. Therefore, no time series graphs will be included in this section.

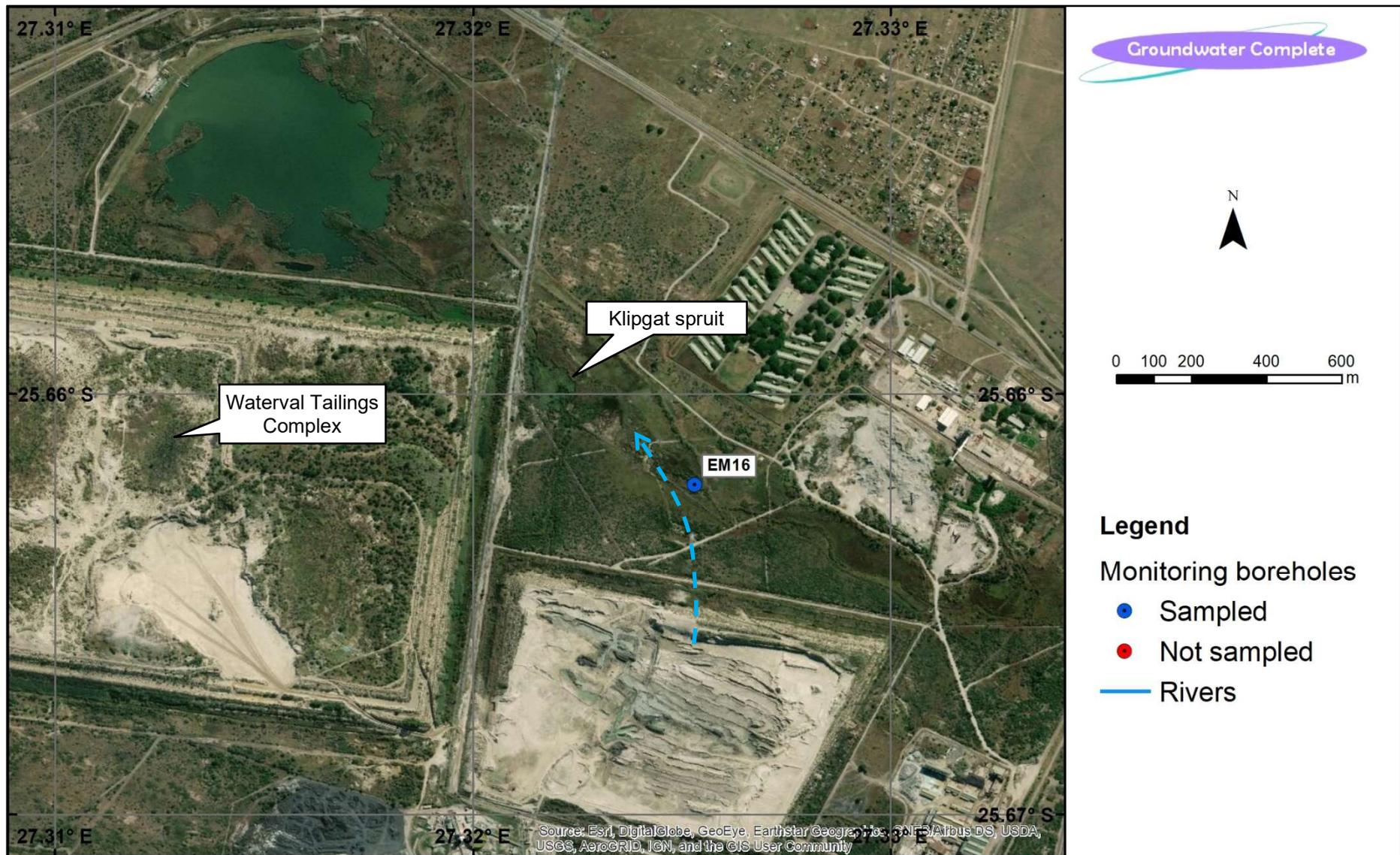


Figure 21: Positions of monitoring boreholes in the Waterval Tailings Complex area

Average groundwater **TDS** concentrations for borehole EM16 during the 2018/2019 monitoring year was 3 440 mg/l, which exceeds the permissible SANS value of 1 200 mg/l. **No guideline concentration is specified for TDS in the Water Use License for RPM.**

The groundwater **sulphate** content measured in the monitoring borehole exceeded the permissible SANS concentration of 500 mg/l and displayed an average of 1018 mg/l (**Table 3**). **The sulphate content of groundwater down gradient from the tailings facility exceeded the RPM WUL guideline concentration of 20 mg/l during the past monitoring year.**

Average groundwater **sodium** concentration for the past monitoring year was 410 mg/l, which exceeded the permissible SANS value of 200 mg/l. **The RPM WUL guideline concentration of 22 mg/l was exceeded in monitoring borehole EM16.**

Groundwater **nitrate** concentrations remained well below the permissible SANS value of 11 mg/l during the past monitoring year. Monitoring borehole EM16 displayed an average of ± 1.1 mg/l. **The RPM WUL guideline concentration of 0.2 mg/l was exceeded in EM16 during the evaluation period.**

Groundwater **chloride** concentrations measured in EM16 exceeded the permissible SANS value of 300 mg/l and displayed an average of 1083 mg/l. **The chloride content of groundwater within the immediate vicinity of the tailings complex exceeded the RPM WUL guideline concentration of 14 mg/l during the 2018/2019 monitoring year.**

According to **Figures 24 and 25** Relatively old, stagnant groundwater that has undergone a significant degree of ion exchange reactions - groundwater is therefore dominated by **magnesium** cations, while the anion content is dominated by **chloride** and **nitrate** is predominant within the immediate vicinity of the Waterval Tailings Complex area:

**The dominant plot positions in field 8 of the EDD confirms definite impacts of the Waterval Tailings Complex on the natural groundwater environment.**

The average **water level** in borehole EM16 is 2 mbs.

#### **Summary:**

- Significant groundwater pollution occurs in the down gradient direction with magnesium and sulphate/chloride being the dominant contaminants.
- Its is recommended that more boreholes be drilled to supplement the monitoring of the tailings complex.
- **The majority of indicator chemical parameters do not comply with the water quality objectives stated in the RPM Water Use License.**

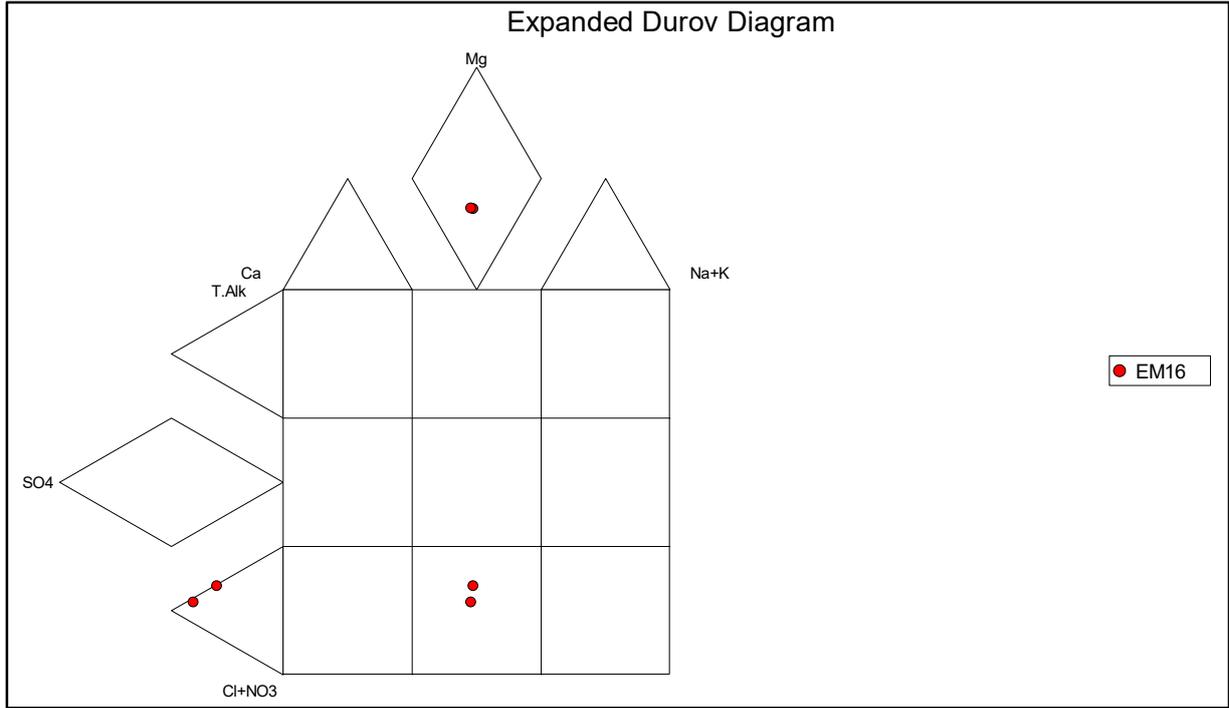


Figure 22: EDD of groundwater chemistry in the Waterval Tailings Complex area

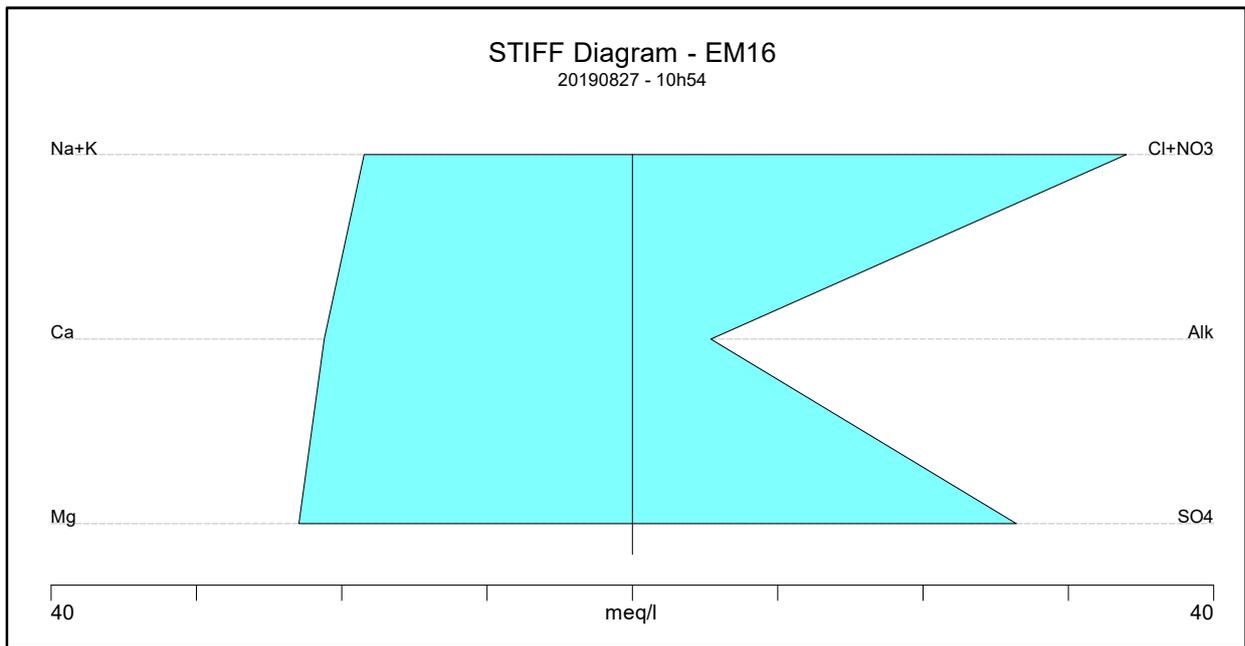


Figure 23: Stiff diagrams of groundwater chemistry in the Waterval Tailings Complex area

### 3 CONCLUSIONS AND RECOMMENDATIONS

The RPM area is a diverse mining and processing area with numerous source areas and varying degrees of impact on the groundwater regime.

- **Sulphate** is a prominent and widespread contaminant in the base metal processing areas such as the concentrators, smelters and refineries. The most significant sulphate pollution occurs at the RBMR, followed by the Waterval Processing Complex. Sulphate is also most commonly the pollutant at the tailings facilities.
- **Magnesium** is generally associated with sulphate-type pollution because magnesium exchanges calcium in the normal geohydrological cycle.
- **Sodium** and **chloride** are usually present in high concentrations in connate water within the crystal structure or matrix of rocks. When blasted, crushed, smelted or processed in some other way, sodium and chloride are liberated. Elevated chloride concentrations occur in groundwater at most of the processing areas like the RBMR and PMR but chloride pollution also occurs at the Waterval tailings facilities.
- **Nitrate** contamination occurs at most of the shaft areas as a result of traces of nitrate-based explosives used in the mining process and dissolving in process and mine water. Nitrate contamination is more prominent in the shaft areas.
- Where groundwater pollution has been confirmed, mitigation and remediation measures should be rolled out as evaluated in the groundwater management plan.
- Due to neutral or slightly basic pH conditions heavy and trace metals are present in very low concentrations in the groundwater of the entire RPM lease area due to the poor solubility.
- Distribution of groundwater monitoring points in the recent monitoring program is mostly adequate, but a number of areas occur where borehole distribution is inadequate for accurate impact characterisation.
- Several boreholes have been blocked or demolished and these boreholes should be reinstated in the monitoring program.
- Monitoring boreholes up gradient from sources are especially important as such monitoring data enables more accurate quantification and delineation of impacts related to specific sources.

#### Summary:

#### **Waterval Smelter and Concentrator and Acid Plant**

- Definite impacts from the Waterval Processing area occur on the down gradient groundwater environment.
- Sulphate is especially of concern, as the majority of groundwater monitoring boreholes indicated elevated concentrations.
- Up gradient groundwater monitoring borehole S407 displayed the highest levels of pollution throughout the 2018/2019 monitoring year, however the pollution is unlikely to originate from the Waterval Processing area.
- S400 also had overall high borehole concentration levels, however it only had a single sample taken and its level of pollution can't be determined definitively.
- Increasing parameter concentrations in the downgradient monitoring borehole, S407 are often observed over the monitoring period.
- **Concentrations of indicator chemical parameters do not comply with the water quality objectives stated in the RPM Water Use License.**
- Water levels vary between 2 and 4 mbs.

### **The Rustenburg Base Metal Refinery**

- Significant pollution impacts from the RBMR occur on the groundwater environment.
- Impacts are by far the most significant in the sodium sulphate solution area to the south-east of the refinery.
- Groundwater iron concentrations measured in monitoring borehole S418 fluctuated significantly throughout the year, which may be the result of varying groundwater pH conditions.
- The most significant impacts from the refinery were measured in groundwater from boreholes S120, S389 and S418.
- The main contaminants of concern are sodium and sulphate.
- The extent of impact (plume) is however limited to within a few meters of the sources due to poor aquifer hydraulic properties.
- **The indicator chemical parameters do not comply with the water quality objectives stated in the RPM Water Use License.**

### **The Precious Metal Refinery (PMR) and Central Deeps Shaft**

- Monitoring borehole EM11 is affected by groundwater contamination, but the PMR is not considered to be the source.
- The Central Deeps Shaft and its discard area north of the Klipfontein Spruit are also not considered to be significant sources of groundwater contamination.
- Exceptionally high levels of sodium and chloride pollution were measured in all boreholes directly down gradient from the PMR, indicating clear impacts from PMR.
- **The majority of indicator chemical parameters do not comply with the water quality objectives stated in the RPM Water Use License.**

### **The Waterval Tailings Complex**

- Significant groundwater pollution occurs in the down gradient direction with magnesium and sulphate/chloride being the dominant contaminants.
- It is recommended that more boreholes be drilled to supplement the monitoring of the tailings complex. One monitoring borehole is not sufficient.

- **The majority of indicator chemical parameters do not comply with the water quality objectives stated in the RPM Water Use License.**

# Appendix B

---

## *Biomonitoring report*

Anglo American Platinum – Rustenburg Operations  
Annual integrated water monitoring report  
Volume I



Report reference: AAPL/A/18

**Prepared by:**

BH Niehaus

*Pri.Sci.Nat.* SACNASP 4000080/13

Clean Stream Biological Services



**Report released:**

2019-02-27

**Tel:** 012-753-2192/3

**Fax:** 086-535-7368

**Email:** brenton@cleanstream-bio.co.za

---

**AMGLO AMERICAN PLATINUM:  
HEX RIVER CATCHMENT  
BIOMONITORING PROGRAMME**

---

**OCTOBER 2018 SURVEY**

## TABLE OF CONTENTS

1. INTRODUCTION.....	3
2. MATERIALS & METHODS .....	4
3. RESULTS & DISCUSSION.....	4
3.1 Study area .....	4
3.2 <i>In-situ</i> water quality (October 2018) .....	7
3.3 Toxicity testing .....	10
3.3.1 May 2018 and October 2018 .....	11
3.3.2 Temporal variation of toxicity results (2008 to 2018).....	13
3.4 Aquatic invertebrate assessment: South African Scoring System 5.....	15
3.5 Fish Assessment.....	20
4 CONCLUSIONS AND RECOMMENDATIONS.....	26
5 REFERENCES .....	28
Appendix 1: Methodology applied during this biomonitoring assessment.....	29
Appendix 2: Site photos of biomonitoring sites (last two surveys).....	32
Appendix 3: Tables.....	36
END OF REPORT .....	37
Addendum 1: Toxicity test report/s (Biotox Laboratory Services).....	38

## LIST OF TABLES

Table 1: Biomonitoring surveys conducted and reports compiled in the period December 1999 to October 2018. ....	3
Table 2: Latitude/Longitude and sampling protocols of selected sampling sites for routine biomonitoring.....	5
Table 3: <i>In-situ</i> water quality variables measured at the time of sampling at the selected biomonitoring sites .....	7
Table 4: Toxicity results and hazard classification for selected pollution facilities (May 2018). ....	12
Table 5: Toxicity results and hazard classification for selected Hex River tributary samples (October 2018). ....	13
Table 6: Integrated Habitat Assessment (IHAS) description of the different biomonitoring sites. ....	16
Table 7: SASS5, ASPT and habitat suitability/availability index scores for different monitoring sites (October 2018). .	17
Table 8: Fish species expected and observed during the last two surveys.....	21
Table 9: Relative FAIL scores calculated at different sampling sites (2017 to 2018). ....	22
Table 10: The relative tolerance of each species towards changes in the environment. ....	23
Table 11: Fish Response Assessment Index (FRAI) results for the Hex River reach (all sites) (2017/8 results). ....	24
Table 12: Descriptive categories used to describe the present ecological status (PES) of biotic components (adapted from Kleynhans, 1999). ....	25

## LIST OF FIGURES

Figure 1: Google Earth image of study area, indicating Hex River and tributary biomonitoring sites. ....	6
Figure 2: Electrical conductivity levels (mS/m) at the time of sampling at the different biomonitoring sites.....	7
Figure 3: pH levels at the time of sampling at the different biomonitoring sites. ....	8
Figure 4: Dissolved oxygen levels (mg/l) at the time of sampling at the different biomonitoring sites. ....	9
Figure 5: Temporal trends of toxicity results (annually tested PCD's and selected streams).....	14
Figure 6: Temporal trends of toxicity results (bi-annually tested tributaries). ....	15
Figure 7: ASPT, SASS5 and total habitat suitability scores at biomonitoring sites during October 2018. ....	17
Figure 8: Linear regression of biotic integrity (as indicated by invertebrate ASPT scores) of the Hex River on a spatial scale (arranged sequentially in a downstream direction) during May 2018 (extended study area). ....	18
Figure 9: Linear regression of biotic integrity (as indicated by invertebrate ASPT scores) of the Hex River on a spatial scale (arranged sequentially in a downstream direction) during October 2018 (reduced study area). ....	18
Figure 10: Long-term trends of biotic integrity in terms of macro-invertebrates at biomonitoring sites. ....	19
Figure 11: Medium-term trends of biotic integrity in terms of macro-invertebrates at biomonitoring sites. ....	19
Figure 12: Relative FAIL scores, HCR's and SHI at the different biomonitoring sites.....	22

## 1. INTRODUCTION

This report is based on the results of the bi-annual biomonitoring survey conducted during October 2018 on the selected sites in the Hex River, Klipfonteinspruit and Klipgatspruit in the Anglo American Platinum (Rustenburg) mining area. Since the sale to Sibanye Stillwater, the study area assigned to Clean Stream Biological Services for biomonitoring has decreased considerably. To avoid confusion with areas tasked by Sibanye Stillwater to other consulting firm/s, the client for the purpose of this report will be referred to as Process Division Services. This long-term monitoring program commenced during December 1999. A comprehensive 20-year temporal database pertaining to the health of aquatic communities, as well as the water quality environment that may be affected by the RPM operations, has been amassed. This continuity of information will be invaluable for any future assessments of impacts to the receiving environment. RPM has thereby diligently maintained their biomonitoring programme on a twice-per-annum schedule (at least) since the inception of the program during 1999. See Table 1 below for a list of surveys performed, with their corresponding report numbers. Report naming will henceforth include the lettering sequence of "AAPL", referring to Anglo American Platinum and in specific the Process Division Services.

Table 1: Biomonitoring surveys conducted and reports compiled in the period December 1999 to October 2018.

Year	Month	Report numbers
1999	December	CS-A-2000
2000	April, July and November	CS-G-2000, CS-K-2000 and CS-A-2001
2001	May and September	CS-H-2001 and CS-L-2001
2002	February, May, August and November	CS-G-2002, CS-I-2002, CS-N-2002 and CS-E 2003
2003	Januaury and May	CS-G2003 and CS-O-2003
2004	April, August and October	CS-H-2004 and AMP-A-05
2005	February, April and November	AMP-B-05, AMP-C-05 and AMP-D-05
2006	April and November	AMP-A-06, AMP-C-06
2007	April and October	ANP-A-07 and ANP-A-08
2008	April and October	ANP-B-08 and ANP-A-09
2009	April and October	ANP-B-09 and RPM-A-09
2009	April and October	RPM-A-10 and RPM-B-10
2011	April and October	RPM-A-11 and RPM-B-11
2012	April and November	RPM-A-12 and RPM-A-13
2013	April and October	RPM-B-13 and RPM-C-13
2014	April and October	RPM-A-14 and RPM-B-14
2015	April and October	RPM-A-15 and RPM-C-15
2016	May and October	RPM-A-16-Ver2 and RPM-B-16
2017	June and November	RPM-A-17 and RPM-B-17
2018	May and October	RPM-A-18 and AAPL-A-18

Rivers are continuum systems, so a river reach can be influenced by activities both upstream and downstream. Pollution incidences upstream of a site will have a negative impact, not only locally, but on the entire ecosystem (depending on the extent of the pollution).

Biological communities reflect overall ecological integrity by integrating different stressors over time, thereby providing a broad measure of their aggregate impact. The monitoring of biological communities hence provides a reliable ecological measure of fluctuating environmental conditions. The biomonitoring protocols applied in this project should give a good reflection of the human impacts on the system under investigation.

The results contained in this report should firstly be interpreted as **spatial** impact monitoring. [Note that spatial impact monitoring in terms of the fish communities considers the last two fish surveys, and not only the last

survey, as in the case of macro-invertebrate communities]. **Temporal** (long- and medium-term trends) impact monitoring is also performed and considers all of the data since 2002 (after initial project design and refinement of the biomonitoring programme between 1999 and 2001).

## 2. MATERIALS & METHODS

Refer to appendix 1 for a description of methodology applied during this assessment.

## 3. RESULTS & DISCUSSION

### 3.1 Study area

Biomonitoring sites were selected to be easily accessible and representative of as many habitats as possible. Four biomonitoring sites were selected within the Hex River (receiving water body) and 3 sites were selected in Hex River tributaries.

The criteria for site selection are as follows:

- The locations should ideally be selected to be both upstream and downstream of potential pollution sources, and as far as possible, exclude other potential impacts not related to the biomonitoring programme (non-AAPL impacts).
- The habitat diversity should be representative of the river/stream being monitored and, as far as possible, be comparable between sites on a spatial scale.
- The habitats should, as far as possible, be suitable for the application of the selected biomonitoring protocols.
- The sampling sites should at least be accessible to off-road vehicle to enable the transport of the required sampling equipment.

Although sampling sites were previously selected to isolate potential and known RPM (Sibanye Stillwater) impacts and hence measure the effect of RPM impacts on the biotic integrity of the receiving water bodies, several additional sites were also selected to illustrate the potential impact of non-RPM related activities. This was done to gain an insight into other potential impacts on the Hex River, in the area upstream of RPM activities as well as up- and downstream of the Klipfonteinspruit (not including the Paardekraal Angling Dam) to isolate the potential impact/s from the Klipfonteinspruit and the Dorpspruit catchment. This approach has now been adapted (since 2018-10) to mainly focus on the possible impact of the AAPL Process Division Services, with possible impacts reaching the final receiving water body (Hex River), via the Klipfonteinspruit and the Klipgatspruit (Table 2; Figure 1).

Various sites/samples were selected for toxicity testing. These include pollution control dams and the Klipfonteinspruit and Klipgatspruit tributaries (included since April 2012, but narrowed down to the two mentioned tributaries since October 2018) joining the Hex River within the study area. Based on the historic electrical conductivity (EC) results (illustrating cumulative water quality deterioration from various sources) and spatial variation in biotic integrity, it is evident that the various tributaries of the Hex River, both upstream and within the newly-refined study area contribute significantly to the spatial variation in ecological integrity of the Hex River. It is therefore of great value for the biomonitoring programme to include DEEEP toxicity testing on all the key tributaries entering the Hex River.

See Table 2 below for sampling site description, its relation to AAPL Process Division Services activities and the frequency of different biomonitoring protocols applied.

Table 2: Latitude/Longitude and sampling protocols of selected sampling sites for routine biomonitoring.

Monitoring site	Description	Potential direct upstream impact	Biomonitoring protocols		GPS coordinates (degrees)	
			Protocol	Frequency per annum	Latitude (South)	Longitude (East)
H1	Hex River, most upstream site.	Not relevant	Discontinued from the Clean Stream Biological Services scope of work, since 2018-10		25.7269	27.3043
H-US-Sand	Hex River, upstream from the Sandspruit confluence.	Non-RPM			25.7148	27.2992
H-DS-Sand	Hex River, downstream from the Sandspruit confluence.	Non-RPM			25.7025	27.3105
Hex00	Hex River, upstream from RPM activities, adjacent to possible future mining activities.	Non-RPM			25.6966	27.3081
H4	Hex River, between sites Hex00 and Hex01, but downstream from H3.	RPM and non-RPM			25.6835	27.2813
Hex01	Hex River, upstream from Klipfonteinspruit.	RPM			25.6765	27.2778
H-US-KF	Hex River, upstream from the Klipfonteinspruit confluence but downstream from the Paardekraal Angling Dam.	AAPL and non-AAPL	*SASS5	Twice	25.6492	27.2906
KF	Klipfonteinspruit, downstream from Waterfall concentrator but upstream from Paardekraal shaft runoff.	AAPL	*SASS5	Twice	25.6578	27.2964
KFD	Klipfonteinspruit, downstream from site KF and the Paardekraal shaft runoff.	AAPL	*SASS5	Twice	25.6496	27.2926
H-DS-KF	Hex River, directly downstream from the Klipfonteinspruit confluence.	AAPL	*SASS5	Twice	25.6473	27.2913
Hex03	Hex River, upstream from Klipgatspruit.	Non-AAPL	*SASS5	Twice	25.6332	27.2903
			**FAIL	Once		
KGT	Klipgatspruit, downstream from tailings complex seepage.	AAPL	*SASS5	Twice	25.6319	27.2951
Hex3B	Hex River, downstream from Klipgatspruit confluence. Newly adopted site (since 2018-10)	AAPL	*SASS5	Twice	25.6237	27.2900
			**FAIL	Once		
DPS	Dorp Spruit, 100m before it confluence with the Hex River	Non-RPM	Discontinued from the Clean Stream Biological Services scope of work, since 2018-10		25.6228	27.2885
Hex04	Hex River, downstream from HEX03.	RPM			25.6081	27.2886
PDK	Paardekraal Spruit, just before confluence with Hex River.	RPM			25.5933	27.2983
Hex4B	Hex River, downstream from Paardekraal Spruit.	RPM			25.5916	27.2993
2	PMR Dam 2	To be confirmed as per AAPL Process Division requirements				
3A	PMR Dam 3A					
3B	PMR Dam 3B					
4&5	PMR Dams 4 and 5					
DPS	Dorpspruit, just upstream from confluence with Hex River					
K035	Klipgat RWD					
K048	Paardekraal Dam 1 RWD					
K064	Paardekraal Dam 3 RWD					
K086	Waterval PCD West					
K098	ACP PCD					
K105	Klipfontain Tailings RWD					
K125	Hoedspruit Tailings RWD					
K133	UG2 PCD					
K176	Paardekraal Phase 4 RWD					
PDKS	Paardekraalspruit just upstream from confluence with Hex River	RPM and non-RPM	Discontinued from the Clean Stream Biological Services scope of work, since 2018-10	25.5933	27.2983	
SS	Sandspruit, just upstream from confluence with Hex River	Non-RPM		25.7115	27.3174	

Key: \* SASS5 = South African Scoring System, version5 (macro-invertebrate index and associated habitat assessment indices i.e. IHAS ver2 and biotope availability and suitability indices)

\*\* FAIL = Fish Assemblage Integrity Index (and associated habitat indices i.e. SHI and HCR)

Site name shaded green = Hex River mainstem	Site name shaded blue = Tributary of Hex River	Site name shaded red = Toxicity testing
Impact shaded gray = Potential RPM and non-RPM impacts (directly upstream)	Impact shaded pink = Potentially impacted by RPM/AAPL (directly upstream)	Impact shaded yellow = No RPM/AAPL impacts (directly upstream)
Site name shaded Orange = Discontinued from Clean Stream Biological Services scope		



Figure 1: Google Earth image of study area, indicating Hex River and tributary biomonitoring sites.

### 3.2 In-situ water quality (October 2018)

Selected water quality variables were measured on-site at the time of biological sampling. The purpose of these measurements is to assist in the interpretation of biological results (refer to Aquatico Scientific’s Water Quality Report for a detailed water quality assessment of the Rustenburg Platinum mining area).

As recorded during most surveys, the EC increased from site H-US-KF to H-DS-KF in the Hex River (104.0 mS/m to 178.3 mS/m) (Table 3; Figure 2). The Klipfonteinspruit joins the Hex River between these sites and probably played a large role in the increased salinity during most preceding surveys (no perceptible surface flow was recorded from the Klipfonteinspruit during many surveys but a subsurface contribution of affected mine water cannot be ruled out). The EC value was relatively high in the Klipfonteinspruit (site KF) during most previous surveys and the latest dataset again confirms this tributary as a potential source of elevated salinity levels, especially during periods of flow (Table 3).

Table 3: In-situ water quality variables measured at the time of sampling at the selected biomonitoring sites

Monitoring site	EC (mS/m)	pH	Oxygen saturation (%)	Dissolved oxygen (mg/l)	Water temp (°C)	Turbidity (visual)	Flow (visual)
H-US-KF	104.0	7.8	79.5	5.2	21.2	Slightly turbid	Moderate
KF	509.0	7.6	84.2	5.5	22.3	Clear	Low
H-DS-KF	178.3	7.7	107.1	7.0	23.2	Slightly turbid	Moderate
Hex-03	195.2	7.5	48.6	3.2	20.9	Slightly turbid	Moderate
KGT	Dry						
Hex-03-B	189.4	7.7	79.9	5.1	21.7	Slightly turbid	Moderate

**Key:**  
 Site name shaded in green = Hex River mainstem  
 Site name shaded in blue = Tributary of the Hex River  
 Values relatively high  
 Values exceeding/below guidelines

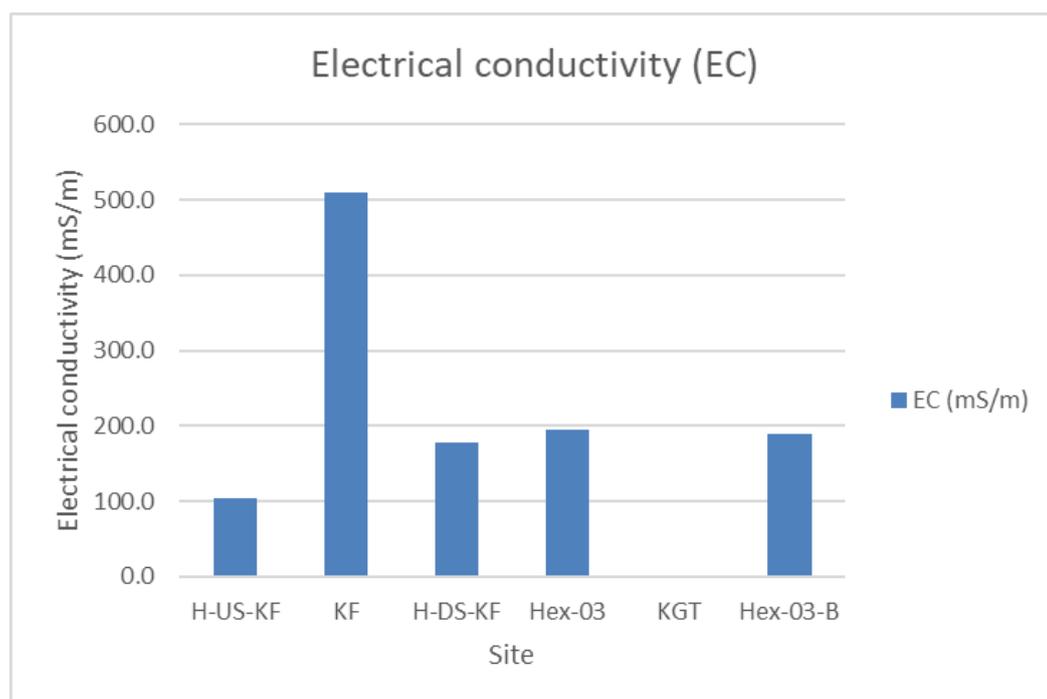


Figure 2: Electrical conductivity levels (mS/m) at the time of sampling at the different biomonitoring sites.

The EC level increased, from site H-DS-KF (178.3 mS/m) to Hex03 (195.2 mS/m). This is a clear indication of non-Anglo Platinum Process Division (APPD) activities impacting on the water quality of the Hex River.

From site Hex03 towards site Hex03B (the most downstream site), the EC values decreased slightly (195.2 mS/m to 189.4 mS/m), thus indicating that the contribution from the Klipgatspruit (dry at the time of sampling) did not affect the salinity of the receiving environment.

The pH fell within the target water quality ranges for fish health (Aquaculture), and marginally within the aquatic ecosystem guideline at all sites during the October 2018 survey (Table 3; Figure 3). The target for fish health is between 6.5 and 9.0. It is expected that most aquatic species will tolerate and reproduce successfully within this pH range (DWAF, 1996).

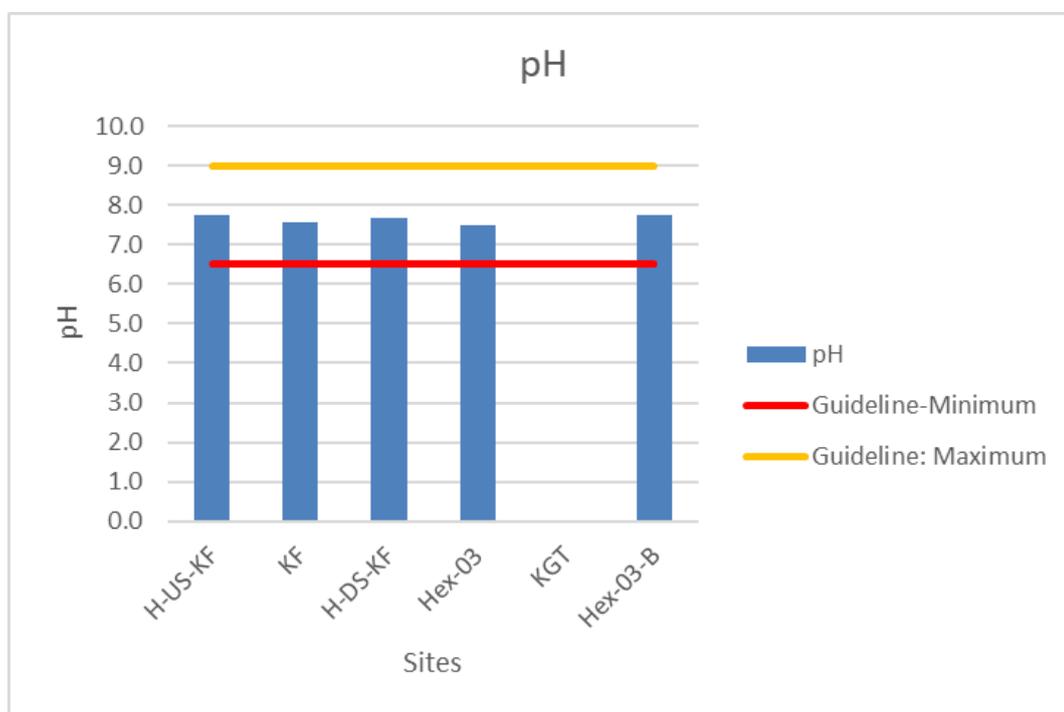


Figure 3: pH levels at the time of sampling at the different biomonitoring sites.

During October 2018, the dissolved oxygen level fell below the target range (> 5 mg/l, as set by Kempster *et.al.*, 1980) at site Hex03 (Table 3; Figure 4). This was also the case during the previous (May 2018) and many preceding surveys. The noted low oxygen levels could therefore possibly have posed a risk to aquatic biota and was probably related to a combination of factors including:

- Elevation and accumulation of organic loads,
- Aquatic vegetation and algal proliferation in response to eutrophication,
- Low flow (all affected sites).

It has to be noted that the cause of lowered dissolved oxygen levels is unlikely to be related to APPD activities because levels were within the guideline at site H-DS-KF and no further APPD activities take place toward site Hex03.

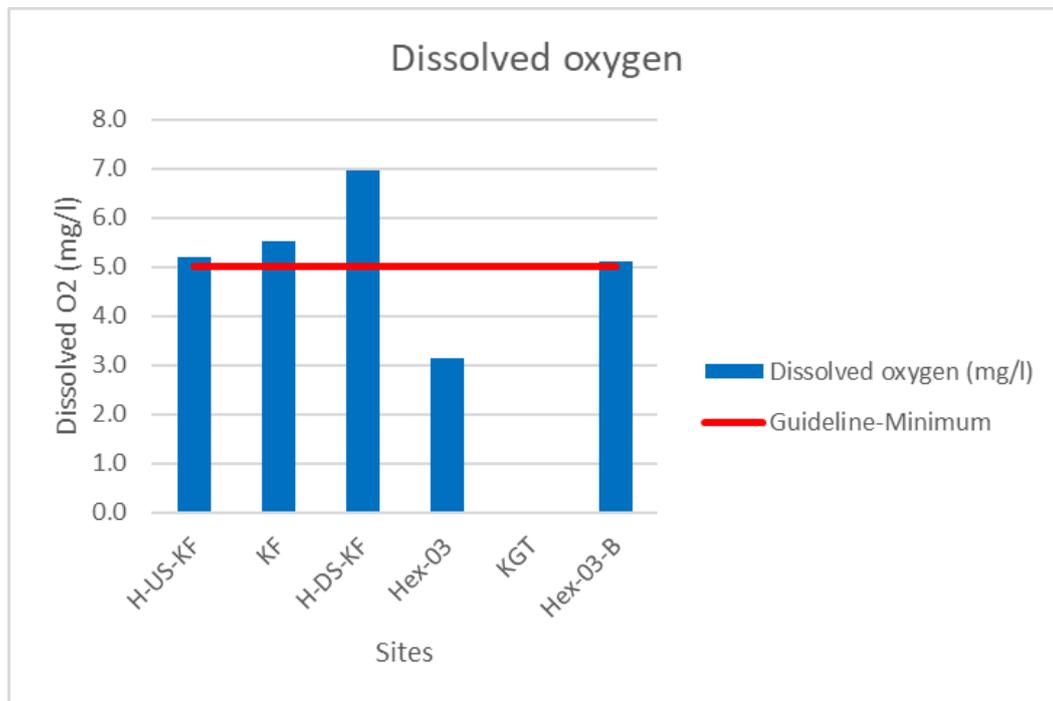


Figure 4: Dissolved oxygen levels (mg/l) at the time of sampling at the different biomonitoring sites.

As a standard management procedure, a full statistical evaluation of water quality data at these biomonitoring localities (as performed by Aquatico Scientific) will be required to conclude whether specific variables or a combination of variables, not included in the biota specific range, are impacting on the aquatic ecosystems.

### 3.3 Toxicity testing

At the time of compiling this biomonitoring report, the latest toxicity testing results for the Pollution Control Facilities available to Clean Stream Biological Services were based on the May 2018 dataset, as submitted as a separate toxicity testing report by Biotox Laboratory Services (Report no. RPM-A-18\_TOX).

The latest tributary toxicity testing report, as performed in conjunction with the October 2018 biomonitoring survey, is also included and discussed in this biomonitoring report.

As per SANAS requirements, the above-mentioned toxicity testing reports were produced independently by Biotox Laboratory Services (Pty) Ltd. All results contained in this section are therefore sourced from the Biotox reports, which are included in Addendum 1.

Toxicity testing (as conducted in this biomonitoring programme) is applied by exposing biota under laboratory conditions to water sources (pollution control dams, effluent streams or streams/rivers) to accurately determine the risk of such water types to the biota of the receiving water bodies. Toxicity results indicate the risk posed to the Hex River and its tributaries in the event of release, seepage or overflow from possible sources of pollution. Up to four trophic levels (at least 3, including acute and chronic approaches) of biota, *i.e.*, vertebrates (*Poecilia reticulata*), invertebrates (*Daphnia magna*), bacteria (*Vibrio fischeri*) and primary producers (*Selenastrum capricornutum*) are exposed to the samples per standard procedures under laboratory conditions and thereafter a risk/hazard category is determined by application of the latest **DEEEP**<sup>1</sup> DWS recommended protocols and hazard classification. The final risk classification is expressed in terms of **acute**<sup>2</sup> and **chronic**<sup>3</sup> toxicity risk. The *Poecilia reticulata* and *Daphnia magna* test results are based on mortality rates over a relatively short period of the lifespan of the organisms, hence allowing for acute interpretation. *Selenastrum capricornutum* and *Vibrio fischeri* individual test results are based on inhibition rates over relatively long periods of the lifespan of the organisms, hence allowing for short-chronic toxicity hazard interpretation.

Selected toxicity samples (Hex River tributaries) were tested on a twice per annum schedule, while the PCD (pollution control dam) samples are tested once per annum, on either a **screening**<sup>4</sup> acute level or a **definitive**<sup>5</sup> acute level, at this stage. The frequency of testing is informed by the level of toxicity. If toxicity levels increase, it may become relevant and useful to increase the frequency of testing. The frequency and type of toxicity testing required (screening vs. definitive) should be revised from time to time based on the outcome of the specific year's assessments.

#### Hazard classification for screening tests (undiluted samples)

After the determination of the percentage effect<sup>6</sup> (EP), obtained with each of the **battery of toxicity screening** tests performed, the sample is ranked into one of the following five classes:

<sup>1</sup> DEEEP = Direct Estimation of Ecological Effect Potential. This is a battery of tests that can measure toxicity of complex mixtures based on a set of parameters stemming from the results of effects, even if all constituents are not known. Thereafter a hazard class is determined based on the resulting parameters of the battery of tests.

<sup>2</sup> Acute = Acute refers to an exposure over a relatively short period of the lifespan of biota, of which the result is generally based on mortality rates.

<sup>3</sup> Chronic = Chronic refers to prolonged exposures over an extended period of the lifespan of test organisms, of which the results are generally based on growth inhibition rates.

<sup>4</sup> Screening = A screening toxicity test refers to an undiluted (100% concentration) sample. This is usually performed on a sample from the biomonitoring sites in the receiving water bodies (river/streams) to determine if any toxicity is present. This is performed both up- and downstream of the potential impacts to enable the determination of downstream increases or decreases in toxicity.

<sup>5</sup> Definitive = A definitive toxicity test refers to the exposure of test organisms to both the 100% concentration as well as a range of dilutions, generally used to determine the risk of a pollution source that may have a toxicity effect on the receiving water body (such as effluents and PCD's). The range of dilutions are therefore useful in the event that the 100% sample concentration presents acute toxicity, and allows for the determination of a safe dilution factor, to negate toxicity effects on the receiving water bodies.

<sup>6</sup> EP (Percentage effect) = an effect measured either as a mortality rate or inhibition rate (depending on the type of test). A 10% effect is regarded as a slight acute toxicity for daphnia and guppies, while a 20% effect is regarded as a slight acute toxicity for algae and bacteria (vibrio). A 50% effect is regarded as an acute toxicity for all of the tests (daphnia, guppies, algae and bacteria)

<b>Screening</b>	<b>Class I</b>	<b>No acute/chronic environmental hazard</b> - none of the tests shows a toxic effect
	<b>Class II</b>	<b>Slight acute/chronic environmental hazard</b> - a statistically significant percentage effect is reached in at least one test, but the effect level is below 50%
	<b>Class III</b>	<b>Acute/chronic environmental hazard</b> - the percentage effect level is reached or exceeded in at least one test, but the effect level is below 100%
	<b>Class IV</b>	<b>High acute/chronic environmental hazard</b> - the 100% percentage effect is reached in at least one test
	<b>Class V</b>	<b>Very high acute/chronic environmental hazard</b> - the 100% percentage effect is reached in all the tests

### Toxicity classification system definitive tests (undiluted samples plus range of dilutions)

The samples are classified into one of the following five classes on the basis of the highest toxicity unit (TUa) found in the **battery of toxicity definitive tests** performed. The toxicity unit is a function of the L(E)C50, where  $(TUa) = 100/L(E)C50$ . The 50% Lethal/Effective concentration (LC50 or LE50) is the linear calculated (derived) concentration at which a 50% mortality or inhibition rate can be expected. Hence, the lower this value is, the higher the acute toxicity level. Conversely, the higher the toxicity unit (TUa) is, the higher the acute toxicity level is. The conversion of L(E)C50 values to TUa values is therefore merely done to achieve a classification scale of increasing values related to increasing toxicity risk:

<b>Definitive</b>	<b>Class I</b>	<b>No acute/chronic environmental hazard</b> - none of the tests shows a toxic effect
	<b>Class II</b>	<b>Slight acute/chronic environmental hazard</b> - the percentage effect observed in at least one toxicity test is significantly higher than in the control, but the effect level is below 50% (TU is <1)
	<b>Class III</b>	<b>Acute/chronic environmental hazard</b> - the L(E)C50 is reached or exceeded in at least one test, but in the 10 fold dilution of the sample the effect level is below 50% (TU is between 1 and 10)
	<b>Class IV</b>	<b>High acute/chronic environmental hazard</b> - the L(E)C50 is reached in the 10 fold dilution for at least one test, but not in the 100 fold dilution (TU is between 10 and 100)
	<b>Class V</b>	<b>Very high acute/chronic environmental hazard</b> - the L(E)C50 is reached in the 100 fold dilution for at least one test (TU is >100)

**Weighting:** Each sample is furthermore weighed according to its relative toxicity levels (out of 100%). Higher values indicate that more of the individual tests indicated toxicity within a specific class.

### 3.3.1 May 2018 and October 2018

#### Toxicity testing of pollution control facilities (May 2018 only)

Various toxicity hazards were identified during the May 2018 survey, ranging from “slight hazard” (Class II) to “very high hazard” (Class III), implying that some effluents/potential effluents could pose a serious risk to the receiving water bodies if released (Table 4).

Sample K035, K086, K105, K125 and K176 showed “no acute/chronic environmental toxicity hazard” (Class I). As a result of macro-invertebrate mortality rates of 20% for samples K046 and K048; as well as 15% mortality recorded for sample K133, these samples were classified as having a “slight acute environmental toxicity hazard” (Class II). Sample K194 showed a “chronic environmental toxicity hazard” (Class II) based on the TU of 2.6.

Table 4: Toxicity results and hazard classification for selected pollution facilities (May 2018).

Results		K035	K046	K048	K086	K105	K125	K133	K176	K194
Water quality	pH @ 25°C (A)	7,8	7,4	7,6	7,7	7,6	7,6	7,7	7,4	7,4
	EC (Electrical conductivity) (mS/m) @ 25°C (A)	584,0	612,0	654,0	537,0	296,0	360,0	209,3	608,0	562,0
	Dissolved oxygen (mg/l) (NA)	6,4	7,2	6,7	6,1	7,4	7,7	5,9	7,1	4,6
V. fischeri (bacteria) (A)	Test started on yy/mm/dd	18/05/17	18/05/17	18/05/17	18/05/17	18/05/17	18/05/17	18/05/17	18/05/17	18/05/18
	% 30min inhibition (-) / stimulation (+) (%)	6	8	2	1	2	-2	-7	-2	-87
	EC:LC20 (30 mins)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	16
	EC:LC50 (30 mins)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	39
	Toxicity unit (TU) / Description	<1	<1	<1	<1	<1	<1	<1	<1	2,6
S. capricornutum (micro-algae) (A)	Test started on yy/mm/dd	18/05/15	18/05/15	18/05/15	18/05/15	18/05/15	18/05/15	18/05/15	18/05/15	18/05/15
	% 72hour inhibition (-) / stimulation (+) (%)	1	0	-8	**	5	-7	-1	-7	-9
	EC:LC20 (72hours)	n.r.	n.r.	n.r.	**	n.r.	n.r.	n.r.	n.r.	n.r.
	EC:LC50 (72hours)	n.r.	n.r.	n.r.	**	n.r.	n.r.	n.r.	n.r.	n.r.
	Toxicity unit (TU) / Description	<1	<1	<1	**	<1	<1	<1	<1	<1
D. magna (water flea) (A)	Test started on yy/mm/dd	18/05/14	18/05/14	18/05/14	18/05/14	18/05/14	18/05/14	18/05/14	18/05/14	18/05/14
	% 48hour mortality rate (-%)	-10	-20	-20	0	0	0	-15	-10	-10
	EC:LC10 (48hours)	n.r.	50	50	n.r.	n.r.	n.r.	83	n.r.	n.r.
	EC:LC50 (48hours)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	Toxicity unit (TU) / Description	<1	<1	<1	<1	<1	<1	<1	<1	<1
P. reticulata (uppy) (A)	Test started on yy/mm/dd	18/05/17	18/05/17	18/05/17	18/05/17	18/05/17	18/05/17	18/05/17	18/05/17	18/05/17
	% 96hour mortality rate (-%)	-8	0	0	0	0	-8	0	0	-8
	EC:LC10 (96hours)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	EC:LC50 (96hours)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	Toxicity unit (TU) / Description	<1	<1	<1	<1	<1	<1	<1	<1	<1
Estimated safe dilution factor (%) (for definitive testing only)	None required	50	50	None required	None required	None required	83	None required	16	
Overall classification - Hazard class***	Class I - No acute/chronic hazard	Class II - Slight acute hazard	Class II - Slight acute hazard	Class I - No acute/chronic hazard	Class I - No acute/chronic hazard	Class I - No acute/chronic hazard	Class II - Slight acute hazard	Class I - No acute/chronic hazard	Class III - Chronic hazard	
Weight (%)	0	25	25	0	0	0	25	0	25	

**Key:**  
 WQ = Water quality at the time of starting the Daphnia magna testing.  
 % = for definitive testing, only the 100% concentration (undiluted) sample mortality/inhibition/stimulation is reflected by this summary table. The dilution series results are considered for EC:LC values and Toxicity unit determinations.  
 n.r. = not relevant, i.e. the 100% concentration caused less than 10/20/50% (effective concentration) mortalities or inhibition.  
 \*\* = Algal test result inconclusive due to interference (caused either by precipitate forming in the sampling during testing). As the degree of inhibition/stimulation is unknown, individual test result was not used for overall hazard classification.  
 \*\*\* = The overall hazard classification takes into account the full battery of tests and is not based on a single test result. Note that the overall hazard classification is expressed as acute/chronic level of toxicity, due to the fact that the S. capricornutum (micro-algae) and the V. fischeri tests are regarded as short-chronic levels of toxicity tests and the overall classification therefore contains a degree of chronic toxicity assessment.  
 Weight (%) = relative toxicity levels (out of 100%), higher values indicate that more of the individual tests indicated toxicity within a specific class.  
 site's sample name shaded in purple = screening test      site's sample name shaded in orange = definitive test

### Toxicity testing of Hex River tributaries (October 2018)

As noted earlier in the report, the electrical conductivity (EC) levels are almost always very high within the Klipfonteinspruit. EC is often an indication of reduced water quality but toxicity testing results revealed that no hazard (Class I) was observed at the Klipfonteinspruit sites (KF), before entering the Hex River (Table 5). This is an encouraging improvement since the October 2014 and April 2015 surveys, when a high hazard (Class IV) and a slight hazard (Class II) was presented by this tributary. It was noted that the Klipfonteinspruit was not flowing during the October 2014 survey and that contamination was therefore contained within isolated pools. It is important to note that APPD activities potentially contribute to this tributary.

It is now recommended to include both site KF and KFD for toxicity testing in the Klipfonteinspruit. The effect of different sources of pollution can then be distinguished more accurately.

Table 5: Toxicity results and hazard classification for selected Hex River tributary samples (October 2018).

		Results	KF
w <sub>Water</sub> quality	pH @ 25°C (A)		7,7
	EC (Electrical conductivity) (mS/m) @ 25°C (A)		501,0
	Dissolved oxygen (mg/l) (NA)		7,6
V. fischeri (bacteria) (A)	Test started on yy/mm/dd		18/11/01
	%30min inhibition (-) / stimulation (+) (%)		<b>44</b>
	EC/LC20 (30 mins)		*
	EC/LC50 (30 mins)		*
	Toxicity unit (TU) / Description		no short-chronic hazard
D. magna (waterflea) (A)	Test started on yy/mm/dd		18/10/29
	%48hour mortality rate (-%)		<b>0</b>
	EC/LC10 (48hours)		*
	EC/LC50 (48hours)		*
	Toxicity unit (TU) / Description		no acute hazard
P. reticulata (guppy) (A)	Test started on yy/mm/dd		18/10/29
	%96hour mortality rate (-%)		<b>0</b>
	EC/LC10 (96hours)		*
	EC/LC50 (96hours)		*
	Toxicity unit (TU) / Description		no acute hazard
<b>Overall classification - Hazard class***</b>		Class I - No acute/short-chronic hazard	
Weight (%)		0	

**Key:**  
 \* = EC/LC values not determined, definitive testing required if a hazard was observed and persists over subsequent sampling runs.  
 \*\*\* = The overall hazard classification takes into account the full battery of tests and is not based on a single test result. Note that the overall hazard classification is expressed as acute/chronic level of toxicity, due to the fact that the *S. capricornutum* (micro-algae) and the *V. fischeri* tests are regarded as short-chronic levels of toxicity tests and the overall classification therefore contains a degree of chronic toxicity assessment.  
**Weight (%)** = relative toxicity levels (out of 100%), higher values indicate that more of the individual tests indicated toxicity within a specific class.  
 site/sample name shaded in purple = screening test  
 site/sample name shaded in orange = definitive test

### 3.3.2 Temporal variation of toxicity results (2008 to 2018)

To determine temporal (over time) trends of increasing/decreasing toxicity levels, the risk class for each sample was plotted for each survey. Thereafter, linear trends over time were determined for the risk class at each site (Figures 5 & 6). It is important to note that these trends were not based on the actual mortalities/inhibition or lethal concentrations, but on the derived risk class for each survey and is merely included to gain a general understanding of increased/decreased risk over time.

#### Annually tested PCD's and selected streams

From the temporal database, it is clear that most samples have varying degrees of toxicity and that almost all of the samples have fallen within the Class II or higher classes from time to time (Figure 5). Samples Dam2 and Dam 4/5 consistently fall into the higher hazard classes and are therefore never suitable for

undiluted release<sup>7</sup> (including uncontrolled releases) to the environment. Some samples have, however, improved notably over time, such as sample K105, which has improved from an acute hazard (Class III) during 2008/2009 to no acute hazard (Class I) over the past five years. Environmental managers should take note of these hazard classes to plan licenced releases and/or contain hazardous water types at the appropriate times.

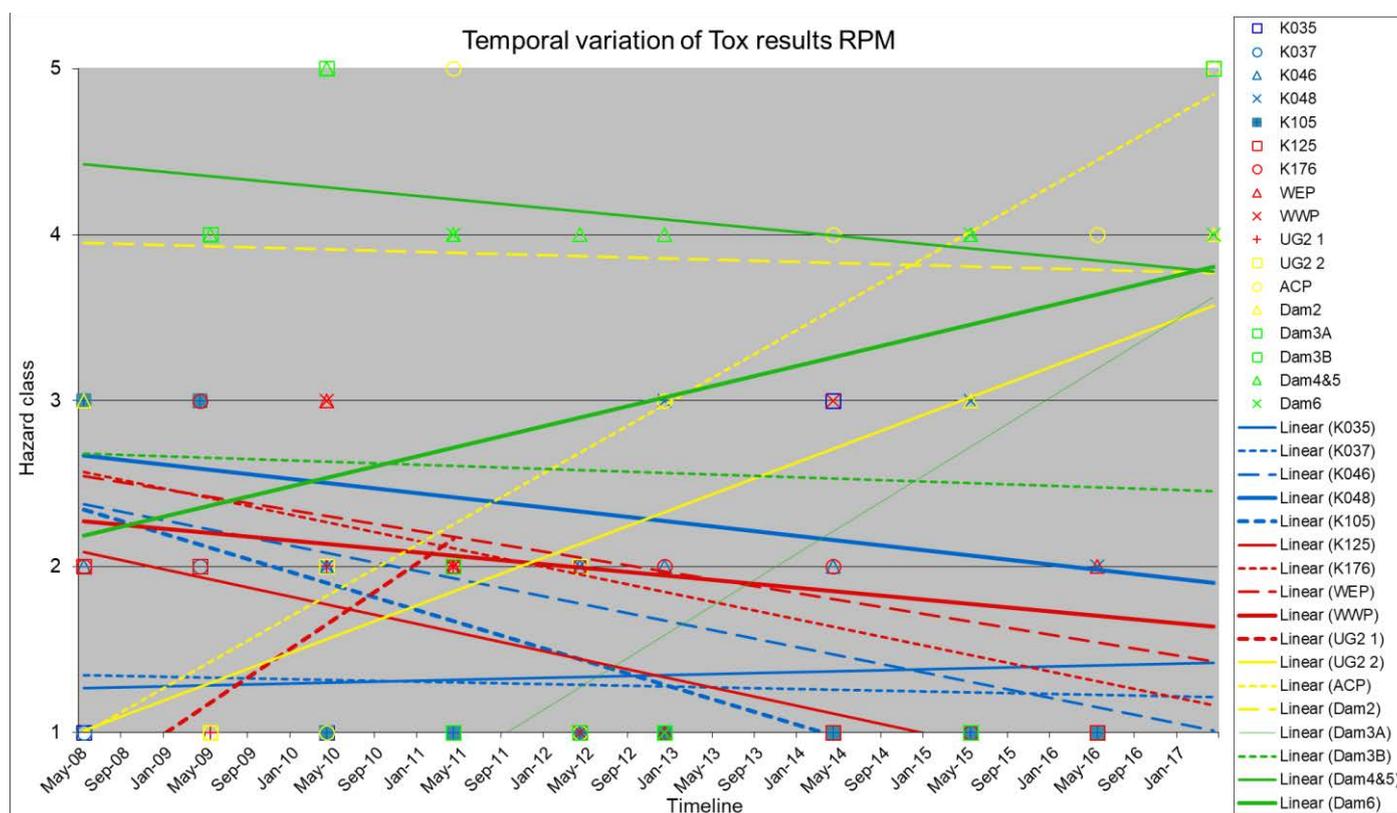


Figure 5: Temporal trends of toxicity results (annually tested PCD's and selected streams).

It is strongly recommended that definitive toxicity testing be continued for the samples that regularly display positive toxicity levels of Class III or higher. Definitive toxicity testing will allow for the estimation of safe dilution factors should the mine wish to apply for a water use licence to legally discharge such water types. These factors will also be essential for environmental managers to predict whether the toxicity of polluted water can be negated by natural dilution if accidentally released (e.g. spills, leaks or seepage) to the receiving environment. Definitive testing will furthermore assist in the suitable scheduling for planned releases (*i.e.* whether water could be released during the dry season and, if not, whether sufficient dilution is only likely to be achieved during the wet season/times of high river flow).

### Bi-annually tested tributaries

From the temporal database, it is clear that the relevant tributaries (Klipfonteinspruit and Klipgatspruit) have displayed toxicity hazards at times (Figure 6). It is encouraging that both tributaries have recently displayed lower toxicity. The Klipfonteinspruit is therefore also displaying a decreasing trend (improved hazard over time) as noted in Figure 6.

<sup>7</sup> Although theoretical predictions in terms of suitability for release are provided, releases remain bound by licensing conditions and are not prescribed/permitted by toxicity testing results.

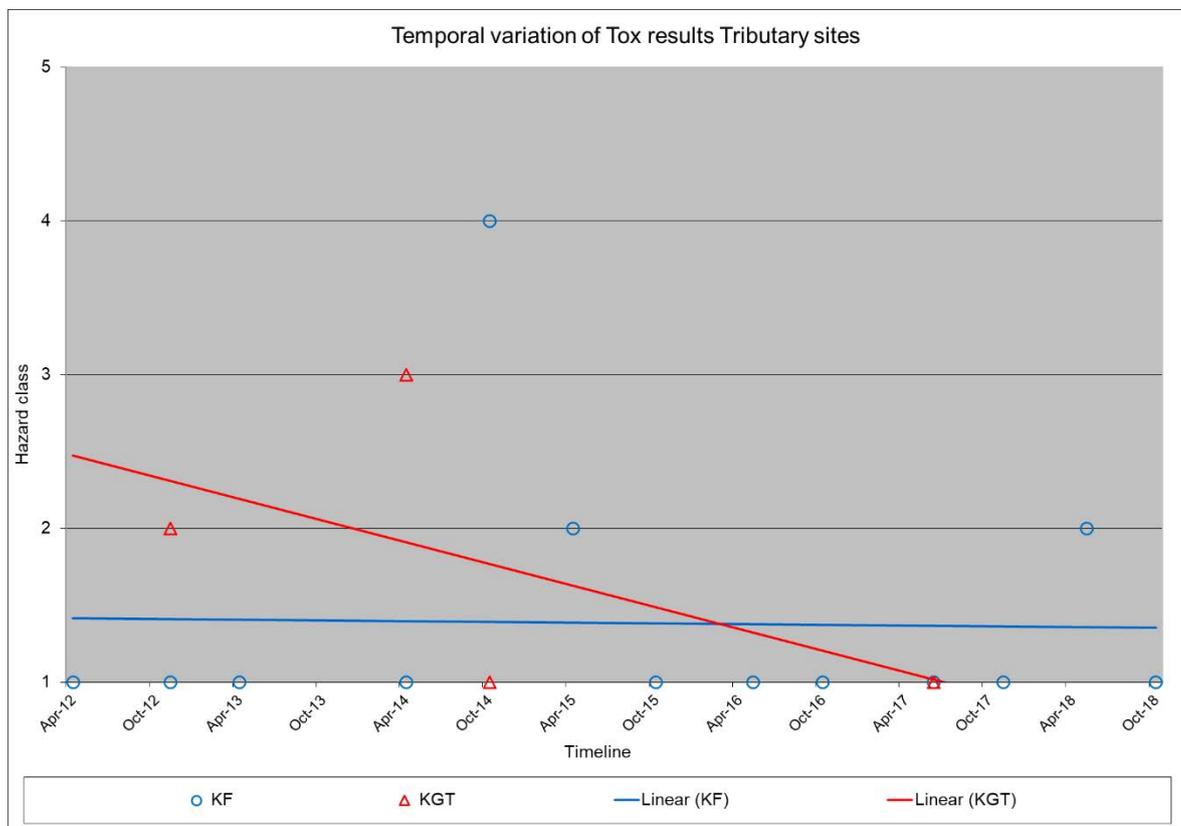


Figure 6: Temporal trends of toxicity results (bi-annually tested tributaries).

### 3.4 Aquatic invertebrate assessment: South African Scoring System 5

The South African Scoring System (Version 5) is a site-specific index which, together with associated habitat index (biotope suitability index), gives a general perspective of the biotic integrity (based on macro-invertebrates) and the impact of water quality on the biotic integrity of the specific sites (Thirion *et.al.*, 1995; Dickens and Graham, 2001). The biotope suitability index considers the suitability of the different sampled biotopes in terms of quality and availability. It thereby firstly assesses whether the total SASS5 scores of two sites are directly comparable by comparing the total biotope suitability scores. If the total biotope suitability scores are very different this would imply that the total SASS5 scores should not be compared, but instead the most comparable SASS biotope scores. The most comparable SASS biotope scores are identified by comparing the various individual biotope suitability scores. In addition to the biotope suitability index, the Integrated Habitat Assessment System version 2 (IHAS) was also applied and included to give the macro-invertebrate specific habitat descriptions (Table 6).

Average score per taxon (ASPT) values are also useful in the assessment and comparison of biotic conditions at different sites. Based on field trials assessed by Dickens and Graham (2001) the ASPT was less variable than total SASS5 scores when conducted within a given river reach by different operators, considering all biotopes. ASPT is therefore included in the discussion below.

Biotic conditions, based on the total SASS5 and ASPT scores, decreased slightly from site H-US-KF to site H-DS-KF (opposite spatial trend observed during the May 2018 survey) (Table 7; Figure 7). This is in contrast with most previous surveys when no spatial deterioration was observed. The most similar biotope<sup>8</sup> between

<sup>8</sup> To compare the effect of water quality on SASS scores on a spatial scale, habitat differences are considered. Therefore, the most comparable SASS<sub>biotope</sub> scores, in terms of habitat are also contrasted to gain insight regarding the effect of water quality on the biotic conditions (biotic integrity)

the two sites was GSM, confirming the probability of downstream deterioration in water quality (Table 7). Although biotic conditions at site KF in the Klipfonteinspruit were relatively low, it appeared to be similar to the adjoining Hex River sites. Furthermore, SASS scores (and hence biotic conditions) are expected to be affected by an intermittent flow regime (as regularly observed in the seasonal Klipfonteinspruit).

Table 6: Integrated Habitat Assessment (IHAS) description of the different biomonitoring sites.

Sampling Habitat	H-US-KF		KF		H-DS-KF		Hex-03		Hex-03-B	
	Desc	Score								
<b>Stones In Current (SIC)</b>										
Total length of white water rapids (ie: bubbling water) (in meters)	0-1	1	0-1	1	none	0	none	0	none	0
Total length of submerged stones in current (run) (in meters)	>2-5	2	>2-5	2	>2-5	2	>2-5	2	>2-5	2
Number of separate SIC area's kicked	2-3	2	6+	4	4-5	3	4-5	3	4-5	3
Average stone sizes kicked (in cm's)	11-20	3	11-20	3	11-20	3	11-20	3	11-20	3
Amount of stone surface clear (in %)	26-50	2	51-75	3	0-25	1	0-25	1	26-50	2
Protocol: time spent actually kicking SIC's (in mins)	2	3	2	3	2	3	2	3	2	3
<b>SIC score (max 20)</b>		<b>13</b>		<b>16</b>		<b>12</b>		<b>12</b>		<b>13</b>
<b>Vegetation (VEG)</b>										
Length of fringing vegetation sampled (banks) (in meters)	2	4	2	4	>1-2	3	2	4	2	4
Amount of aquatic vegetation/algae sampled (in square meters)	0-0.5	1	>0.5-1	2	0-0.5	1	>0.5-1	2	>0.5-1	2
Fringing vegetation sampled in	mix	5	mix	5	run	2	mix	5	mix	5
Type of veg. (percent leafy as apposed to stems/shoots)	26-50	3	26-50	3	26-50	3	26-50	3	26-50	3
<b>Veg score (max 15)</b>		<b>13</b>		<b>14</b>		<b>9</b>		<b>14</b>		<b>14</b>
<b>Other Habitat / General (O.H.)</b>										
Stones Out Of Current (SOOC) sampled (in square meters)	0-0.5	1	0-0.5	1	0-0.5	1	0-0.5	1	0-0.5	1
Sand sampled (in minutes)	none	0	none	0	none	0	0-0.5	2	0-0.5	2
Mud sampled (in minutes)	0.5	3	0.5	3	0.5	3	0-0.5	2	0-0.5	2
Gravel sampled (in minutes)	none	0	none	0	none	0	0-0.5	1	0-0.5	1
Bedrock sampled (all = no SIC, sand, gravel)	none	0	some	1	some	1	some	1	some	1
Algal presence (m <sup>2</sup> )	>1sqm	3	>1-2sqm	2	rocks	1	>2sqm	0	>1-2sqm	2
Tray identification	correct	3								
<b>O.H. score (max 20)</b>		<b>10</b>		<b>10</b>		<b>9</b>		<b>10</b>		<b>12</b>
<b>Sampling habitat totals (max 55)</b>		<b>36</b>		<b>40</b>		<b>30</b>		<b>36</b>		<b>39</b>
<b>Stream Condition</b>										
<b>Physical</b>										
River make up	2 mix	4	2 mix	4	run	2	2 mix	4	2 mix	4
Average width of stream (in meters)	1-2	4	>2-5	5	>5-10	2	>2-5	5	>2-5	5
Average depth of stream (in meters)	>0.5	5	0.5	4	>0.5	5	0.5	4	>0.5	5
Approximate velocity of stream	mix	5	mix	5	medium	3	mix	5	mix	5
Water colour	discoloured	3								
Recent disturbances	none	5								
Bank/Riparian vegetation	mix	4	mix	4	grass	2	mix	4	mix	4
Surrounding impacts	farming	1	other	3	erosion	0	farming	1	farming	1
Left bank cover (rocks and vegetation) (in %)	51-80	1	51-80	1	0-50	0	51-80	1	51-80	1
Right bank cover (rocks and vegetation) (in %)	51-80	1	51-80	1	0-50	0	51-80	1	51-80	1
<b>Stream condition total (max 45)</b>		<b>33</b>		<b>35</b>		<b>22</b>		<b>33</b>		<b>34</b>
<b>Total IHAS score (%)</b>		<b>69</b>		<b>75</b>		<b>52</b>		<b>69</b>		<b>73</b>

Biotic conditions, based on the total SASS5 scores and ASPT values, decreased largely from site H-DS-KF to Hex03 (Table 7; Figure 5). This was not habitat related as availability and suitability was better at the downstream site. A comparison of similar SASS-biotopes confirmed lowered biotic conditions, suggesting that the water quality was further affected between these sites, during October 2018. It has to be noted that organic enrichment and solid waste disposal appears extensively at this site and will likely affect biotic integrity if not mitigated (Plate 2). This was further supported by low dissolved oxygen levels (Refer to Section 3.2). It is again noted that the reason for lowered dissolved oxygen levels are unlikely to be related to APPD

activities because levels were within the guideline at site H-US-KF and no further APPD activities take place towards site Hex03.

Table 7: SASS5, ASPT and habitat suitability/availability index scores for different monitoring sites (October 2018).

Monitoring site	SASS5 score	ASPT	SASS5-score per biotope			Biotope availability and suitability (Scores)			
			SASS <sub>Stones</sub>	SASS <sub>Vegetation</sub>	SASS <sub>GSM</sub>	Stones	Vegetation	GSM	Combined
H-US-KF	49	3.77	7	46	21	3	6	3	12
KF	46	3.54	19	45	6	5	11	3	19
H-DS-KF	37	3.70	19	22	10	6	5	3	14
Hex-03	14	2.80	11	14	11	4	9	6	19
KGT	Dry								
Hex-03-B	28	4.00	28	20	8	5	14	5	24

**Key:**  
 ASPT - Average Score Pre Taxon      S-Stones      Veg-Vegetation      GSM-Gravel, sand & mud

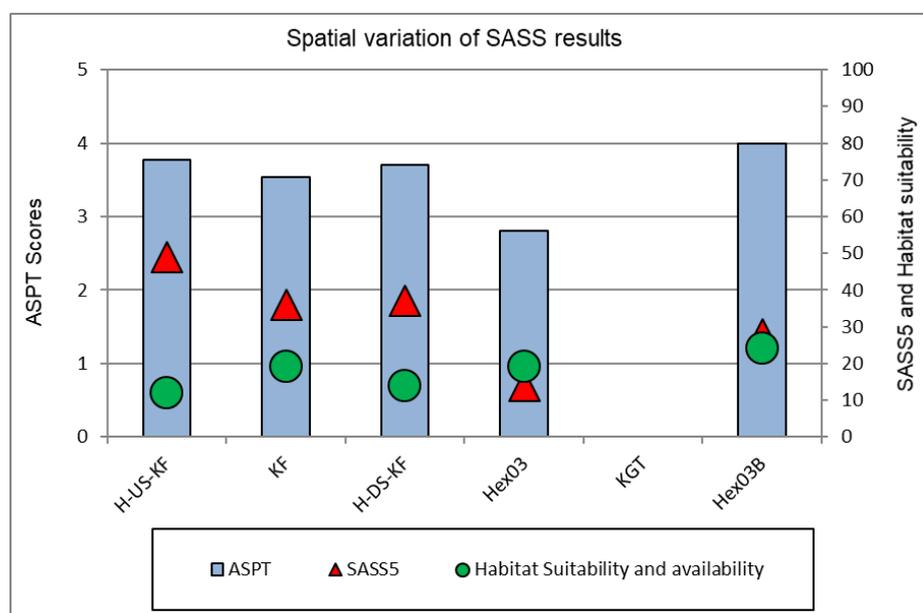


Figure 7: ASPT, SASS5 and total habitat suitability scores at biomonitoring sites during October 2018.

Biotic conditions, based on the total SASS5 scores and ASPT values, recovered largely from site Hex03 to Hex03-B (Table 7; Figure 5). This was at least partly habitat related as availability and suitability was better at the downstream site. Comparison of similar SASS-biotopes confirmed improved biotic conditions, suggesting that the water quality was not further affected between these sites, during October 2018. Site Hex03-B was included for the first time during the October 2018 survey. This was done to gauge the point-source effect, on the spatial integrity of the Hex River taking into consideration the Klipgatspruit. APPD is a potential contributor to pollution of the Klipgatspruit and continued monitoring (comparison of sites Hex03 and Hex03-B) will be essential to verify any possible impact and the severity thereof.

In conclusion, it can be stated that various sections of the Hex River within the study area show clear signs of reduced biotic integrity, based on macro-invertebrates. This was especially evident with the previous extended study area (now reduced due to Sibanye Stillwater sale and the complete scope no longer tasked to Clean Stream Biological Services). As such, a steady deterioration in biotic integrity in a downstream direction has consistently been recorded (Figure 8). However, the biotic integrity of the Hex River currently does improve on a spatial scale at certain sites (Figure 9) and appears to be more stable within the recently adopted reduction of the study area.



Plate 2: Indication of organic enrichment (algal proliferation) and solid waste disposal at site Hex03.

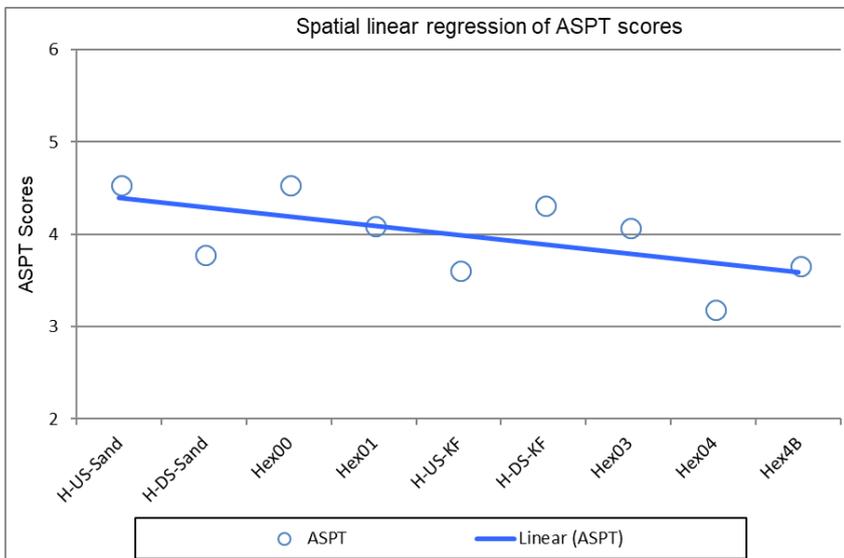


Figure 8: Linear regression of biotic integrity (as indicated by invertebrate ASPT scores) of the Hex River on a spatial scale (arranged sequentially in a downstream direction) during May 2018 (extended study area).

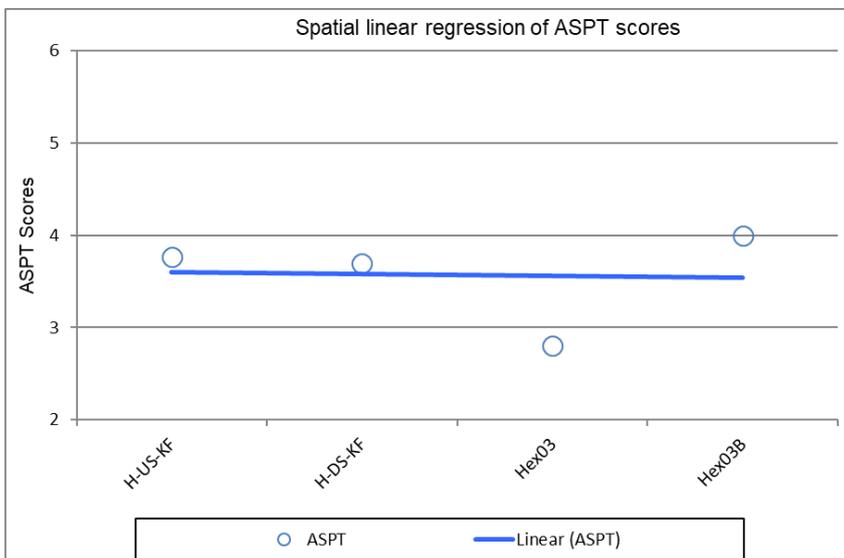


Figure 9: Linear regression of biotic integrity (as indicated by invertebrate ASPT scores) of the Hex River on a spatial scale (arranged sequentially in a downstream direction) during October 2018 (reduced study area).

### Temporal (long- and medium-term) trends

All of the datasets collected since May 2002 were compared to evaluate long-term and medium-term temporal trends in the biotic condition of the Hex River (Figures 10 & 11). Linear regression of historic ASPT values were calculated and plotted in order to achieve this. For the purpose of this monitoring programme, temporal trends are differentiated into long-term (more than four years) and medium-term (last 4 years) trends. The long-term trend gives a perspective on whether the biotic integrity (at the different sites) has improved or deteriorated since the inception of the monitoring programme. The medium-term trend confirms whether observed long-term trends are likely to continue or are in the process of being reversed.

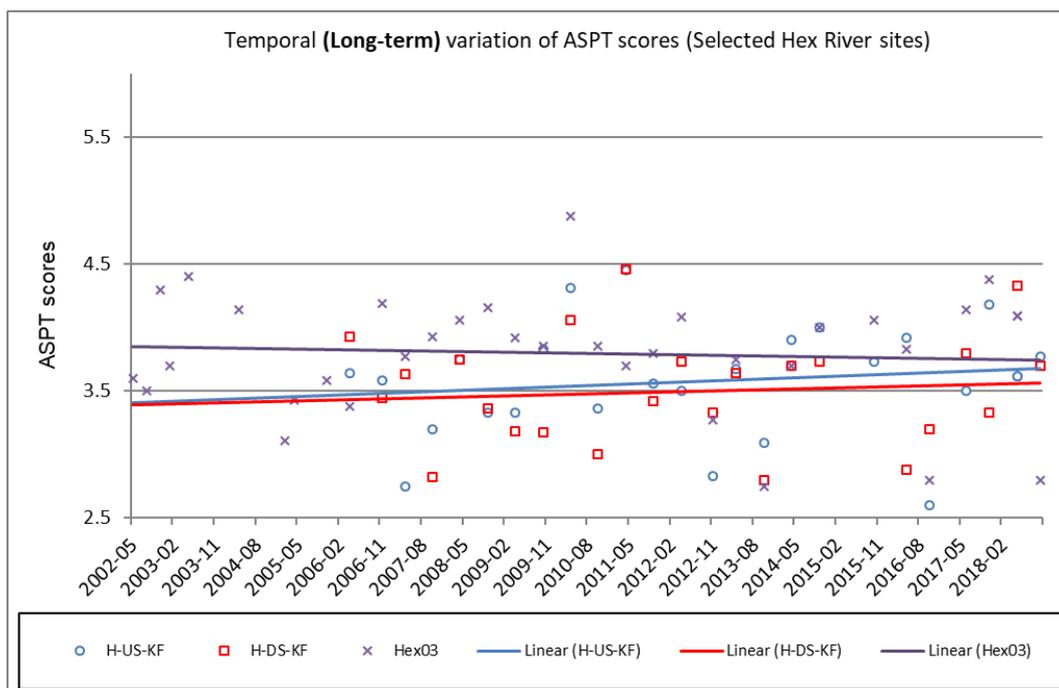


Figure 10: Long-term trends of biotic integrity in terms of macro-invertebrates at biomonitoring sites.

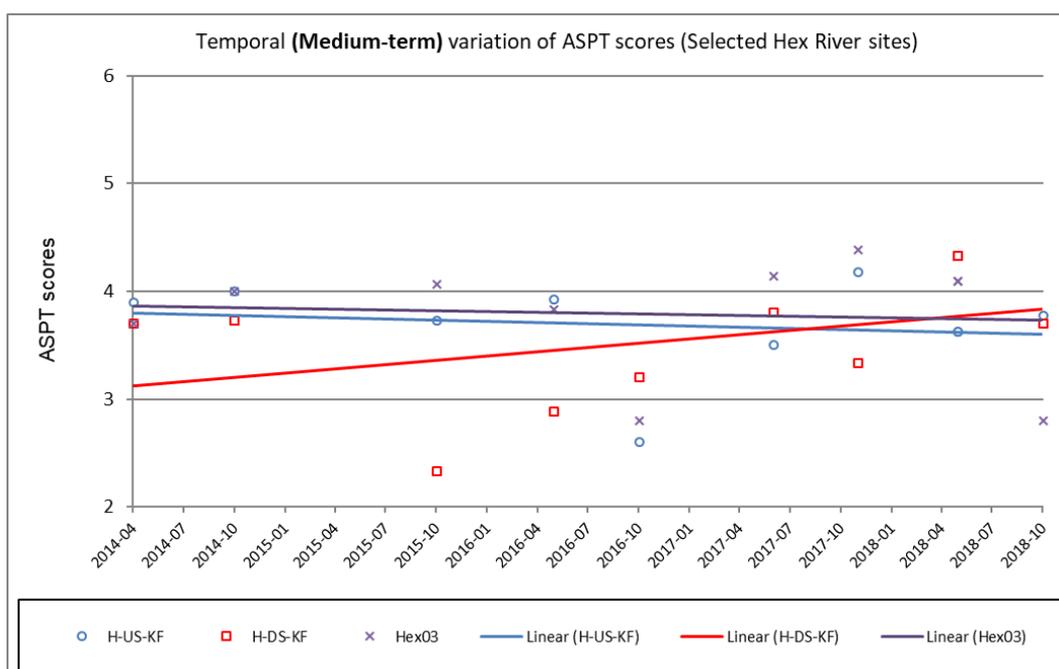


Figure 11: Medium-term trends of biotic integrity in terms of macro-invertebrates at biomonitoring sites.

Long-term trends indicated fair but stable biotic conditions at the three relevant Hex River sites (Figure 10). A slight improvement is in fact visible at sites H-US-KF and H-DS-KF. Medium term analyses (Figure 11) confirm generally lower biotic conditions at the latter site but, encouragingly, also eludes to recent improvement.

The long-term trend at site Hex03 is slightly negative as confirmed by the medium-term trends. The recently observed organic pollution is almost certainly the cause, being **unrelated** to APPD activities.

Continued monitoring will be essential to amass a database at the newly adopted downstream site (Hex03-B). This will serve to gauge the temporal effect of water users (including APPD) within the Klipgatspruit catchment, on the receiving environment (Hex River).

### 3.5 Fish Assessment

***Fish sampling is only scheduled once per annum and was last performed during May 2018, based on the extended scope (prior to Sibanye Stillwater sale). The approach and study area will therefore change in future, taking into consideration the reduced scope of this specific study, as performed by Clean Stream Biological Services. The complete extract (report RPM-A-18) of the fish results, is again repeated below for reference value and the sake of comprehensiveness. It should be kept in mind that various sampled sites are no longer part of this scope of work (since October 2018).***

The state and health of fish communities have been noted to give a reliable indication of short and long-term stress on aquatic systems. Fish communities possess various characteristics that render them important in the assessment of river health. They occupy positions throughout the aquatic food web, and are typically present in all but the most polluted of waters. Because fish often move over considerable distances, they have the potential to integrate diverse aspects of relatively large-scale habitats. Fish can therefore provide an integrated view of watershed conditions. Compared to other aquatic organisms, fish are furthermore relatively long-lived, and are therefore useful in providing a temporal dimension. They are also relatively easy to identify and after data is gathered, they can be released again. For the general public, fish are also the most well-known of aquatic organisms, and they are more likely to understand information about the condition of the fish community than about other taxa such as invertebrates. There are, however, some difficulties in using fish as biomonitoring indicators. Amongst these problems is the selective sampling attained by certain sampling equipment (for specific biotopes and for certain sizes and species of fish), the mobility of fish on spatial and temporal time scales, and the labour intensity of fish sampling.

Seven naturally occurring (native) fish species (*Barbus*<sup>9</sup> *paludinosus*; *Barbus trimaculatus*; *Barbus unitaeniatus*; *Clarias gariepinus*; *Oreochromis mossambicus*; *Pseudocrenilabrus philander* and *Tilapia sparrmanii*) were sampled at the five sampling sites in the Hex River during the 2017 to 2018 period (Table 8). The diversity of observed fish species was lower than expected at all of the sampling sites, indicating lowered biotic integrity (when compared to natural expected conditions). Possible reasons for lowered species diversity are outlined in the paragraphs below, which deal with the Fish Assemblage Integrity Index (FAII) and Fish Response Assessment Index (FRAI) results.

<sup>9</sup> Recent literature (Yang *et al.*, 2015) recommend a name change of the genus '*Barbus*' to '*Enteromius*'. This was however contested and rejected by various authors (i.e. Schmidt and Bart, 2015) and requires further verification. Skelton (2016) supports the recommended name change and started implementing this in recent studies and literature.

### The Fish Assemblage Integrity Index (FAII) and Fish Response Assessment Index (FRAI)

For the purpose of this study, a simplified version of the FAII was used (presence / absence) to enable comparisons between each site (spatial analyses), while the FRAI was used to determine the estimated biotic integrity, based on fish, of the entire Hex River reach under investigation which would provide a valuable tool to provide an overall status of the reach under investigation and to determine long-term (temporal) changes.

Table 8: Fish species expected and observed during the last two surveys.

Species	Native/Exotic	Sites									
		Hex00		Hex01		Hex03		Hex04		Hex4B	
		Exp	Obs								
<i>Amphillius uranoscopus</i>	Native										
<i>Enteromius<sup>#</sup> paludinosus</i>	Native		Obs		Obs		Obs		Obs		
<i>Enteromius<sup>#</sup> trimaculatus</i>	Native						Obs				
<i>Enteromius<sup>#</sup> unitaeniatus</i>	Native										
<i>Chiloglanis pretoriae</i>	Native										
<i>Clarias gariepinus</i>	Native		Obs				Obs		Obs		Obs
<i>Cyprinus carpio<sup>*</sup></i>	Exotic										
<i>Labeobarbus marequensis</i>	Native										
<i>Labeo cylindricus</i>	Native										
<i>Labeo molybdinus</i>	Native										
<i>Mesobola brevianalis</i>	Native										
<i>Oreochromis mossambicus</i>	Native						Obs		Obs		Obs
<i>Pseudocrenilabrus philander</i>	Native		Obs								
<i>Tilapia sparrmanii</i>	Native		Obs		Obs						
No. of naturally occurring species expected/present		9	4	10	3	10	5	13	4	13	3
% expected / observed		44		30		50		31		23	

**Key:** sampled previous survey, sampled this survey, sampled last two surveys

\* Exotic species are by definition not expected to occur under natural conditions and therefore not taken into account for FAII calculations

# Previous genus name: Barbus

### Fish Assemblage Integrity Index (FAII)

Based on morphological characteristics and the limited number of sites, each sampling site was classified as a separate fish habitat segment. Therefore, the “frequency of occurrence of fish within segments” was omitted from FAII for separate monitoring sites. Comparison of relative FAII scores for different sites would firstly give a perspective on the relative condition of the fish community at different sites and secondly indicate the impact of various anthropogenic activities up- and downstream of the different sites. Scores should however be treated with circumspection because the *frequency of occurrence* criterion was not considered, and the FAII scores are therefore less accurate. The list of fish species expected to occur at the sites under investigation is based on information from Skelton (1993) and Le Roux & Steyn (1968), as well as experience from previous surveys (this biomonitoring programme as well as various other mining related biomonitoring programmes, research and Department of Water Affairs’ reserve determination studies). The expected species list is also updated with the knowledge gained from this biomonitoring programme. The species intolerance ratings used in the calculation of the FAII were taken from Kleynhans (2002) and were based on specialisation of preferences towards habitat, food, flowing water and water quality.

The composition of the fish community and the relative FAII (Fish Assemblage Integrity Index) are based on the last two surveys. This is done to increase the accuracy of the results and to avoid the incidental omission of a particular species at a particular site. Furthermore, fish generally take longer to react to stressors

(compared with macro-invertebrates) and are therefore more applicable as an indicator over a period of time (as opposed to a snapshot at any given time).

The biotic integrity (as reflected by the fish assemblage integrity index) increased slightly from site Hex00 (23%) to Hex01 (27%) (Table 9 & Appendix tables; Figure 12). This is an indication that the biotic integrity (based on the fish communities) was not recently deteriorated due to by impacts in the area between these two sites. This is a similar trend as observed with the macro-invertebrate results, which indicated stable biotic conditions between these sites.

Table 9: Relative FAIL scores calculated at different sampling sites (2017 to 2018).

Locality	Relative FAIL (%)
Hex00	23
Hex01	27
Hex03	46
Hex04	22
Hex4B	22

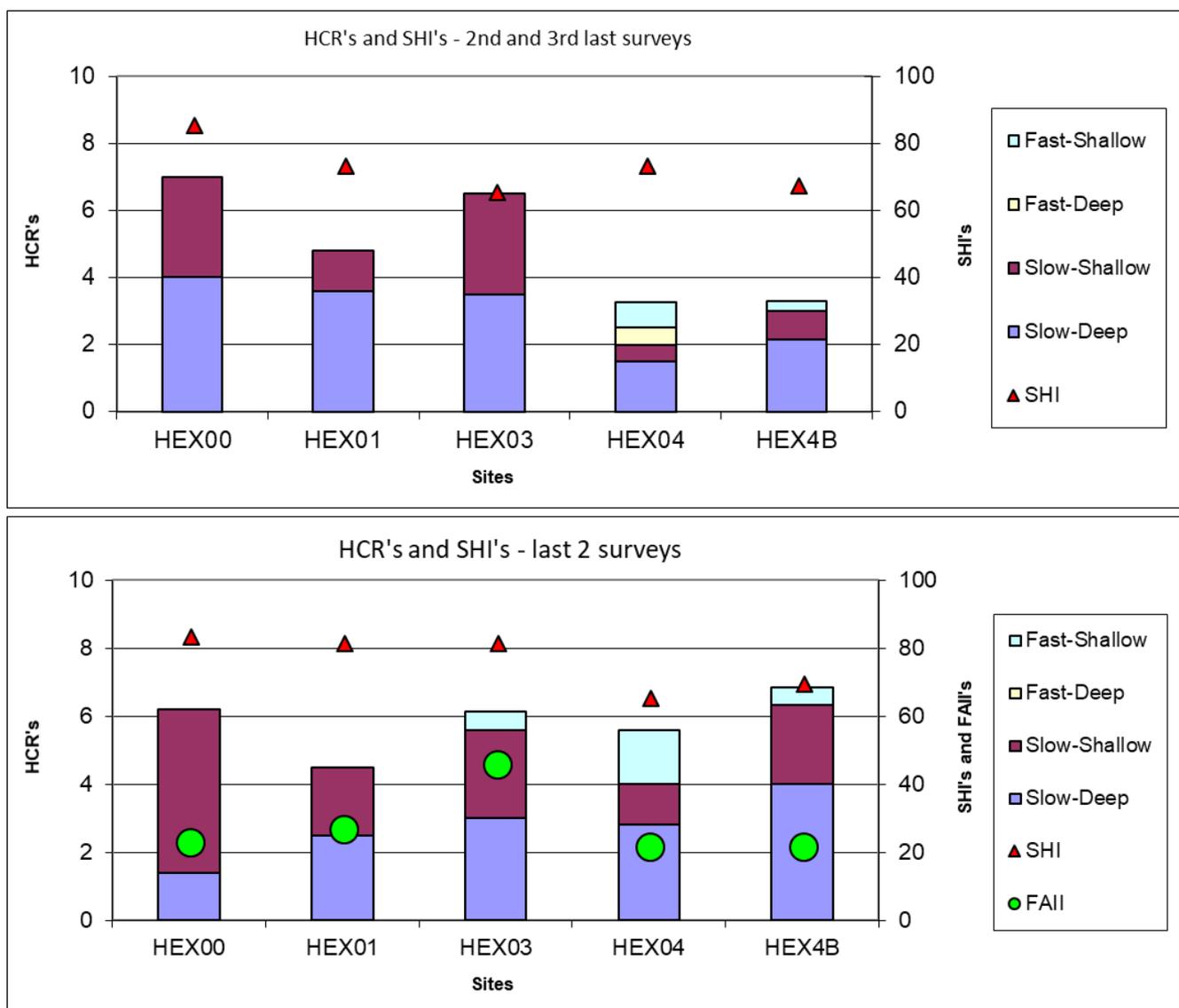


Figure 12: Relative FAIL scores, HCR's and SHI at the different biomonitoring sites.

A spatial improvement was observed from site Hex01 to Hex03, as shown by the FAIL scores increasing from 27% to 46% (Table 9; Figure 12). The spatial improvement was mainly attributed to the presence of *Enteromius trimaculatus* and *Enteromius unitaeniatus* at site Hex03. Both species are tolerant to moderately intolerant to water quality changes (Table 10) and their absence from site Hex01 is therefore not likely to be water quality related. Its absence from site Hex 00 during the 2017 to 2018 period is likely to be a response to lower habitat diversity and availability at this site. Based on these results, it appears that biotic integrity (based on fish) was probably not reduced by deteriorating water quality originating from the Klipfonteinspruit (possibly RPM-related) and/or from the sewage plant (non-RPM-related). This deduction is similar to the macro-invertebrate based deduction between these sites.

Table 10: The relative tolerance of each species towards changes in the environment.

SPECIES NAME	Common name	Trophic specialisation	Habitat specialisation	Flow dependance	Requirement for high water quality	Total intolerance ratings
<i>Amphilius uranoscopus</i>	Stargazer	4.7	4.9	4.8	4.8	4.8
<i>Chiloglanis pretoriae</i>	Shortspine suckermouth	4.4	4.8	4.8	4.5	4.6
<i>Labeo molybdinus</i>	Leadeo labeo	3.3	3.0	3.3	3.2	3.2
<i>Labeo cylindricus</i>	Redeye labeo	3.3	3.0	3.1	3.1	3.1
<i>Labeobarbus marequensis</i>	Largescale yellowfish	2.4	2.8	3.2	2.1	2.6
<i>Mesobola brevianalis</i>	River sardine	3.1	2.2	1.1	2.8	2.3
<i>Enteromius trimaculatus</i>	Threespot barb	3.1	1.4	2.7	1.8	2.2
<i>Enteromius paludinosus</i>	Straightfin barb	1.6	1.4	2.3	1.8	1.8
<i>Enteromius unitaeniatus</i>	Longbeard barb	1.1	1.3	2.3	2.2	1.7
<i>Oreochromis mossambicus</i>	Mazambique tilapia	1.2	1.9	0.9	1.3	1.3
<i>Pseudocrenilabrus philander</i>	Southern mouthbrooder	1.3	1.4	1.0	1.4	1.3
<i>Tilapia sparrmanii</i>	Banded tilapia	1.6	1.4	0.9	1.4	1.3
<i>Clarias gariepinus</i>	Sharptooth catfish	1.0	1.2	1.7	1.0	1.2

**Key:**

Intolerance ratings are colour shaded on a scale from green to red, with green being least intolerant and red being most intolerant

Species are sorted in descending order from most intolerant (total intolerance rating) to least intolerant

1-2 = Tolerant 2-3 = Moderate tolerant 3-4 = Moderately intolerant 4-5 = Intolerant

As also observed previously, the FAIL scores were largely reduced from site Hex03 towards site Hex04 (Table 9). The potential impact of water quality on the biotic integrity of the Hex River (as measured by the FAIL at site Hex04) should therefore not be disregarded as both macro-invertebrates (during 2016 and 2018) and fish (last 4 years) are now indicating as such. Potential sources of reduced water quality between sites Hex03 and Hex04 include the Klipgatspruit and the Dorpspruit (see also previous discussions regarding potential Dorpspruit impacts).

The biotic integrity (based on fish) was similar between site Hex04 to site Hex4B (Table 9 & Appendix tables; Figure 12), being very poor at both sites. The same poor conditions (albeit spatially increased) was indicated by the macro-invertebrate assessment for these sites.

**Fish Response Assessment Index (FRAI)**

As mentioned earlier, the Fish Response Assessment Index was adopted to assist in the classification of the Ecological Status, based on fish, of the entire Hex River reach under investigation. The results are therefore

pooled for all sites. The resulting classification is therefore not a reflection of RPM mining impacts, but rather a reflection of the overall cumulative impact/s derived from the catchment.

The section below shows the individual metric driver results (Velocity-Depth, Cover, Flow, Physico-chemical, Migration and Introduced species), as well as the overall FRAI categories and category descriptions for the Hex River (Table 11).

Table 11: Fish Response Assessment Index (FRAI) results for the Hex River reach (all sites) (2017/8 results).

METRIC GROUP	METRIC	*RATING (CHANGE)	METRIC GROUP WEIGHT (%)
VELOCITY-DEPTH CLASSES METRICS	Response of species with high to very high preference for FAST-DEEP conditions	-5	97
	Response of species with high to very high preference for FAST-SHALLOW conditions	-5	
	Response of species with high to very high preference for SLOW-DEEP conditions	-2.5	
	Response of species with high to very high preference for SLOW-SHALLOW conditions	-2	
COVER METRICS	Response of species with a very high to high preference for overhanging vegetation	-1.5	100
	Response of species with a very high to high preference for undercut banks and root wads	-0.5	
	Response of species with a high to very high preference for a particular substrate type	-5	
	Response of species with a high to very high preference for instream vegetation	-0.5	
	Response of species with a very high to high preference for the water column	-3	
FLOW DEPENDANCE METRICS	Response of species intolerant of no-flow conditions	-5	94
	Response of species moderately intolerant of no-flow conditions	-5	
	Response of species moderately tolerant of no-flow conditions	-2	
	Response of species tolerant of no-flow conditions	-1.5	
PHYSICO-CHEMICAL METRICS	Response of species intolerant of modified physico-chemical conditions	-5	64
	Response of species moderately intolerant of modified physico-chemical conditions	-5	
	Response of species moderately tolerant of modified physico-chemical conditions	-4	
	Response of species tolerant of modified physico-chemical conditions	-1	
MIGRATION METRICS	Response in terms of distribution/abundance of spp with catchment scale movements	0	61
	Response in terms of distribution/abundance of spp with requirement for movement between reaches or fish habitat segments	4	
	Response in terms of distribution/abundance of spp with requirement for movement within reach or fish habitat segment	2	
INTRODUCED SPECIES METRICS	The impact/potential impact of introduced competing/predaceous spp?	0	45
	How widespread (frequency of occurrence) are introduced competing/predaceous spp?	0	
	The impact/potential impact of introduced habitat modifying spp?	2	
	How widespread (frequency of occurrence) are habitat modifying spp?	1	
FRAI SCORE (%)		32.3	
FRAI CATEGORY		E	
FRAI CATEGORY DESCRIPTION		Seriously modified	

- Reduced flows and altered flooding regime of the river.
- Cover metrics: Seriously deterioration in substrate as cover, most probably associated with extensive algal growth (as described earlier in this report), flow modification (decreased riffle/rapid habitats) and sedimentation.
- Flow dependence metrics: Serious modification of fish species intolerant to moderately intolerant to no-flow conditions, again indicating on altered hydrological regime (altered flows and floods).
- Physico-chemical metrics: Seriously modified conditions indicated by fish species that are intolerant to moderately intolerant of modified water quality, indicating on seriously deteriorated water quality prevailing in this river reach.
- Migration metrics: Indicating seriously modified migratory impacts, associated with various physical and potentially also chemical migration barriers within this reach.
- Introduced species metrics: Slight impacts associated with the presence of the habitat modifying alien Common carp (*Cyprinus carpio*).

Table 12: Descriptive categories used to describe the present ecological status (PES) of biotic components (adapted from Kleynhans, 1999).

CATEGORY	BIOTIC INTEGRITY	DESCRIPTION OF GENERALLY EXPECTED CONDITIONS
A	Excellent	Unmodified, or approximates natural conditions closely. The biotic assemblages compares to that expected under natural, unperturbed conditions.
B	Good	Largely natural with few modifications. A change in community characteristics may have taken place but species richness and presence of intolerant species indicate little modifications. Most aspects of the biotic assemblage as expected under natural unperturbed conditions.
C	Fair	Moderately modified. A lower than expected species richness and presence of most intolerant species. Most of the characteristics of the biotic assemblages have been moderately modified from its naturally expected condition. Some impairment of health may be evident at the lower end of this class.
D	Poor	Largely modified. A clearly lower than expected species richness and absence or much lowered presence of intolerant and moderately intolerant species. Most characteristics of the biotic assemblages have been largely modified from its naturally expected condition. Impairment of health may become evident at the lower end of this class.
E	Very Poor	Seriously modified. A strikingly lower than expected species richness and general absence of intolerant and moderately tolerant species. Most of the characteristics of the biotic assemblages have been seriously modified from its naturally expected condition. Impairment of health may become very evident.
F	Critical	Critically modified. Extremely lowered species richness and an absence of intolerant and moderately tolerant species. Only intolerant species may be present with complete loss of species at the lower end of the class. Most of the characteristics of the biotic assemblages have been critically modified from its naturally expected conditions. Impairment of health generally very evident.

## 4 CONCLUSIONS AND RECOMMENDATIONS

The following conclusions are based on the aquatic macro-invertebrate assessments performed during October 2018. Reference is not made to fish based conclusions since the new scope of work (study area) has invalidated spatial and temporal findings, which will be refined when fish assessment are once again performed (scheduled once per annum).

The most important **spatial** conclusions are as follows:

- Biotic conditions, based on the total SASS5 and ASPT scores, **decreased slightly** from site H-US-KF to site H-DS-KF (opposite spatial trend observed during the May 2018 survey). This is in contrast with most previous surveys when no spatial deterioration was observed. The most similar biotope between the two sites was GSM, confirming the probability of downstream deterioration in water quality. Although biotic conditions at site KF in the Klipfonteinspruit were relatively low, it appeared to be similar to the adjoining Hex River sites. Furthermore, SASS scores (and hence biotic conditions) are expected to be affected by an intermittent flow regime (as regularly observed in the seasonal Klipfonteinspruit).
- Biotic conditions, based on the total SASS5 scores and ASPT values, **decreased largely from site H-DS-KF to Hex03**. This was not habitat related as availability and suitability was better at the downstream site. A comparison of similar SASS-biotopes confirmed lowered biotic conditions, suggesting that the water quality was further affected between these sites, during October 2018. It has to be noted that organic enrichment and solid waste disposal appears extensively at this site and will likely affect biotic integrity if not mitigated. This was further supported by low dissolved oxygen levels. It is again noted that the reason for lowered dissolved oxygen levels are unlikely to be related to APPD activities because levels were within the guideline at site H-DS-KF and no further APPD activities take place towards site Hex03.
- Biotic conditions, based on the total SASS5 scores and ASPT values, **recovered largely from site Hex03 to Hex03-B**. This was at least partly habitat related as availability and suitability was better at the downstream site. Comparison of similar SASS-biotopes confirmed improved biotic conditions, suggesting that the water quality was not further affected between these sites, during October 2018. Site Hex03-B was included for the first time during the October 2018 survey. This was done to gauge the point-source effect, on the spatial integrity of the Hex River taking into consideration the Klipgatspruit. APPD is a potential contributor to pollution of the Klipgatspruit and continued monitoring (comparison of sites Hex03 and Hex03-B) will be essential to verify any possible impact and the severity thereof.

The most important **temporal (long- and medium-term)** conclusions regarding the biotic integrity of the Hex River are as follows:

- Long-term trends indicated fair but **stable biotic conditions** at the three relevant Hex River sites. A slight improvement is in fact visible at sites H-US-KF and H-DS-KF. Medium term analyses confirm generally lower biotic conditions at the latter site but, encouragingly, also eludes to recent improvement.
- The long-term trend at site **Hex03 is slightly negative** as confirmed by the medium-term trends. The recently observed organic pollution is almost certainly the cause, being **unrelated** to APPD activities.
- Continued monitoring will be essential to amass a database at the newly adopted downstream site (Hex03-B). This will serve to gauge the temporal effect of water users (including APPD) within the Klipgatspruit catchment, on the receiving environment (Hex River).

### General conclusions and recommendations

In conclusion, it can be stated that various sections of the Hex River within the study area show clear signs of reduced biotic integrity, based on macro-invertebrates. This was especially evident with the previous extended study area (now reduced due to Sibanye Stillwater sale and the complete scope no longer tasked

to Clean Stream Biological Services). As such, a steady deterioration in biotic integrity in a downstream direction has consistently been recorded. However, the biotic integrity of the Hex River currently does improve on a spatial scale at certain sites and appears to be more stable within the recently adopted reduction of the study area.

Future biomonitoring should be maintained on at least a biannual interval to gauge the trend of deterioration/improvement. This would facilitate the identification of possible impacts by APPD (and others) to this aquatic ecosystem. Early identification of impacts to the biota should prompt the identification of contaminants and the implementation of mitigation measures to reduce or prevent continued risk to the aquatic ecosystem.

It is strongly recommended that definitive toxicity testing be continued for the PCDs that regularly display toxicity levels of Class III or higher. Definitive toxicity testing will allow for the calculation of safe dilution ratios and will allow for the process of risk assessment. The risk assessment involves predicting the amount of a substrate that may enter the environment and comparing this with definitive toxicity results.

Calculated dilution ratios will be essential for environmental managers to predict whether the toxicity of polluted water will be negated if released or accidentally spilled into the receiving environment. Definitive testing will furthermore assist with scheduling planned licenced releases (*i.e.* whether water could be released during the dry season and, if not, whether sufficient dilution is likely to be achieved during the wet season/times of high river flow). All discharges should fall within the ambit of an approved water use licence, with biomonitoring and toxicity data being essential for the licensing process. In addition, increasing the frequency of testing of the pollution control facilities to at least twice a year should be considered. The confidence of results is relatively low if testing is only performed once a year, especially since toxicity hazards could conceivably change on a daily basis. More regular testing will therefore increase the confidence of results and lead to more informed management decisions.

It is now recommended to include both site KF and KFD (in the Klipfonteinspruit) for toxicity testing (in addition to the Klipgatspruit; site KGT). The effect of different sources of pollution can then be distinguished more accurately.

## 5 REFERENCES

- ABOATOX Oy. 2012. BO1243-500 BioTox™ Kit. Instructions for use. Savikuja 2. FIN-21250, Masku Finland. [www.aboatox.com](http://www.aboatox.com)
- BARBOUR MT and STRIBLING JB (1994) A technique for assessing stream habitat structure. Pp. 156-178, In: Proceedings of the conference "Riparian Ecosystems of the Humid U.S. Management, functions, and Values". National Association of Conservation Districts. Washington, DC
- DEPARTMENT OF WATER AFFAIRS AND FORESTRY. 1996. South African Water Quality Guidelines (second edition). Volume 6: Agricultural water use: Aquaculture.
- DEPARTMENT OF WATER AFFAIRS AND FORESTRY, 2003. The Management of Complex Industrial Waste Water Discharges. Introducing the Direct Estimation of Ecological Effect Potential (DEEEP) approach, a discussion document. Institute of Water Quality Studies, Pretoria.
- DICKENS C and GRAHAM M (2001) South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers. River Health Programme Web Page.
- EUROPEAN Standard, 1998. "Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) – Part 3 for the method using freeze-dried bacteria", EN ISO 11348-3. European Committee for Standardization, Brussels.
- KEMPSTER, P. L. HATTINGH, W. H. J. & VAN VLIET, H. R. 1982. Summarised water quality criteria. Technical report NR. Tr 108. Department of Environmental Affairs.
- KILIAN V. 1996. Fish Pathology (FHA): A biomonitoring procedure for rapid evaluation of fish health and condition. Report used during Field biosurveys and Integrated ecological assessment course, Institute of Water Quality Studies, DWAF.
- KLEYNHANS, C. J. 1997. *An exploratory investigation of the Instream Biological Integrity of the Crocodile River, Mpumalanga, as based on the Assessment of Fish Communities*. Draft Report, Department of Water Affairs and Forestry, Institute for Water Quality Studies. 61 pp.
- KLEYNHANS, C. J. 1997. *An exploratory investigation of the Instream Biological Integrity of the Crocodile River, Mpumalanga, as based on the Assessment of Fish Communities*. Draft Report, Department of Water Affairs and Forestry, Institute for Water Quality Studies. 61 pp.
- KLEYNHANS, C.J. 2002. Fish Intolerance ratings. Personal electronic communication of proceedings resulting from the national fish workshop held at the WRC during 2001.
- LE ROUX, P & STEYN, S. 1968. *Visse van Transvaal*. Kaap & Transvaal Drukkers Beperk, Kaapstad. 108pp.
- McMILLAN, P. H. 1998. *An Integrated Habitat Assessment System (IHAS v2), for the Rapid Biological Assessment of Rivers and Streams*. A CSIR research project. Number ENV-P-I 98132 for the Water Resources Management Programme. CSIR. ii + 44 pp.
- MICROBIOTEST INC. 2012. DAPHTOXKIT F™ MAGNA. Crustacean Toxicity Screening Test for freshwater. Standard Operational Procedure. Kleimoer 15, 9030 Mariakerke (Gent), Belgium. [www.microbiotest.be](http://www.microbiotest.be).
- ODUM EP (1971). *Fundamentals of Ecology*. Third Edition. W. B. Saunders Co. London.
- PERSOONE G, BLAHOŠLAV M, BLINOVA I, TÖRÖKNE A, ZARINA T, MANUSADZIANAS L, NALECZ-JAWECKI G, TOFAN L, STEPANOVA L, TOTHOVA L, KOLAR B. A practical and user-friendly toxicity classification system with Microbiotests for natural waters and wastewaters (personal communication).
- ROUX DJ (1999). Incorporating technologies for the monitoring and assessment of biological indicators into a holistic resource-based water quality management approach- conceptual models and some case studies. Ph.D Thesis. Rand Afrikaans University, JHB, SA.
- SKELTON P. H., 1993. *A complete guide to freshwater fishes of Southern Africa*. Southern Book Publishers (Pty) Ltd., Halfway House. 388pp.
- THIRION, C. A.; MOCKE, A & WOEST, R. 1995. *Biological Monitoring of Streams and Rivers using SASS4: A User Manual*. Final Report, No. N 000/00/REQ/1195. Institute of Water Quality Studies, Department of Water Affairs and Forestry.
- UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (US EPA), 1996. *Ecological effects test guidelines. Fish acute toxicity test – Freshwater and marine*. OPPTS 850.1075. Report number EPA-712-c-96-118.
- UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (US EPA), 1993. *Method for measuring the acute toxicity of effluent and receiving waters to freshwater and marine organisms*. EPA/600/4-90/027F, 4<sup>th</sup> edition. Office of Research and Development, Washington.
- YANG L, SADO T, HIRT MV, PASCO-VEIL E, ARUNACHALAM M, Li J, WANG X, FREYHOF J, SAOTOH K, SIMINS AM, MIYA M, He S, MAYDEN RL. 2015. Phylogeny and polypoidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). *Mol. Phylogenet. Evol.* 85, 97 – 116.

## Appendix 1: Methodology applied during this biomonitoring assessment.

### 1. *In-situ* water quality

The following surface water quality variables were measured on site: pH, Conductivity, water temperature, dissolved oxygen and oxygen saturation (Hach HQ40d Multimeter; Serial Number: 130300086148).

### 2. Habitat assessment

An evaluation of habitat quality and availability to biota is critical to any assessment of ecological integrity and should be conducted at each site at the time of biological sampling. On site habitat assessments were conducted by using existing habitat evaluation indices.

#### 2.1 Habitat condition

The United States Environment Protection Agency Habitat Assessment Index (HAI) index was used to determine the general physical habitat condition at each site. Habitat parameters used by this index in this assessment of habitat integrity include the following: Epifaunal substrate/Available cover, Pool substrate characteristics, Pool variability, Channel alteration, Sediment deposition, Channel sinuosity, Channel flow status, Bank vegetative protection, Bank stability and Riparian vegetative zone width. Each of the above mentioned criteria was assessed and according to its condition, rated in one of the following classes, namely: Optimal/Excellent, Sub optimal/Good, Marginal/Fair or Poor. For each criterion, a score was given within the selected class. The sum of these scores gives a final score for this Index, and can be used in comparison to other sites or, if possible, to the baseline or reference condition to indicate its physical integrity (Barbour *et al.*, 1999).

#### 2.2 Fish Habitat Assessment

This assessment is aimed at the determination of the potential of a site to provide habitat for fish (Fish habitat cover ratings) and to identify the potential human impact on the fish habitat (Site fish habitat integrity) (Kleynhans, 1997).

##### *Fish Habitat Cover Rating (HCR)*

This approach was developed to assess habitats according to different attributes that are surmised to satisfy the habitat requirements of various fish species (Kleynhans, 1997). At each site, the following depth-flow (df) classes are identified, namely:

Slow (<0.3m/s), shallow (<0.5m) - Shallow pools and backwaters.

Slow, deep (>0.5m) - Deep pools and backwaters.

Fast (>0.3m/s), shallow - Riffles, rapids and runs.

Fast, deep - Usually rapids and runs.

The relative contribution of each of the above mentioned classes at a site was estimated and indicated as:

0 = Absent

1 = Rare (<5%)

2 = Sparse (5-25%)

3 = Moderate (25-75%)

4 = Extensive (>75%)

For each depth-flow class, the following cover features (cf), considered to provide fish with the necessary cover to utilise a particular flow and depth class, were investigated:

- Overhanging vegetation
- Undercut banks and root wads
- Stream substrate
- Aquatic macrophytes

The amount of cover present at each of these cover features (cf) was noted as:

0 = absent

1 = Rare/very poor (<5%)

2 = Sparse/poor (5-25%)

3 = Moderate/good (25-75%)

4 = Extensive/excellent (>75%)

The fish habitat cover rating (HCR) was calculated as follows:

- The contribution of each depth-flow class at the site was calculated ( $df/\Sigma df$ ).

- For each depth-flow class, the fish cover features (cf) were summed ( $\Sigma cf$ ).

$HCR = df/\Sigma df \times \Sigma cf$ .

##### *Site fish habitat integrity (SHI)*

This approach is based on the assessment of physical habitat disturbance and is directed towards the indirect qualitative evaluation of fish habitat integrity, compared to the expected natural condition (Kleynhans, 1997). The following impacts (cause for fish habitat integrity degradation) is investigated, namely: Water abstraction, flow modification, bed modification, channel modification, inundation, exotic macrophytes, solid waste disposal, indigenous vegetation removal, exotic vegetation encroachment and bank erosion. Estimation of the impact of each of these modifications on the fish habitat integrity at a site is scored as follows:

No Impact = 0

Small impact = 1

Moderate Impact = 3

Large impact = 5

### 3. Aquatic invertebrate assessment: South African Scoring System, Version 5.

Benthic macro-invertebrate communities of the selected sites were investigated according to the South African Scoring System, version 5 (SASS5) approach (Dickens & Graham, 2001). This method is based on the British Biological Monitoring Working Party (BMWP) method and has been adapted for South

African conditions by Dr. F. M. Chutter (Thirion *et al.*, 1995). The SASS method is a rapid, simple and cost effective method, which has progressed through four different upgrades/versions. The current upgrade is Version 5, which is specifically designed to comply with international accreditation protocols.

#### **Sample Collection**

An invertebrate net (30 x 30cm square with 1mm mesh netting) was used for the collection of the organisms. The available biotopes at each site were identified on arrival. Each of the biotopes was sampled by different methods explained later (samples should not be collected when the river is in flood).

The biotopes were combined into three different groups, which were sampled and assessed separately:

#### **a) Stone (S) Biotopes:**

**Stones in current (SIC) or any solid object:** *Movable stones of at least cobble size (3 cm diameter) to approximately 20 cm in diameter, within the fast and slow flowing sections of the river.* Kicksampling is used to collect organisms in this biotope. This is done by putting the net on the bottom of the river, just downstream of the stones to be kicked, in a position where the current will carry the dislodged organisms into the net. The stones are then kicked over and against each other to dislodge the invertebrates (kicksampling) for  $\pm 2$  minutes.

**Stones out of current (SOOC):** *Where the river is still, such as behind a sandbank or ridge of stones or in backwaters.* Collection is again done by the method of kicksampling, but in this case the net is swept across the area sampled to catch the dislodged biota. Approximately 1 m<sup>2</sup> is sampled in this way.

**Bedrock or other solid substrate:** Bedrock includes stones greater than 30cm, which are generally immovable, including large sheets of rock, waterfalls and chutes. The surfaces are scraped with a boot or hand and the dislodged organisms collected. Sampling effort is included under SIC and SOOC above.

#### **b) Vegetation (VG) Biotopes:**

**Marginal vegetation (MV):** *This is the overhanging grasses, bushes, twigs and reeds growing on the edge of the stream, often emergent, both in current (MvegIC) and out of current (MvegOOC).* Sampling is done by holding the net perpendicular to the vegetation (half in and half out of the water) and sweeping back and forth in the vegetation ( $\pm 2$ m of vegetation).

**Submerged vegetation (AQV):** *This vegetation is totally submerged and includes Filamentous algae and the roots of floating aquatics such as water hyacinth.* Sampled by pushing the net (under the water) against and amongst the vegetation in an area of approximately one square meter.

#### **c) Gravel, Sand and Mud (GSM) biotopes:**

**Sand:** *This includes sandbanks within the river, small patches of sand in hollows at the side of the river or sand between the stones at the side of the river.* This biotope is sampled by stirring the substrate by shuffling or scraping of the feet, which is done for half a minute, whilst the net is continuously swept over the disturbed area.

**Gravel:** *Gravel typically consists of smaller stones (2-3 mm up to 3 cm).* Sampling similar to that of sand.

**Mud:** *It consists of very fine particles, usually as dark-collared sediment.* Mud usually settles to the bottom in still or slow flowing areas of the river. Sampling similar to that of sand.

#### **d) Hand picking and visual observation:**

Before and after disturbing the site, approximately 1 minute of "hand-picking" for specimens that may have been missed by the sampling procedures was carried out.

#### **Sample preparation**

The organisms sampled in each biotope group were identified and their relative abundance also noted on the SASS5 datasheet.

#### **SASS-Habitat Assessment**

A SASS-habitat assessment index, according to the habitats sampled, was performed due to the fact that changes in habitat can be responsible for changes in SASS5 scores. This was done by the application of Integrated Habitat Assessment System (IHAS version 2) (McMillan, 1998).

#### **4. Fish Assemblage Integrity Index (FAII) (Kleynhans, 1997)**

Due to the difficulty of applying the generally used Index of Biotic Integrity (IBI) in rivers of South Africa, Kleynhans (1997) developed an alternative approach. The following procedures were used in the application of the FAII:

##### *Species tolerance ratings*

The species intolerance ratings used in the calculation of the FAII were taken from Kleynhans (2002). Four components are taken into account in estimating the intolerance of the relevant fish species, namely habitat preferences and specialisation (HS), food preference and specialisation (TS), requirements for flowing water during different life-stages (FW) and water quality requirements (WQ). Each of these aspects are scored for a species according to low requirement/specialisation (rating=1), moderate requirement/ specialisation (rating=3) and high requirement/specialisation (rating=5). The total intolerance (IT) of a fish species is estimated as follows:

$$IT = (HS+TS+FW+WQ)/4$$

##### *Health*

The percentage of fish with externally evident disease or other anomalies are used to score this metric. The following procedure is used to score the health of individual species:

Frequency of affected fish >5%, score = 1

Frequency of affected fish 2 - 5%, score = 3

Frequency of affected fish <2%, score = 5

The expected health for a species living under unperturbed conditions is assumed to be unimpaired and would score 5.

##### **The FAII is calculated as follows:**

The *expected index score* [FAII (exp.)] per segment:

$$FAII (exp.) = \sum(TxH)$$

where: T = Tolerance rating for individual species

H = Expected health rating for individual species.

The *observed index score* [FAII (obs)] is calculated on a similar basis but is based on the information collected during the survey:

$$FAII (obs) = \sum(TxH)$$

The observed fish assemblage index score for a segment is expressed as a percentage of the expected total FAII score to arrive at a relative FAII rating:

$$FAII (obs) / FAII (exp.) \times 100$$

**Fish Response Assessment Index (FRAI)**

The determination and description of the present ecological status (PES) of the aquatic ecosystems in the study area, in terms of fish, was done according to the methodology described for River EcoClassification during Reserve Determinations (Kleynhans & Louw, 2008) using the Fish Response Assessment Index (FRAI) (Kleynhans, 2008). The results were then used to classify the present state of the fish assemblage into a specific descriptive category (A to F) (Table A1).

The FRAI is not in its conventional form designed for the application per site, but rather to a reach with a few sites. Metrics are therefore based on spatial frequency of occurrence of a species within the reach.

Table A1: Descriptive categories used to describe the present ecological status (PES) of biotic components (adapted from Kleynhans, 1999).

CATEGORY	BIOTIC INTEGRITY	DESCRIPTION OF GENERALLY EXPECTED CONDITIONS
A	Excellent	Unmodified, or approximates natural conditions closely. The biotic assemblages compares to that expected under natural, unperturbed conditions.
B	Good	Largely natural with few modifications. A change in community characteristics may have taken place but species richness and presence of intolerant species indicate little modifications. Most aspects of the biotic assemblage as expected under natural unperturbed conditions.
C	Fair	Moderately modified. A lower than expected species richness and presence of most intolerant species. Most of the characteristics of the biotic assemblages have been moderately modified from its naturally expected condition. Some impairment of health may be evident at the lower end of this class.
D	Poor	Largely modified. A clearly lower than expected species richness and absence or much lowered presence of intolerant and moderately intolerant species. Most characteristics of the biotic assemblages have been largely modified from its naturally expected condition. Impairment of health may become evident at the lower end of this class.
E	Very Poor	Seriously modified. A strikingly lower than expected species richness and general absence of intolerant and moderately tolerant species. Most of the characteristics of the biotic assemblages have been seriously modified from its naturally expected condition. Impairment of health may become very evident.
F	Critical	Critically modified. Extremely lowered species richness and an absence of intolerant and moderately tolerant species. Only intolerant species may be present with complete loss of species at the lower end of the class. Most of the characteristics of the biotic assemblages have been critically modified from its naturally expected conditions. Impairment of health generally very evident.

It must be emphasized that the A→F scale represents a continuum, and that the boundaries between categories are notional, artificially-defined points along the continuum (as presented below). This situation falls within the concept of a fuzzy boundary, where a particular entity may potentially have membership of both classes (Robertson *et al.* 2004). For practical purposes, these situations are referred to as boundary categories and are denoted as B/C, C/D, and so on.



**Appendix 2: Site photos of biomonitoring sites (last two surveys)**





Plate 9: Upstream view of KFD (2018-05)



Plate 10: Downstream view of KFD (2018-05)



Plate 11: Upstream view of KFD (2018-10)

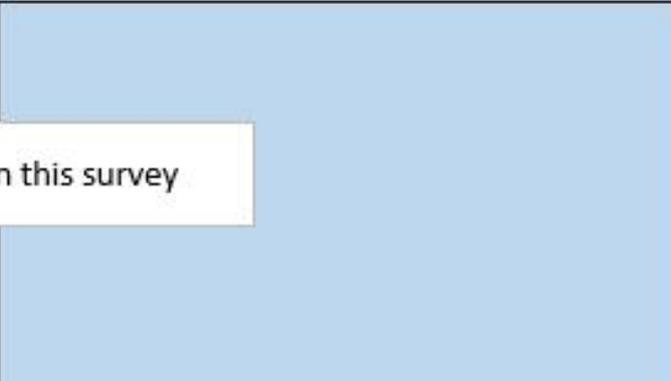


Plate 12: Downstream view of KFD (2018-10)

Not included in this survey



Plate 13: Upstream view of H-DS-KF (2018-05)



Plate 14: Downstream view of H-DS-KF (2018-05)



Plate 15: Upstream view of H-DS-KF (2018-10)



Plate 16: Downstream view of site H-DS-KF (2018-10)



Plate 16: Upstream view of Hex03 (2018-05)



Plate 17: Downstream view of Hex03 (2018-05)



Plate 18: Upstream view of Hex03 (2018-10)



Plate 19: Downstream view of site Hex03 (2018-10)



Plate 20: Upstream view of KGT (2018-05)



Plate 21: Downstream view of KGT (2018-05)



Plate 22: Upstream view of KGT (2018-10)



Plate 23: Downstream view of site KGT (2018-10)

Not included in this survey

Plate 24: Upstream view of Hex03B (2018-05)

Plate 25: Downstream view of Hex03B (2018-05)



Plate 26: Upstream view of Hex03B (2018-10)



Plate 27: Downstream view of site Hex03B (2018-10)

### Appendix 3: Tables

**Table A1: SASS5 analysis including macro-invertebrate families sampled and habitat suitability scores calculated for the various sites (December 2018).**

Taxon	H-US-KF				KF				H-DS-KF				Hex-03				Hex-03-B			
	Stones	Veg	GSM	Total	Stones	Veg	GSM	Total	Stones	Veg	GSM	Total	Stones	Veg	GSM	Total	Stones	Veg	GSM	Total
TURBELLARIA	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-
Oligochaeta	B	-	A	B	-	-	A	A	-	-	B	B	A	1	A	A	A	-	-	A
Leeches	A	A	A	B	-	-	-	-	-	-	-	-	-	1	-	1	A	-	A	A
Baetidae 1 sp.	-	1	A	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coenagrionidae	-	1	-	1	A	B	-	B	-	A	1	A	-	-	-	-	A	B	-	B
Aeshnidae	-	-	-	-	-	A	-	A	-	-	-	-	-	-	-	-	-	-	-	-
Libellulidae	-	-	-	-	1	B	-	B	-	-	-	-	-	-	-	-	-	-	-	-
Belostomatidae*	-	-	-	-	-	B	-	B	-	-	-	-	-	-	-	-	-	-	-	-
Corixidae*	B	B	A	B	B	B	B	B	B	B	B	B	1	1	1	A	-	-	-	-
Gerridae*	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Naucoridae*	-	1	-	1	-	-	-	-	-	A	-	A	-	-	-	-	-	-	-	-
Notonectidae*	-	-	-	-	-	-	-	-	-	A	-	A	-	-	-	-	-	-	-	-
Pleidae*	-	-	-	-	-	A	-	A	-	-	-	-	-	-	-	-	-	-	-	-
Veliidae*	-	-	-	-	-	A	-	A	-	-	-	-	-	-	-	-	-	-	-	-
Hydropsychidae 1sp.	-	B	-	B	-	-	-	-	B	-	-	B	-	-	-	-	A	-	-	A
Hydroptilidae	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Leptoceridae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
Dytiscidae (adults*)	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-
Gyrinidae (adults*)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Chironomidae	-	-	B	B	B	B	B	C	A	B	B	B	B	B	B	B	A	A	B	B
Culicidae*	-	1	-	1	-	A	-	A	-	-	-	-	-	-	-	-	-	-	-	-
Muscidae	-	-	-	-	1	A	-	A	-	-	-	-	-	-	-	-	1	-	-	1
Simuliidae	-	B	B	B	B	A	-	B	A	-	-	A	A	A	1	B	B	B	-	C
Ancylidae	-	A	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Physidae*	-	A	A	A	-	-	-	-	-	-	-	-	-	-	-	-	B	B	B	B
Total SASS5 score	7	46	21	49	19	45	6	46	19	22	10	37	11	14	11	14	28	20	8	34
No. of families	3	11	7	13	6	12	3	13	5	6	4	10	4	5	4	5	9	5	3	10
ASPT	2.33	4.18	3.00	3.77	3.17	3.75	2.00	3.54	3.80	3.67	2.50	3.70	2.75	2.80	2.75	2.80	3.11	4.00	2.67	3.40
Total IHAS				69				75				52				69				73
IHAS - Habs sampled				36				40				30				36				39
IHAS - Stream condition				33				35				22				33				34
Suitability score	3	6	3	12	5	11	3	19	6	5	3	14	4	9	6	19	0	0	0	0

Key: High requirement for unmodified water quality Veg=Vegetation

Moderate requirement for unmodified water quality

Low requirement for unmodified water quality

Very low requirement for unmodified water quality

A = 1-10 individuals; B = 11-100 individuals; C = 101-1000 individuals; ASPT = Average score per taxon.

**Table A3: Fish Assemblage Integrity Index (FAII) scores calculated for the various sampling sites (2017-2018).**

SPECIES	Intolerance rating					Health rating					SCORE				
	HEX00	HEX01	HEX03	HEX04	HEX4B	HEX00	HEX01	HEX03	HEX04	HEX4B	HEX00	HEX01	HEX03	HEX04	HEX4B
<i>Amphillius uranoscopus</i>	4.8	4.8	4.8	4.8	4.8	5	5	5	5	5	24.0	24.0	24.0	24.0	24.0
<i>Barbus paludinosus</i>	1.8	1.8	1.8	1.8	1.8	5	5	5	5	5	9.0	9.0	9.0	9.0	9.0
<i>Barbus trimaculatus</i>	2.2	2.2	2.2	2.2	2.2	5	5	5	5	5	11.0	11.0	11.0	11.0	11.0
<i>Barbus unitaeniatus</i>		1.7	1.7	1.7	1.7		5	5	5	5	0.0	8.5	8.5	8.5	8.5
<i>Chiloglanis pretoriae</i>				4.6	4.6				5	5	0.0	0.0	0.0	23.0	23.0
<i>Clarias gariepinus</i>	1.2	1.2	1.2	1.2	1.2	5	5	5	5	5	6.0	6.0	6.0	6.0	6.0
<i>Labeobarbus marequensis</i>	2.6	2.6	2.6	2.6	2.6	5	5	5	5	5	13.0	13.0	13.0	13.0	13.0
<i>Labeo cylindricus</i>				3.1	3.1				5	5	0.0	0.0	0.0	15.5	15.5
<i>Labeo molybdinus</i>				3.2	3.2				5	5	0.0	0.0	0.0	16.0	16.0
<i>Mesobola brevianalis</i>	2.3	2.3	2.3	2.3	2.3	5	5	5	5	5	11.5	11.5	11.5	11.5	11.5
<i>Oreochromis mossambicus</i>	1.3	1.3	1.3	1.3	1.3	5	5	5	5	5	6.5	6.5	6.5	6.5	6.5
<i>Pseudocrenilabrus philander</i>	1.3	1.3	1.3	1.3	1.3	5	5	5	5	5	6.5	6.5	6.5	6.5	6.5
<i>Tilapia sparrmanii</i>	1.3	1.3	1.3	1.3	1.3	5	5	5	5	5	6.5	6.5	6.5	6.5	6.5
	<b>Total Expected</b>										<b>94.0</b>	<b>102.5</b>	<b>102.5</b>	<b>157.0</b>	<b>157.0</b>
<i>Amphillius uranoscopus</i>											0.0	0.0	0.0	0.0	0.0
<i>Barbus paludinosus</i>	1.8	1.8	1.8	1.8	1.8	5	5	5	5	5	9.0	9.0	9.0	9.0	9.0
<i>Barbus trimaculatus</i>			2.2					5			0.0	0.0	11.0	0.0	0.0
<i>Barbus unitaeniatus</i>			1.7					5			0.0	0.0	8.5	0.0	0.0
<i>Chiloglanis pretoriae</i>											0.0	0.0	0.0	0.0	0.0
<i>Clarias gariepinus</i>		1.2	1.2	1.2	1.2		5	5	5	5	0.0	6.0	6.0	6.0	6.0
<i>Labeobarbus marequensis</i>											0.0	0.0	0.0	0.0	0.0
<i>Labeo cylindricus</i>											0.0	0.0	0.0	0.0	0.0
<i>Labeo molybdinus</i>											0.0	0.0	0.0	0.0	0.0
<i>Mesobola brevianalis</i>											0.0	0.0	0.0	0.0	0.0
<i>Oreochromis mossambicus</i>			1.3	1.3	1.3			5	5	5	0.0	0.0	6.5	6.5	6.5
<i>Pseudocrenilabrus philander</i>	1.3	1.3	1.3	1.3	1.3	5	5	5	5	5	6.5	6.5	6.5	6.5	6.5
<i>Tilapia sparrmanii</i>	1.3	1.3		1.3	1.3	5	5		5	5	6.5	6.5	0.0	6.5	6.5
	<b>Total Observed</b>										<b>22.0</b>	<b>28.0</b>	<b>47.5</b>	<b>34.5</b>	<b>34.5</b>
	<b>Relative FAII (%)</b>										<b>23</b>	<b>27</b>	<b>46</b>	<b>22</b>	<b>22</b>

**END OF REPORT**

**Addendum 1: Toxicity test report/s (Biotox Laboratory Services)**

Submitted as separate PDF document/s

Report reference: AAPL/A/19

**Prepared by:**

BH Niehaus  
*Pri.Sci.Nat.* SACNASP 4000080/13  
Clean Stream Biological Services



**Report released:**

2019-08-06

**Tel:** 012-753-2192/3

**Fax:** 086-535-7368

**Email:** [brenton@cleanstream-bio.co.za](mailto:brenton@cleanstream-bio.co.za)

---

**AMGLO AMERICAN PLATINUM:  
HEX RIVER CATCHMENT  
BIOMONITORING PROGRAMME**

---

**JUNE 2019 SURVEY**

## TABLE OF CONTENTS

1. INTRODUCTION.....	3
2. MATERIALS & METHODS .....	4
3. RESULTS & DISCUSSION.....	4
3.1 Study area .....	4
3.2 <i>In-situ</i> water quality (June 2019) .....	7
3.3 Toxicity testing .....	10
3.3.1 May 2019 and June 2019 .....	12
3.3.2 Temporal variation of toxicity results (2008 to 2019) .....	14
3.4 Aquatic invertebrate assessment: South African Scoring System 5.....	16
3.5 Fish Assessment.....	22
4 CONCLUSIONS AND RECOMMENDATIONS.....	28
5 REFERENCES .....	30
Appendix 1: Methodology applied during this biomonitoring assessment.....	31
Appendix 2: Site photos of biomonitoring sites (last two surveys) .....	34
Appendix 3: Tables.....	38
END OF REPORT .....	39
Addendum 1: Toxicity test report/s (Biotox Laboratory Services).....	40

## LIST OF TABLES

Table 1: Biomonitoring surveys conducted and reports compiled in the period December 1999 to June 2019. ....	3
Table 2: Latitude/Longitude and sampling protocols of selected sampling sites for routine biomonitoring. ....	5
Table 3: <i>In-situ</i> water quality variables measured at the time of sampling at the selected biomonitoring sites .....	7
Table 4: Toxicity results and hazard classification for selected pollution facilities (May 2019). ....	13
Table 5: Toxicity results and hazard classification for selected Hex River tributary samples (June 2019). ....	14
Table 6: Integrated Habitat Assessment (IHAS) description of the different biomonitoring sites. ....	17
Table 7: SASS5, ASPT and habitat suitability/availability index scores for different monitoring sites (June 2019). ....	18
Table 8: Fish species expected and observed during the last two surveys.....	23
Table 9: Relative FAIL scores calculated at different sampling sites (2017 to 2018). ....	24
Table 10: The relative tolerance of each species towards changes in the environment. ....	25
Table 11: Fish Response Assessment Index (FRAI) results for the Hex River reach (all sites) (2017/8 results). ....	26
Table 12: Descriptive categories used to describe the present ecological status (PES) of biotic components (adapted from Kleynhans, 1999). ....	27

## LIST OF FIGURES

Figure 1: Google Earth image of study area, indicating Hex River and tributary biomonitoring sites. ....	6
Figure 2: Electrical conductivity levels (mS/m) at the time of sampling at the different biomonitoring sites.....	7
Figure 3: pH levels at the time of sampling at the different biomonitoring sites. ....	8
Figure 4: Dissolved oxygen levels (mg/l) at the time of sampling at the different biomonitoring sites. ....	9
Figure 5: Temporal trends of toxicity results (annually tested PCD's and selected streams).....	15
Figure 6: Temporal trends of toxicity results (bi-annually tested tributaries). ....	16
Figure 7: ASPT, SASS5 and total habitat suitability scores at biomonitoring sites during June 2019. ....	18
Figure 8: Linear regression of biotic integrity (as indicated by invertebrate ASPT scores) of the Hex River on a spatial scale (arranged sequentially in a downstream direction) during May 2018 (extended study area). ....	20
Figure 9: Linear regression of biotic integrity (as indicated by invertebrate ASPT scores) of the Hex River on a spatial scale (arranged sequentially in a downstream direction) during June 2019 (reduced study area). ....	20
Figure 10: Long-term trends of biotic integrity in terms of macro-invertebrates at biomonitoring sites. ....	21
Figure 11: Medium-term trends of biotic integrity in terms of macro-invertebrates at biomonitoring sites. ....	21
Figure 12: Relative FAIL scores, HCR's and SHI at the different biomonitoring sites.....	24

## 1. INTRODUCTION

This report is based on the results of the bi-annual biomonitoring survey conducted during June 2019 on the selected sites in the Hex River, Klipfonteinspruit and Klipgatspruit in the Anglo American Platinum (Rustenburg) mining area. Since the sale to Sibanye Stillwater, the study area assigned to Clean Stream Biological Services for biomonitoring has decreased considerably. To avoid confusion with areas tasked by Sibanye Stillwater to other consulting firm/s, the client for the purpose of this report will be referred to as Process Division Services. This long-term monitoring program commenced during December 1999. A comprehensive 20-year temporal database pertaining to the health of aquatic communities, as well as the water quality environment that may be affected by the RPM operations, has been amassed. This continuity of information will be invaluable for any future assessments of impacts to the receiving environment. RPM has thereby diligently maintained their biomonitoring programme on a twice-per-annum schedule (at least) since the inception of the program during 1999. See Table 1 below for a list of surveys performed, with their corresponding report numbers. Report naming will henceforth include the lettering sequence of "AAPL", referring to Anglo American Platinum and in specific the Process Division Services.

Table 1: Biomonitoring surveys conducted and reports compiled in the period December 1999 to June 2019.

Year	Month	Report numbers
1999	December	CS-A-2000
2000	April, July and November	CS-G-2000, CS-K-2000 and CS-A-2001
2001	May and September	CS-H-2001 and CS-L-2001
2002	February, May, August and November	CS-G-2002, CS-I-2002, CS-N-2002 and CS-E 2003
2003	Januaury and May	CS-G2003 and CS-O-2003
2004	April, August and October	CS-H-2004 and AMP-A-05
2005	February, April and November	AMP-B-05, AMP-C-05 and AMP-D-05
2006	April and November	AMP-A-06, AMP-C-06
2007	April and October	ANP-A-07 and ANP-A-08
2008	April and October	ANP-B-08 and ANP-A-09
2009	April and October	ANP-B-09 and RPM-A-09
2009	April and October	RPM-A-10 and RPM-B-10
2011	April and October	RPM-A-11 and RPM-B-11
2012	April and November	RPM-A-12 and RPM-A-13
2013	April and October	RPM-B-13 and RPM-C-13
2014	April and October	RPM-A-14 and RPM-B-14
2015	April and October	RPM-A-15 and RPM-C-15
2016	May and October	RPM-A-16-Ver2 and RPM-B-16
2017	June and November	RPM-A-17 and RPM-B-17
2018	May and October	RPM-A-18 and AAPL-A-18
2019	June	AAPL-A-19

Rivers are continuum systems, so a river reach can be influenced by activities both upstream and downstream. Pollution incidences upstream of a site will have a negative impact, not only locally, but on the entire ecosystem (depending on the extent of the pollution).

Biological communities reflect overall ecological integrity by integrating different stressors over time, thereby providing a broad measure of their aggregate impact. The monitoring of biological communities hence

provides a reliable ecological measure of fluctuating environmental conditions. The biomonitoring protocols applied in this project should give a good reflection of the human impacts on the system under investigation.

The results contained in this report should firstly be interpreted as **spatial** impact monitoring. [Note that spatial impact monitoring in terms of the fish communities considers the last two fish surveys, and not only the last survey, as in the case of macro-invertebrate communities]. **Temporal** (long- and medium-term trends) impact monitoring is also performed and considers all of the data since 2002 (after initial project design and refinement of the biomonitoring programme between 1999 and 2001).

## 2. MATERIALS & METHODS

Refer to appendix 1 for a description of methodology applied during this assessment.

## 3. RESULTS & DISCUSSION

### 3.1 Study area

Biomonitoring sites were selected to be easily accessible and representative of as many habitats as possible. Four biomonitoring sites were selected within the Hex River.

The criteria for site selection are as follows:

- The locations should ideally be selected to be both upstream and downstream of potential pollution sources, and as far as possible, exclude other potential impacts not related to the biomonitoring programme (non-AAPL impacts).
- The habitat diversity should be representative of the river/stream being monitored and, as far as possible, be comparable between sites on a spatial scale.
- The habitats should, as far as possible, be suitable for the application of the selected biomonitoring protocols.
- The sampling sites should at least be accessible to off-road vehicle to enable the transport of the required sampling equipment.

Although sampling sites were previously selected to isolate potential and known RPM (Sibanye Stillwater) impacts and hence measure the effect of RPM impacts on the biotic integrity of the receiving water bodies, several additional sites were also selected to illustrate the potential impact of non-RPM related activities. This was done to gain an insight into other potential impacts on the Hex River, in the area upstream of RPM activities as well as up- and downstream of the Klipfonteinspruit (not including the Paardekraal Angling Dam) to isolate the potential impact/s from the Klipfonteinspruit and the Dorpspruit catchment. This approach has now been adapted (since 2018-10) to mainly focus on the possible impact of the AAPL Process Division Services, with possible impacts reaching the final receiving water body (Hex River), via the Klipfonteinspruit and the Klipgatspruit (Table 2; Figure 1).

Various sites/samples were selected for toxicity testing. These include pollution control dams and the Klipfonteinspruit and Klipgatspruit tributaries (included since April 2012 but narrowed down to the two mentioned tributaries since October 2018) joining the Hex River within the study area. Based on the historic electrical conductivity (EC) results (illustrating cumulative water quality deterioration from various sources) and spatial variation in biotic integrity, it is evident that the various tributaries of the Hex River, both upstream and within the newly-refined study area contribute significantly to the spatial variation in ecological integrity of the Hex River. It is therefore of great value for the biomonitoring programme to include DEEEP toxicity testing on all the key tributaries entering the Hex River.

See Table 2 below for sampling site description, its relation to AAPL Process Division Services activities and the frequency of different biomonitoring protocols applied.

Table 2: Latitude/Longitude and sampling protocols of selected sampling sites for routine biomonitoring.

Monitoring site	Description	Potential direct upstream impact	Biomonitoring protocols		GPS coordinates (degrees)	
			Protocol	Frequency per annum	Latitude (South)	Longitude (East)
H-US-KF	Hex River, upstream from the Klipfonteinspruit confluence but downstream from the Paardekraal Angling Dam.	AAPL and non-AAPL	*SASS5	Twice	25.6492	27.2906
KF	Klipfonteinspruit, downstream from Waterfall concentrator but upstream from Paardekraal shaft runoff.	AAPL	Toxicity testing	Twice	25.6578	27.2964
KFD	Klipfonteinspruit, downstream from site KF and the Paardekraal shaft runoff.	AAPL	Toxicity testing	Twice	25.6496	27.2926
H-DS-KF	Hex River, directly downstream from the Klipfonteinspruit confluence.	AAPL	*SASS5	Twice	25.6473	27.2913
Hex03	Hex River, upstream from Klipgatspruit.	Non-AAPL	*SASS5	Twice	25.6332	27.2903
			**FAII	Once		
KGT	Klipgatspruit, downstream from tailings complex seepage.	AAPL	Toxicity testing	Twice	25.6319	27.2951
Hex3B	Hex River, downstream from Klipgatspruit confluence. Newly adopted site (since 2018-10)	AAPL	*SASS5	Twice	25.6237	27.2900
			**FAII	Once		
K209	PMR Dam 2	To be confirmed as per AAPL Process Division requirements				
K210	PMR Dam 3A					
K211	PMR Dam 3B					
K212	PMR Dams 4 and 5					
K213	PMR Dam 6					
K194						
K160	RBMR Dam 3A					
K161	RBMR Dam 3B					
K162	RBMR Triangular Dam					
K035	Klipgat RWD					
K098	ACP PCD					

**Key:** \* SASS5 = South African Scoring System, version5 (macro-invertebrate index and associated habitat assessment indices i.e. IHAS ver2 and biotope availability and suitability indices)

\*\* FAII = Fish Assemblage Integrity Index (and associated habitat indices i.e. SHI and HCR)

Site name shaded green = Hex River mainstem	Site name shaded blue = Tributary of Hex River	Site name shaded red = Toxicity testing
Impact shaded gray = Potential RPM and non-RPM impacts (directly upstream)	Impact shaded pink = Potentially impacted by RPM/AAPL (directly upstream)	Impact shaded yellow = No RPM/AAPL impacts (directly upstream)
Site name shaded Orange = Discontinued from Clean Stream Biological Services scope		



### 3.2 In-situ water quality (June 2019)

Selected water quality variables were measured on-site at the time of biological sampling. The purpose of these measurements is to assist in the interpretation of biological results (refer to Aquatico Scientific's Water Quality Report for a detailed water quality assessment of the Rustenburg Platinum mining area).

As recorded during most surveys, the EC increased from site H-US-KF to H-DS-KF in the Hex River (119.4 mS/m to 142.0 mS/m) (Table 3; Figure 2). The Klipfonteinspruit joins the Hex River between these sites and probably played a large role in the increased salinity during most preceding surveys (no perceptible surface flow was recorded from the Klipfonteinspruit during many surveys, but a subsurface contribution of affected mine water cannot be ruled out). The EC value was high in the Klipfonteinspruit sites KF (565.0 mS/m) and KFD (765.0 mS/m), as during most previous surveys, again confirming this tributary as a potential source of elevated salinity levels, especially during periods of flow (see section 3.3).

Table 3: In-situ water quality variables measured at the time of sampling at the selected biomonitoring sites

Monitoring site	EC (mS/m)	pH	Oxygen saturation (%)	Dissolved oxygen (mg/l)	Water temp (°C)	Turbidity (visual)	Flow (visual)	Time	Date
H-US-KF	119.4	7.2	40.5	3.3	14.8	Slightly turbid	Moderate	15:30	18/06/2019
H-DS-KF	142.0	7.4	73.9	6.0	14.5	Clear	Moderate	12:45	19/06/2019
HEX03	139.5	7.4	53.4	4.5	13.1	Slightly turbid	Low	10:30	19/06/2019
HEX3B	26.4	7.1	63.5	5.5	12.7	Slightly turbid	Moderate	10:02	19/06/2019

Value outside general guideline.

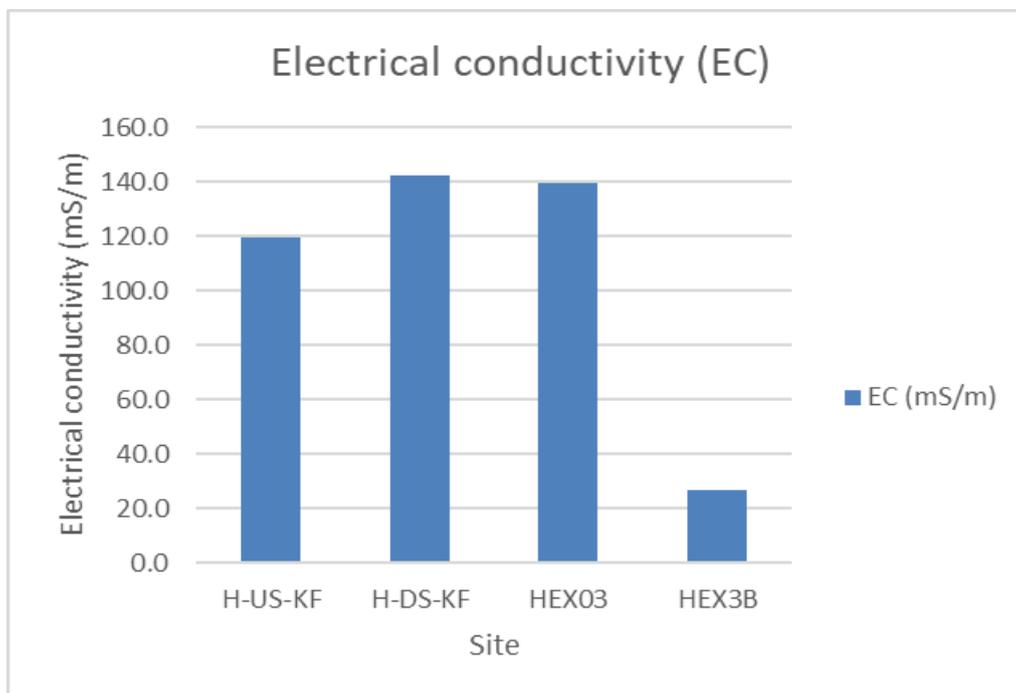


Figure 2: Electrical conductivity levels (mS/m) at the time of sampling at the different biomonitoring sites.

The EC level remained stable from site H-DS-KF (142.0 mS/m) to Hex03 (139.5 mS/m) (Table 3; Figure 2), therefore not indicating on impacts from non-Anglo Platinum Process Division (APPD) activities on the salinity of the Hex River at the time of sampling. This contrasts with the previous survey that showed an increase in salinity from site H-DS-KF to Hex03 (see report AAPL-A-18).

From site Hex03 towards site Hex03B (the most downstream site), the EC values decreased notably (195.2 mS/m to 26.4 mS/m) (Table 3; Figure 2), thus indicating that the contribution from the Klipgatspruit (dry at the time of sampling) did not lead to an increase in the salinity of the receiving environment.

The pH fell within the target water quality ranges for fish health (Aquaculture), which is between 6.5 and 9.0, at all sites during June 2019. It is expected that most aquatic species will tolerate and reproduce successfully within this pH range (DWAF, 1996), and the pH values recorded should therefore not be limiting to aquatic biota.

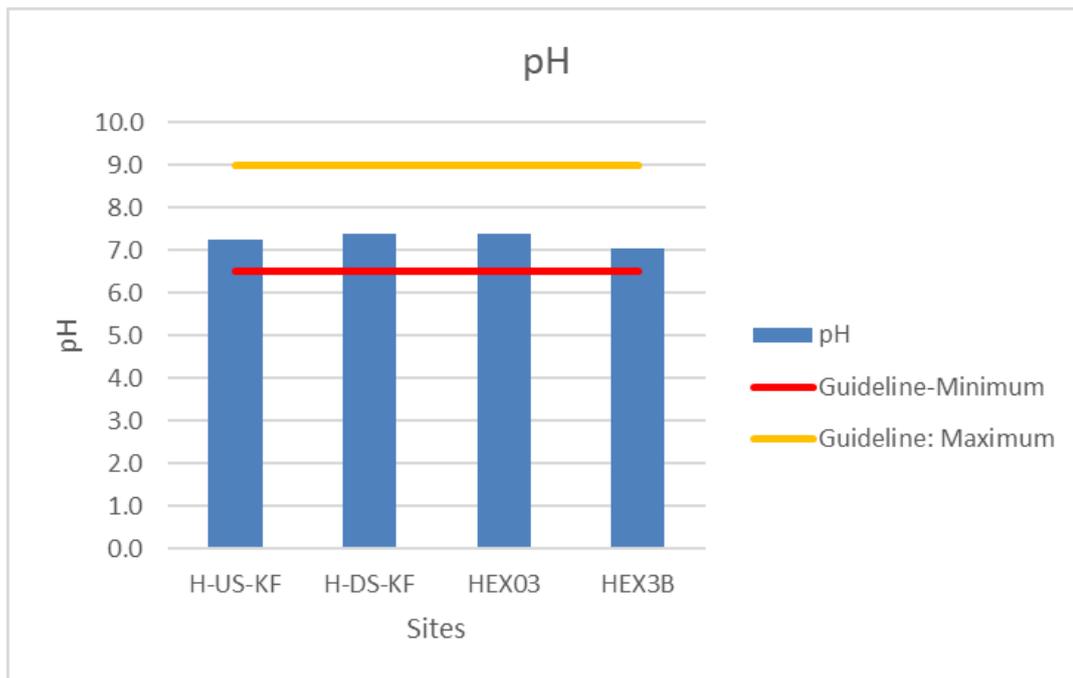


Figure 3: pH levels at the time of sampling at the different biomonitoring sites.

During June 2019, the dissolved oxygen level fell below the target range (> 5 mg/l, as set by Kempster *et al.*, 1980) at site H-US-KF (3.3 mg/l) (Table 3; Figure 4). These low levels of dissolved oxygen will be limiting to aquatic biota, particularly if persistent or frequently occurring.

As during the previous (October 2018) and many preceding surveys, dissolved oxygen levels were again below the target range at site Hex03 (4.5 mg/l) during the present survey (Table 3; Figure 4). The noted low oxygen levels could therefore possibly have posed a risk to aquatic biota and was probably related to a combination of factors including:

- Elevation and accumulation of organic loads,
- Aquatic vegetation and algal proliferation in response to eutrophication,
- Low flow (all affected sites).

It has to be noted that the cause of lowered dissolved oxygen levels is unlikely to be related to APPD activities because levels were within the guideline at site H-DS-KF and no further APPD activities take place toward site Hex03. Dissolved oxygen levels improved to above the target range towards site Hex03B (5.5 mg/l) (Table 3; Figure 4) and should therefore not be limiting to aquatic biota at this site.

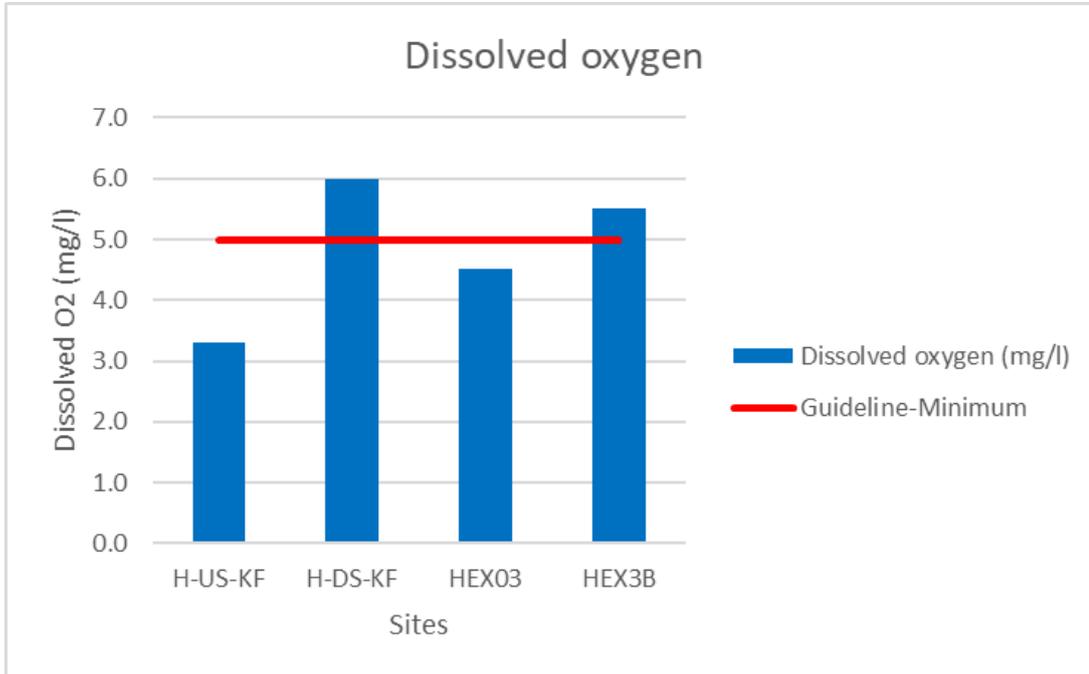


Figure 4: Dissolved oxygen levels (mg/l) at the time of sampling at the different biomonitoring sites.

As a standard management procedure, a full statistical evaluation of water quality data at these biomonitoring localities (as performed by Aquatico Scientific) will be required to conclude whether specific variables or a combination of variables, not included in the biota specific range, are impacting on the aquatic ecosystems.

### 3.3 Toxicity testing

At the time of compiling this biomonitoring report, the latest toxicity testing results for the Pollution Control Facilities available to Clean Stream Biological Services were based on the May 2019 dataset, as submitted as a separate toxicity testing report by Biotox Laboratory Services (Report no. RPM-A-19\_TOX).

The latest tributary toxicity testing report, as performed in conjunction with the June 2019 biomonitoring survey, is also included and discussed in this biomonitoring report.

As per SANAS requirements, the above-mentioned toxicity testing reports were produced independently by Biotox Laboratory Services (Pty) Ltd. All results contained in this section are therefore sourced from the Biotox reports, which are included in Addendum 1.

Toxicity testing (as conducted in this biomonitoring programme) is applied by exposing biota under laboratory conditions to water sources (pollution control dams, effluent streams or streams/rivers) to accurately determine the risk of such water types to the biota of the receiving water bodies. Toxicity results indicate the risk posed to the Hex River and its tributaries in the event of release, seepage or overflow from possible sources of pollution. Up to four trophic levels (at least 3, including acute and chronic approaches) of biota, *i.e.*, vertebrates (*Poecilia reticulata*), invertebrates (*Daphnia magna*), bacteria (*Allivibrio fischeri*) and primary producers (*Selenastrum capricornutum*) are exposed to the samples per standard procedures under laboratory conditions and thereafter a risk/hazard category is determined by application of the latest **DEEEP**<sup>1</sup> DWS recommended protocols and hazard classification. The final risk classification is expressed in terms of **acute**<sup>2</sup> and **chronic**<sup>3</sup> toxicity risk. The *Poecilia reticulata* and *Daphnia magna* test results are based on mortality rates over a relatively short period of the lifespan of the organisms, hence allowing for acute interpretation. *Selenastrum capricornutum* and *Vibrio fischeri* individual test results are based on inhibition rates over relatively long periods of the lifespan of the organisms, hence allowing for short-chronic toxicity hazard interpretation.

Selected toxicity samples (Hex River tributaries) were tested on a twice per annum schedule, while the PCD (pollution control dam) samples are tested once per annum, on either a **screening**<sup>4</sup> acute level or a **definitive**<sup>5</sup> acute level, at this stage. The frequency of testing is informed by the level of toxicity. If toxicity levels increase, it may become relevant and useful to increase the frequency of testing. The frequency and type of toxicity testing required (screening vs. definitive) should be revised from time to time based on the outcome of the specific year's assessments.

<sup>1</sup> DEEEP = Direct Estimation of Ecological Effect Potential. This is a battery of tests that can measure toxicity of complex mixtures based on a set of parameters stemming from the results of effects, even if all constituents are not known. Thereafter a hazard class is determined based on the resulting parameters of the battery of tests.

<sup>2</sup> Acute = Acute refers to an exposure over a relatively short period of the lifespan of biota, of which the result is generally based on mortality rates.

<sup>3</sup> Chronic = Chronic refers to prolonged exposures over an extended period of the lifespan of test organisms, of which the results are generally based on growth inhibition rates.

<sup>4</sup> Screening = A screening toxicity test refers to an undiluted (100% concentration) sample. This is usually performed on a sample from the biomonitoring sites in the receiving water bodies (river/streams) to determine if any toxicity is present. This is performed both up- and downstream of the potential impacts to enable the determination of downstream increases or decreases in toxicity.

<sup>5</sup> Definitive = A definitive toxicity test refers to the exposure of test organisms to both the 100% concentration as well as a range of dilutions, generally used to determine the risk of a pollution source that may have a toxicity effect on the receiving water body (such as effluents and PCD's). The range of dilutions are therefore useful in the event that the 100% sample concentration presents acute toxicity, and allows for the determination of a safe dilution factor, to negate toxicity effects on the receiving water bodies.

## Hazard classification for screening tests (undiluted samples)

After the determination of the percentage effect<sup>6</sup> (EP), obtained with each of the **battery of toxicity screening** tests performed, the sample is ranked into one of the following five classes:

### Hazard classification system for screening tests

Class I	No acute/short-chronic environmental toxicity hazard - none of the tests shows a toxic effect (i.e. an effect value significantly higher than that in the control)
Class II	Slight acute/short-chronic environmental toxicity hazard - a statistically significant ( $P < 0,05$ ) percentage effect is reached in at least one test, but the effect level is below 50%
Class III	Acute/short-chronic environmental toxicity hazard - the percentage effect level is reached or exceeded in at least one test, but the effect level is 50-99%
Class IV	High acute/short-chronic environmental toxicity hazard - the 100% percentage effect is reached in at least one test
Class V	Very high acute/short-chronic environmental toxicity hazard - the 100% percentage effect is reached in all the tests

**Weighting:** Each sample is furthermore weighted according to its relative toxicity levels (out of 100%). Higher values indicate that more of the individual tests indicated toxicity within a specific class.

## Toxicity classification system definitive tests (undiluted samples plus range of dilutions)

The samples are classified into one of the following five classes on the basis of the highest toxicity unit (TUa) found in the **battery of toxicity definitive tests** performed. The toxicity unit is a function of the L(E)C50, where  $(TUa) = 100/L(E)C50$ . The 50% Lethal/Effective concentration (LC50 or LE50) is the linear calculated (derived) concentration at which a 50% mortality or inhibition rate can be expected. Hence, the lower this value is, the higher the acute toxicity level. Conversely, the higher the toxicity unit (TUa) is, the higher the acute toxicity level is. The conversion of L(E)C50 values to TUa values is therefore merely done to achieve a classification scale of increasing values related to increasing toxicity risk:

### Hazard classification system for definitive tests

Class I	No acute/short-chronic environmental toxicity hazard - none of the tests shows a toxic effect (i.e. an effect value significantly higher than that in the control)
Class II	Slight acute/short-chronic environmental toxicity hazard - the percentage effect observed in at least one toxicity test is significantly higher than in the control, but the effect level is below 50% ( $TU < 1$ )
Class III	Acute/short-chronic environmental toxicity hazard - the L(E)C50 is reached or exceeded in at least one test, but in the 10 fold dilution of the sample the effect level is less than 50% ( $1 \leq TU \leq 9,99$ )
Class IV	High acute/short-chronic environmental toxicity hazard - the L(E)C50 is reached in the 10 fold dilution for at least one test, but not in the 100 fold dilution ( $10 \leq TU \leq 99,99$ )
Class V	Very high acute/short-chronic environmental toxicity hazard - the L(E)C50 is reached in the 100 fold dilution for at least one test ( $TU \geq 100$ )

**Note:**

The samples are classified into one of the above five classes on the basis of the highest toxicity unit (TU) found in the battery of toxicity definitive tests performed

**Weighting:** Each sample is furthermore weighted according to its relative toxicity levels (out of 100%). Higher values indicate that more of the individual tests indicated toxicity within a specific class.

<sup>6</sup> EP (Percentage effect) = an effect measured either as a mortality rate or inhibition rate (depending on the type of test). A 10% effect is regarded as a slight acute toxicity for daphnia and guppies, while a 20% effect is regarded as a slight acute toxicity for algae and bacteria (vibrio). A 50% effect is regarded as an acute toxicity for all of the tests (daphnia, guppies, algae and bacteria)

### 3.3.1 May 2019 and June 2019

#### Toxicity testing of pollution control facilities (May 2019 only)

Various toxicity hazards were identified during the May 2019 survey, ranging from “no acute/short-chronic environmental toxicity hazard” (Class I) to “very high acute/short-chronic environmental toxicity hazard” (Class V), implying that some effluents/potential effluents could pose a serious risk to the receiving water bodies if released (Table 4).

Sample K035 (Klipgat Dam) was tested as posing “no acute/short-chronic environmental toxicity hazard” (Class I) using the normal range of dilutions at a macro-invertebrate level (Table 4). **However, from a 0.1% dilution level, significant mortalities (100%) were noted potentially indicating sources of pollution affecting organisms more severely at lower concentration, e.g. nano materials, and further testing is recommended (see Addendum 1).**

Sample K211 (PMR Dam 3B) displayed a “slight acute/short-chronic hazard” (Class II) based on the 31% bacterial light emission inhibition effect noted during testing (Table 4). A safe dilution factor of 89% (ratio of 89 parts K211 water to 11 parts unpolluted water) was calculated for this sample. Sample K210 (PMR Dam 3A) showed an “acute/short chronic hazard” (Class III) based on the highest toxicity unit (2.2) calculated on a bacterial testing level (Table 4). A safe dilution factor of 36% was estimated for this sample.

Samples K098 (ACP Dam), K162 (RBMR Triangular Dam), K212 (PMR Dam 4+5), and K213 (PMR Dam 6E) showed a “high acute/short-chronic hazard” (Class IV), based on the 100% mortality effects on at least one trophic level test and toxicity units ranging from 5.4 - >100 (Table 4). A safe dilution factor of 2% was calculated for samples K098 (ACP Dam) and K162 (RBMR Triangular Dam). Very low safe dilution factors (<1%) were calculated for samples K212 (PMR Dam 4+5), and K213 (PMR Dam 6E) and **water from these facilities should not be allowed to reach the natural environment.**

Samples K160 (RBMR Dam 3A), K161 (RBMR Dam 3B) and K209 (PMR Dam 2) showed a “very high acute/short-chronic environmental toxicity hazard” (Class V) based on the toxicity units >100 calculated for these samples during testing at all 3 trophic levels (Table 4). Very low safe dilution factors (<1%) were calculated for these samples and **water from these facilities should not be allowed to reach the natural environment.** The toxicity effects observed for sample K160 (RBMR Dam 3A) were particularly severe and could not be diluted out (tested to dilutions of 0.195% of original sample).

Table 4: Toxicity results and hazard classification for selected pollution facilities (May 2019).

Results		K035 (Klipgat Dam)	K098 (ACP Dam)	K160 (RBMR Dam 3A)	K161 (RBMR Dam 3B)	K162 (RBMR Triangular Dam)	K209 (PMR Dam 2)	K210 (PMR Dam 3A)	K211 (PMR Dam 3B)	K212 (PMR Dam 4+5)	K213 (PMR Dam 6E)
w <sub>o</sub> Water quality	pH @ 25°C (A)	7.3	2.7	3.2	5.2	10.5	1.2	9.8	9.4	5.5	5.6
	EC (Electrical conductivity) (mS/m) @ 25°C (A)	539.0	186.9	6340.0	3880.0	6090.0	10650.0	42.4	63.7	18940.0	24000.0
	Dissolved oxygen (mg/l) (NA)	9.4	7.5	7.4	7.5	7.3	7.6	8.6	7.8	7.6	7.5
A. fischeri (bacterial) (A)	Test started on yy/mm/dd	19/05/29	19/05/29	19/05/29	19/05/29	19/05/30	19/05/30	19/06/05	19/06/05	19/06/05	19/06/06
	%30min inhibition (-) / stimulation (+) (%)	55	-100	-100	-100	-100	-100	-97	-31	-100	-100
	EC/LC20 (30 mins)	n.r	2	n.c	n.c.	2	n.c	36	89	n.c	n.c
	EC/LC50 (30 mins)	n.r	9	n.c	0.5	3	n.c	45	n.r.	4	3
	Toxicity unit (TU) / Description	<1	11.8	>100	25.6	29.5	>100	2.2	<1	27.1	30.6
D. magna (waterflea) (A)	Test started on yy/mm/dd	19/05/27	19/05/27	19/05/27	19/05/27	19/05/27	19/05/27	19/05/27	19/05/27	19/05/27	19/05/27
	%48hour mortality rate (-%)	-5	-100	-100	-100	-100	-100	-5	0	-100	-100
	EC/LC10 (48hours)	n.r	10	n.c	n.c	2	n.c	n.r	n.r	n.c	n.c
	EC/LC50 (48hours)	n.r	19	n.c	n.c	8	0.3	n.r	n.r	4	3
	Toxicity unit (TU) / Description	<1	5.4	>100	>100	12.9	>100	<1	<1	28.0	32.5
P. reticulata (snail) (A)	Test started on yy/mm/dd	19/05/30	19/05/30	19/05/30	19/05/30	19/05/30	19/05/30	19/05/30	19/05/30	19/05/30	19/05/30
	%96hour mortality rate (-%)	0	-100	-100	-100	-100	-100	-25	0	-100	-100
	EC/LC10 (96hours)	n.r	12	n.c	n.c	10	2	70	n.r	5	2
	EC/LC50 (96hours)	n.r	18	n.c	0.3	17	6	n.r.	n.r	7	6
	Toxicity unit (TU) / Description	<1	5.7	>100	>100	5.9	18.2	<1	<1	14.3	18.2
Estimated safe dilution factor (%) [for definitive testing only]	None required	2	<1	<1	2	<1	36	89	<1	<1	
Overall classification - Hazard class***	Class I - No acute/short-chronic hazard	Class IV - High acute/short-chronic hazard	Class V - Very high acute/short-chronic hazard	Class V - Very high acute/short-chronic hazard	Class IV - High acute/short-chronic hazard	Class V - Very high acute/short-chronic hazard	Class III - Acute/short-chronic hazard	Class II - Slight short-chronic hazard	Class IV - High acute/short-chronic hazard	Class IV - High acute/short-chronic hazard	
Weight (%)	0	78	100	100	89	100	67	33	100	100	

**Key:**  
 % = for definitive testing, only the 100% concentration (undiluted) sample mortality/inhibition/stimulation is reflected by this summary table. The dilution series results are considered for EC/LC values and Toxicity unit determinations  
 n.r. = not relevant, i.e. the 100% concentration caused less than 10/20/50% (effective concentration) mortalities or inhibition  
 n.c. = not calculable, although the 100% concentration led to more than 10/20/50% mortalities/inhibition, the 10/20/50% mortality/inhibition rate was exceeded throughout the test  
 \*\*\* = The overall hazard classification takes into account the full battery of tests and is not based on a single test result. Note that the overall hazard classification is expressed as acute/short-chronic level of toxicity, due to the fact that the A. fischeri test is regarded as short-chronic level of toxicity test and the overall classification therefore contains a degree of short-chronic toxicity assessment.  
 Weight (%) = relative toxicity levels (out of 100%), higher values indicate that more of the individual tests indicated toxicity within a specific class  
 site/sample name shaded in purple = screening test  
 site/sample name shaded in orange = definitive test

### Toxicity testing of Hex River tributaries (June 2019)

Toxicity testing was scheduled for the Hex River tributaries of the Klipfonteinspruit (samples KF and KFD) and the Klipgatspruit (sample KGT) for the June 2019 survey. The Klipgatspruit site was however dry at time of the survey precluding sampling.

As noted earlier in the report, the electrical conductivity (EC) levels are almost always very high within the Klipfonteinspruit. EC is often an indication of reduced water quality, but toxicity testing results revealed that no hazard (**Class I**) was observed at the Klipfonteinspruit sites (KF and KFD), before entering the Hex River (Table 5). This is an encouraging improvement since the October 2014 and April 2015 surveys, when a high hazard (Class IV) and a slight hazard (Class II) was presented by this tributary. It was noted that the Klipfonteinspruit was not flowing during the October 2014 survey and that contamination was therefore contained within isolated pools. It is important to note that APPD activities potentially contribute to this tributary.

Table 5: Toxicity results and hazard classification for selected Hex River tributary samples (June 2019).

	Results	KF	KFD
w <sub>0</sub> Water quality	pH @ 25°C (A)	8.4	8.4
	EC (Electrical conductivity) (mS/m) @ 25°C (A)	565.0	765.0
	Dissolved oxygen (mg/l) (NA)	7.8	8.1
A. fischeri (bacteria) (A)	Test started on yy/mm/dd	19/06/28	19/06/28
	%30min inhibition (-) / stimulation (+) (%)	21	33
	EC/LC20 (30 mins)	*	*
	EC/LC50 (30 mins)	*	*
	Toxicity unit (TU) / Description	no short-chronic hazard	no short-chronic hazard
D. magna (waterflea) (A)	Test started on yy/mm/dd	19/07/01	19/07/01
	%48hour mortality rate (-%)	-5	0
	EC/LC10 (48hours)	*	*
	EC/LC50 (48hours)	*	*
	Toxicity unit (TU) / Description	no acute hazard	no acute hazard
P. reticulata (guppy) (A)	Test started on yy/mm/dd	19/06/24	19/06/24
	%96hour mortality rate (-%)	0	0
	EC/LC10 (96hours)	*	*
	EC/LC50 (96hours)	*	*
	Toxicity unit (TU) / Description	no acute hazard	no acute hazard
Overall classification - Hazard class***	Class I - No acute/short-chronic hazard	Class I - No acute/short-chronic hazard	
Weight (%)	0	0	

**Key:**

\* = EC/LC values not determined, definitive testing required if a hazard was observed and persists over subsequent sampling runs  
 \*\*\* = The overall hazard classification takes into account the full battery of tests and is not based on a single test result. Note that the overall hazard classification is expressed as acute/short-chronic level of toxicity, due to the fact that the *A. fischeri* test is regarded as a short-chronic level of toxicity test and the overall classification therefore contains a degree of short-chronic toxicity assessment.  
 Weight (%) = relative toxicity levels (out of 100%), higher values indicate that more of the individual tests indicated toxicity within a specific class  
 site/sample name shaded in purple = screening test  
 site/sample name shaded in orange = definitive test

**3.3.2 Temporal variation of toxicity results (2008 to 2019)**

To determine temporal (over time) trends of increasing/decreasing toxicity levels, the risk class for each sample was plotted for each survey. Thereafter, linear trends over time were determined for the risk class at each site (Figures 5 & 6). It is important to note that these trends were not based on the actual mortalities/inhibition or lethal concentrations, but on the derived risk class for each survey and is merely included to gain a general understanding of increased/decreased risk over time.

**Annually tested PCD's and selected streams**

From the temporal database, most samples show varying degrees of toxicity and almost all the samples have fallen within the Class II or higher classes from time to time (Figure 5). Samples Dam2 and Dam 4/5

consistently fall into the higher hazard classes and are therefore never suitable for undiluted release<sup>7</sup> (including uncontrolled releases) to the environment. Some samples have, however, improved notably over time, such as sample K105, which has improved from an acute hazard (Class III) during 2008/2009 to no acute hazard (Class I) over the past five years. Samples from Dam6 and ACP have shown notably increasing trends in toxicity hazard over time. Environmental managers should take note of these hazard classes to plan licensed releases and/or contain hazardous water types at the appropriate times.

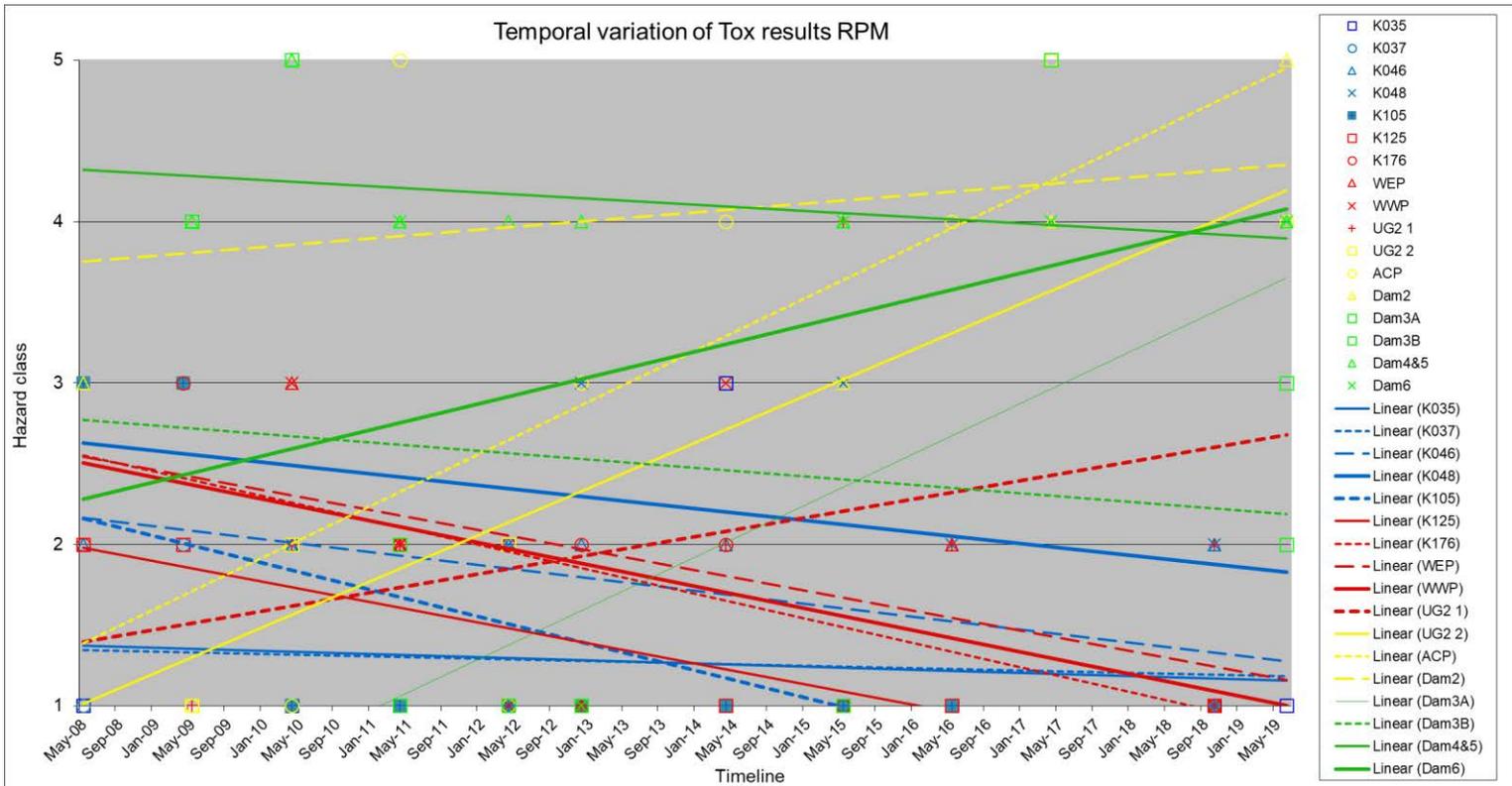


Figure 5: Temporal trends of toxicity results (annually tested PCD's and selected streams).

It is strongly recommended that definitive toxicity testing be continued for the samples that regularly display positive toxicity levels of Class III or higher. Definitive toxicity testing will allow for the estimation of safe dilution factors should the mine wish to apply for a water use licence to legally discharge such water types. These factors will also be essential for environmental managers to predict whether the toxicity of polluted water can be negated by natural dilution if accidentally released (e.g. spills, leaks or seepage) to the receiving environment. Definitive testing will furthermore assist in the suitable scheduling for planned releases (*i.e.* whether water could be released during the dry season and, if not, whether sufficient dilution is only likely to be achieved during the wet season/times of high river flow).

<sup>7</sup> Although theoretical predictions in terms of suitability for release are provided, releases remain bound by licensing conditions and are not prescribed/permitted by toxicity testing results.

### Bi-annually tested tributaries

From the temporal database, it is clear that the relevant tributaries (Klipfonteinspruit and Klipgatspruit) have displayed toxicity hazards at times (Figure 6). It is encouraging that both tributaries have recently displayed lower toxicity. The Klipfonteinspruit is therefore also displaying a decreasing trend (improved hazard over time) as noted in Figure 6.

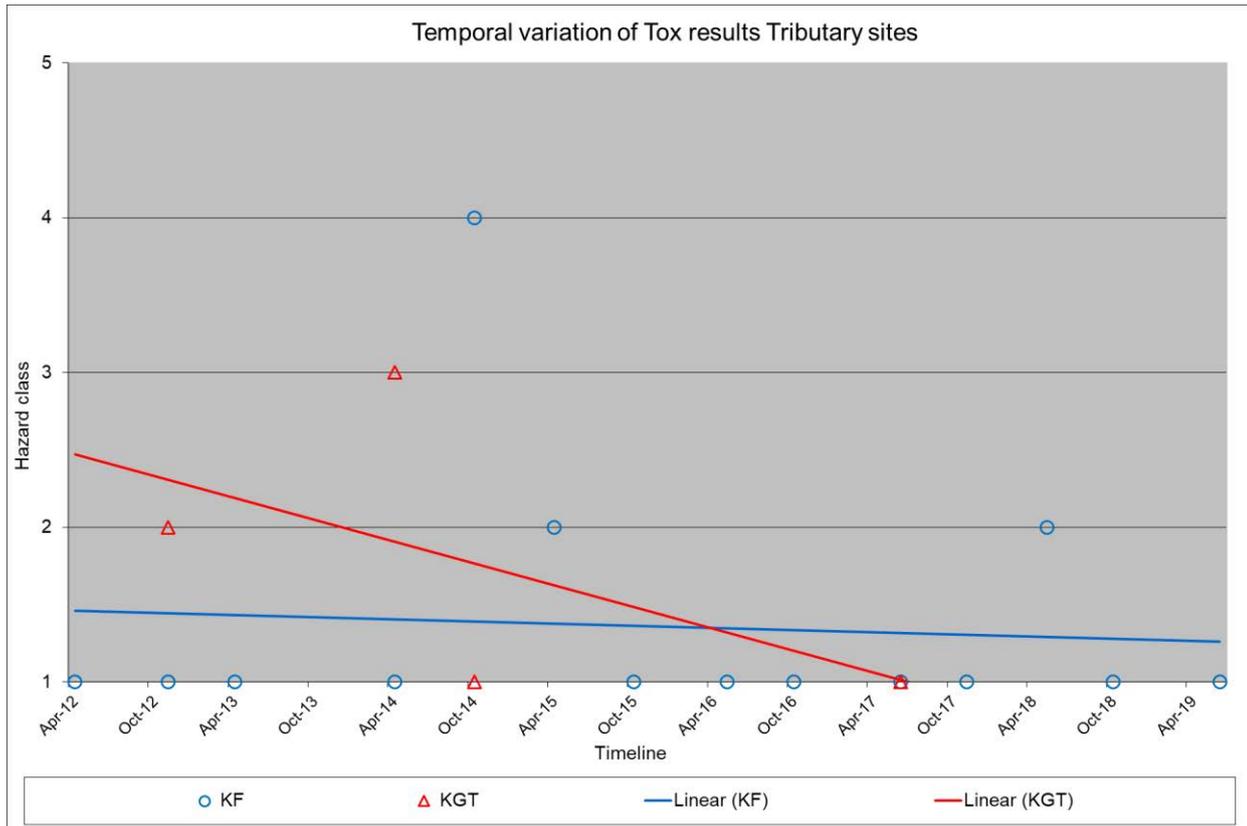


Figure 6: Temporal trends of toxicity results (bi-annually tested tributaries).

### 3.4 Aquatic invertebrate assessment: South African Scoring System 5

The South African Scoring System (Version 5) is a site-specific index which, together with associated habitat index (biotope suitability index), gives a general perspective of the biotic integrity (based on macro-invertebrates) and the impact of water quality on the biotic integrity of the specific sites (Thirion *et al.*, 1995; Dickens and Graham, 2001). The biotope suitability index considers the suitability of the different sampled biotopes in terms of quality and availability. It thereby firstly assesses whether the total SASS5 scores of two sites are directly comparable by comparing the total biotope suitability scores. If the total biotope suitability scores are very different this would imply that the total SASS5 scores should not be compared, but instead the most comparable SASS biotope scores. The most comparable SASS biotope scores are identified by comparing the various individual biotope suitability scores. In addition to the biotope suitability index, the Integrated Habitat Assessment System version 2 (IHAS) was also applied and included to give the macro-invertebrate specific habitat descriptions (Table 6).

Average score per taxon (ASPT) values are also useful in the assessment and comparison of biotic conditions at different sites. Based on field trials assessed by Dickens and Graham (2001) the ASPT was less variable than total SASS5 scores when conducted within a given river reach by different operators, considering all biotopes. ASPT is therefore included in the discussion below.

Table 6: Integrated Habitat Assessment (IHAS) description of the different biomonitoring sites.

Sampling Habitat	H-US-KF		H-DS-KF		HEX03		HEX3B	
	Desc	Score	Desc	Score	Desc	Score	Desc	Score
<b>Stones In Current (SIC)</b>								
Total length of white water rapids (ie: bubbling water) (in meters)	0-1	1	0-1	1	none	0	none	0
Total length of submerged stones in current (run) (in meters)	0-2	1	>2-5	2	none	0	>2-5	2
Number of separate SIC area's kicked	2-3	2	2-3	2	0	0	1	1
Average stone sizes kicked (in cm's)	11-20	3	11-20	3	none	0	>2-10	2
Amount of stone surface clear (in %)	0-25	1	0-25	1	n/a	0	0-25	1
Protocol: time spent actually kicking SIC's (in mins)	2	3	2	3	none	0	>1-2	2
<b>SIC score (max 20)</b>		<b>11</b>		<b>12</b>		<b>0</b>		<b>8</b>
<b>Vegetation (VEG)</b>								
Length of fringing vegetation sampled (banks) (in meters)	2	4	2	4	2	4	2	4
Amount of aquatic vegetation/algae sampled (in square meters)	>1	3	none	0	0-0.5	1	>1	3
Fringing vegetation sampled in	mix	5	mix	5	pool	3	mix	5
Type of veg. (percent leafy as apposed to stems/shoots)	26-50	3	1-25	2	1-25	2	26-50	3
<b>Veg score (max 15)</b>		<b>15</b>		<b>11</b>		<b>10</b>		<b>15</b>
<b>Other Habitat / General (O.H.)</b>								
Stones Out Of Current (SOOC) sampled (in square meters)	>0.5-1	2	>0.5-1	2	none	0	0-0.5	1
Sand sampled (in minutes)	0-0.5	2	0-0.5	2	none	0	0-0.5	2
Mud sampled (in minutes)	0-0.5	2	0-0.5	2	0.5	3	0-0.5	2
Gravel sampled (in minutes)	0.5	2	none	0	none	0	none	0
Bedrock sampled (all = no SIC, sand, gravel)	some	1	some	1	none	0	none	0
Algal presence (m <sup>2</sup> )	isolated	4	>1sqm	3	>1sqm	3	>1-2sqm	2
Tray identification	correct	3	correct	3	correct	3	correct	3
<b>O.H. score (max 20)</b>		<b>16</b>		<b>13</b>		<b>9</b>		<b>10</b>
<b>Sampling habitat totals (max 55)</b>		<b>42</b>		<b>36</b>		<b>19</b>		<b>33</b>
<b>Stream Condition</b>								
<b>Physical</b>								
River make up	2 mix	4	run	2	pool	0	2 mix	4
Average width of stream (in meters)	1-2	4	>2-5	5	1-2	4	1-2	4
Average depth of stream (in meters)	>0.5-1	3	0.5	4	>0.5-1	3	1-2	1
Approximate velocity of stream	medium	3	medium	3	medium	3	mix	5
Water colour	discoloured	3	discoloured	3	discoloured	3	discoloured	3
Recent disturbances	other	3	other	3	other	3	other	3
Bank/Riparian vegetation	mix	4	mix	4	mix	4	mix	4
Surrounding impacts	other	3	other	3	other	3	other	3
Left bank cover (rocks and vegetation) (in %)	51-80	1	51-80	1	51-80	1	51-80	1
Right bank cover (rocks and vegetation) (in %)	51-80	1	51-80	1	51-80	1	51-80	1
<b>Stream condition total (max 45)</b>		<b>29</b>		<b>29</b>		<b>25</b>		<b>29</b>
<b>Total IHAS score (%)</b>		<b>71</b>		<b>65</b>		<b>44</b>		<b>62</b>

Biotic conditions, based on the total SASS5 and ASPT scores, remained fairly stable from site H-US-KF to site H-DS-KF (Table 7; Figure 7), in contrast to the October 2018 survey when a clear downstream deterioration was observed. The findings are in line with most previous surveys when no spatial deterioration was observed. The two most similar biotopes<sup>8</sup> between the two sites (GSM and Vegetation), showed contrasting results therefore no conclusions on water quality related differences in biotic integrity could be

<sup>8</sup> To compare the effect of water quality on SASS scores on a spatial scale, habitat differences are considered. Therefore, the most comparable SASS<sub>biotope</sub> scores, in terms of habitat are also contrasted to gain insight regarding the effect of water quality on the biotic conditions (biotic integrity)

made. *In-situ* water quality measures showed improvement in terms of dissolved oxygen levels towards site H-DS-KF, but deterioration in terms of salinity (see section 3.2). Overall, macroinvertebrate-based biotic integrity in the Hex River did not appear to deteriorate after the inflow of the Klipfonteinspruit tributary (and potentially associated APPD impacts).

As in the October 2018 survey, biotic conditions, based on the total SASS5 scores and ASPT values, decreased from site H-DS-KF to Hex03 (Table 7; Figure 7). Habitat likely played a role, with site Hex03 having lower biotope availability and suitability (no stones biotope), and IHAS scores. Comparison of the most similar SASS-biotope (Vegetation) however indicated that water quality deterioration likely also contributed to the reduction in biotic integrity (Tables 6 and 7), and *in-situ* water quality measures likewise showed downstream deterioration in dissolved oxygen levels with levels below the median guideline recorded at site Hex03 (see section 3.2). It must be noted that organic enrichment and solid waste disposal appears extensively at this site and will likely affect biotic integrity if not mitigated (Plate 2). It is again noted that the reason for lowered dissolved oxygen levels are unlikely to be related to APPD activities because levels were within the guideline at site H-DS-KF and no further APPD activities take place towards site Hex03.

Table 7: SASS5, ASPT and habitat suitability/availability index scores for different monitoring sites (June 2019).

Monitoring site	SASS5 score	ASPT	SASS5-score per biotope			Biotope availability and suitability (Scores)			
			SASS <sub>Stones</sub>	SASS <sub>Vegetation</sub>	SASS <sub>GSM</sub>	Stones	Vegetation	GSM	Combined
H-US-KF	50	3.85	31	42	13	3	4	3	10
H-DS-KF	46	3.83	28	30	20	5	4	3	12
HEX03	22	3.14	0	21	3	0	3	1	4
HEX3B	26	3.25	2	23	8	1	4	2	7

**Key:**

ASPT - Average Score Pre Taxon      S-Stones      Veg-Vegetation      GSM-Gravel, sand & mud

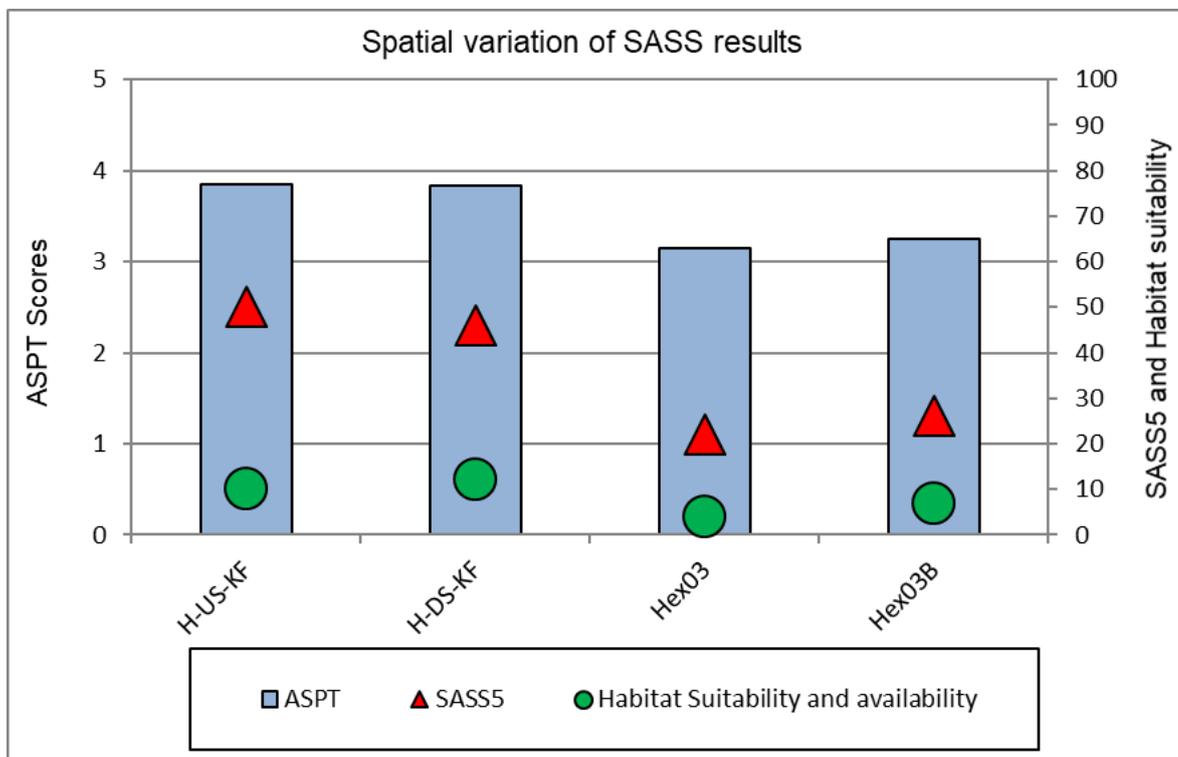


Figure 7: ASPT, SASS5 and total habitat suitability scores at biomonitoring sites during June 2019.

Biotic conditions, based on the total SASS5 scores and ASPT values, were stable to slightly improved from site Hex03 to Hex03-B (Table 7; Figure 7). This was at least partly habitat related as availability and suitability, and IHAS scores were better at the downstream site (Tables 6 and 7). None of the biotopes were directly comparable but all showed stable to improving scores, potentially indicating improved water quality towards the downstream site. *In-situ* water quality measures indicated considerable water quality improvements with a reduction in salinity and increase in dissolved oxygen levels, supporting the notion of improved water quality contributing to increased biotic integrity (see section 3.2). Comparisons between sites Hex03 and Hex03B were done to gauge the point-source effect, on the spatial integrity of the Hex River taking into consideration the Klipgatspruit. APPD is a potential contributor to pollution of the Klipgatspruit and continued monitoring (comparison of sites Hex03 and Hex03-B) will be essential to verify any possible impact and the severity thereof. The Klipgatspruit was dry at the time of sampling in June 2019 (although subsurface flow and impacts cannot be excluded), precluding water quality and toxicity testing, but did not appear to cause a deterioration in biotic integrity of the Hex River.



Plate 2: Indication of organic enrichment (algal proliferation) and solid waste disposal at site Hex03.

In conclusion, various sections of the Hex River within the study area show clear signs of reduced biotic integrity, based on macroinvertebrates. This was especially evident with the previous extended study area (now reduced due to Sibanye Stillwater sale and the complete scope no longer tasked to Clean Stream Biological Services). As such, a steady deterioration in biotic integrity in a downstream direction has consistently been recorded (Figure 8). However, the biotic integrity of the Hex River currently does improve on a spatial scale at certain sites and appears to be more stable within the recently adopted reduction of the study area (Figure 9).

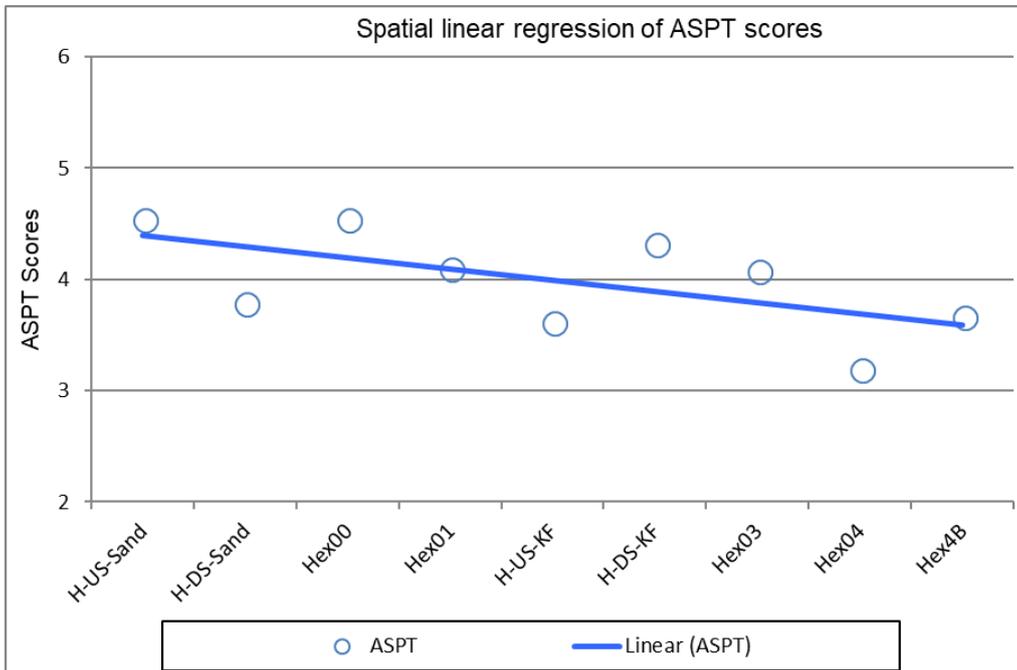


Figure 8: Linear regression of biotic integrity (as indicated by invertebrate ASPT scores) of the Hex River on a spatial scale (arranged sequentially in a downstream direction) during May 2018 (extended study area).

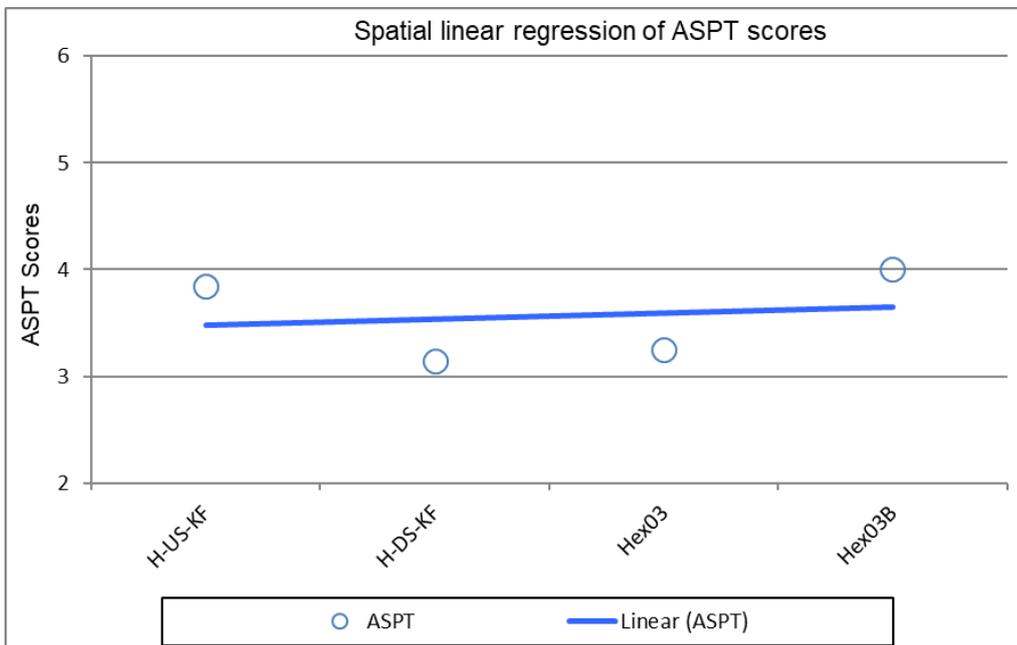


Figure 9: Linear regression of biotic integrity (as indicated by invertebrate ASPT scores) of the Hex River on a spatial scale (arranged sequentially in a downstream direction) during June 2019 (reduced study area).

### Temporal (long- and medium-term) trends

All of the datasets collected since May 2002 were compared to evaluate long-term and medium-term temporal trends in the biotic condition of the Hex River (Figures 10 & 11). Linear regression of historic ASPT values were calculated and plotted in order to achieve this. For the purpose of this monitoring programme, temporal

trends are differentiated into long-term (more than four years) and medium-term (last 4 years) trends. The long-term trend gives a perspective on whether the biotic integrity (at the different sites) has improved or deteriorated since the inception of the monitoring programme. The medium-term trend confirms whether observed long-term trends are likely to continue or are in the process of being reversed.

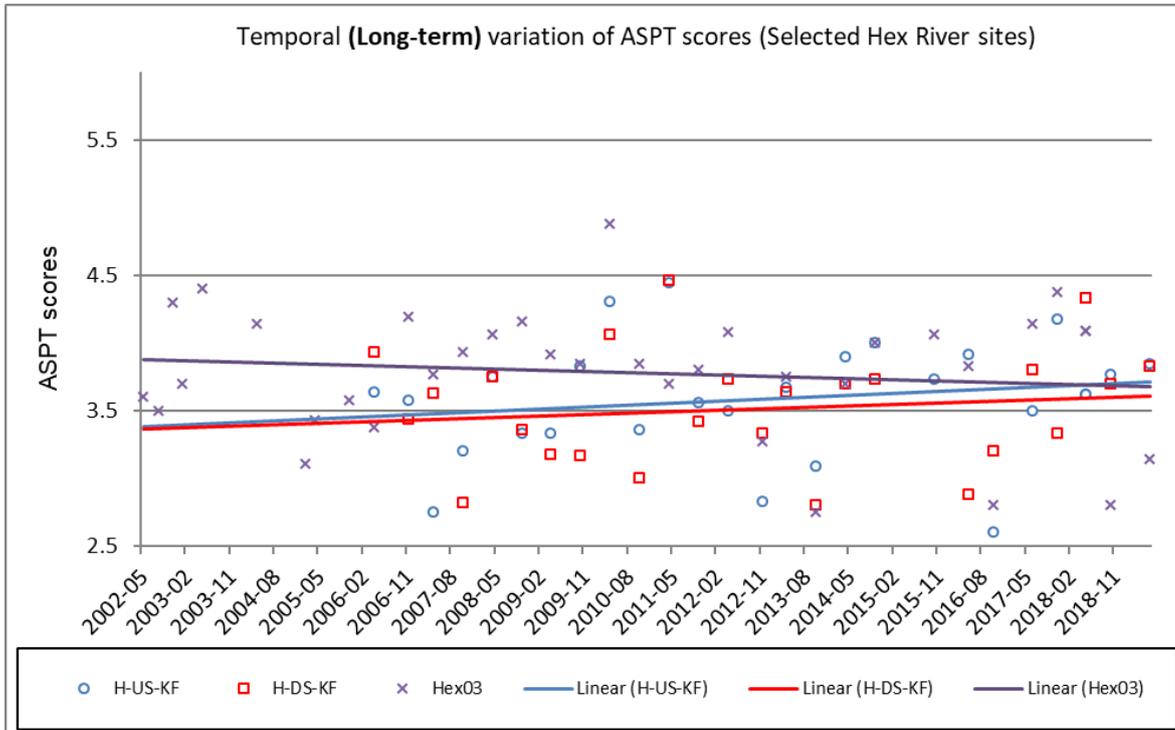


Figure 10: Long-term trends of biotic integrity in terms of macro-invertebrates at biomonitoring sites.

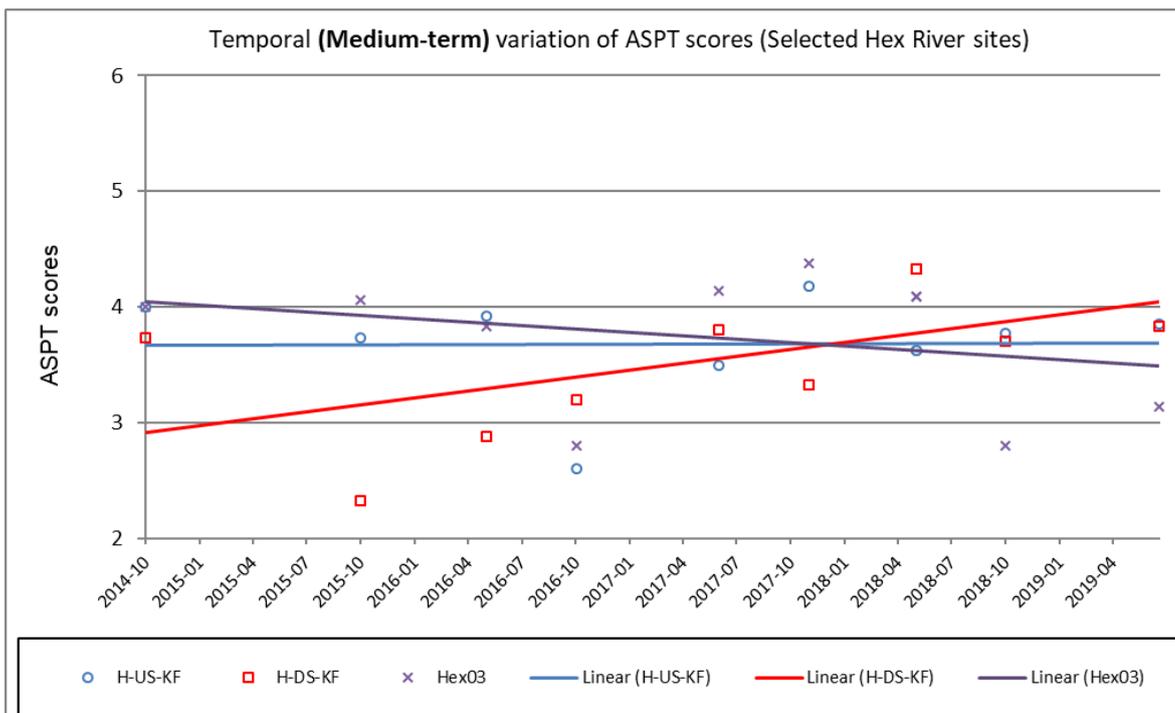


Figure 11: Medium-term trends of biotic integrity in terms of macro-invertebrates at biomonitoring sites.

Long-term trends indicated fair but stable (to slightly improving) biotic conditions at sites H-US-KF and H-DS-KF (Figure 10). However, long-term and medium-term trends at site Hex03 show a steady deterioration in biotic integrity (Figures 10 and 11). The recently observed organic pollution is almost certainly the cause, being **unrelated** to APPD activities.

Medium-term analyses confirm initially lower biotic conditions at site H-DS-KF (downstream of potential APPD impacts) but, encouragingly, biotic conditions at this site has improved at a faster rate than at the upstream site, with biotic integrity now being better at site H-DS-KF than at site H-US-KF (Figure 11). The inflow of the Klipfonteinspruit (and potential associated APPD impacts), therefore do not appear to have affected the macroinvertebrate-based biotic integrity of this reach of the Hex River over the medium to long term.

Continued monitoring will be essential to amass a database at the newly adopted downstream site (Hex03-B). This will serve to gauge the temporal effect of water users (including APPD) within the Klipgatspruit catchment, on the receiving environment (Hex River).

### 3.5 Fish Assessment

***Fish sampling was scheduled for the present survey but, due to safety risks posed to samplers (hostile behaviour from a crowd in the vicinity), could not be performed and sampling will again be attempted during the next scheduled survey.***

***Fish sampling is only scheduled once per annum and was last performed during May 2018, based on the extended scope (prior to Sibanye Stillwater sale). The approach and study area will therefore change in future, taking into consideration the reduced scope of this specific study, as performed by Clean Stream Biological Services. The complete extract (report RPM-A-18) of the fish results, is again repeated below for reference value and the sake of comprehensiveness. It should be kept in mind that various sampled sites are no longer part of this scope of work (since October 2018).***

The state and health of fish communities have been noted to give a reliable indication of short and long-term stress on aquatic systems. Fish communities possess various characteristics that render them important in the assessment of river health. They occupy positions throughout the aquatic food web, and are typically present in all but the most polluted of waters. Because fish often move over considerable distances, they have the potential to integrate diverse aspects of relatively large-scale habitats. Fish can therefore provide an integrated view of watershed conditions. Compared to other aquatic organisms, fish are furthermore relatively long-lived, and are therefore useful in providing a temporal dimension. They are also relatively easy to identify and after data is gathered, they can be released again. For the general public, fish are also the most well-known of aquatic organisms, and they are more likely to understand information about the condition of the fish community than about other taxa such as invertebrates. There are, however, some difficulties in using fish as biomonitoring indicators. Amongst these problems is the selective sampling attained by certain sampling equipment (for specific biotopes and for certain sizes and species of fish), the mobility of fish on spatial and temporal time scales, and the labour intensity of fish sampling.

Seven naturally occurring (native) fish species (*Barbus*<sup>9</sup> *paludinosus*; *Barbus trimaculatus*; *Barbus unitaeniatus*; *Clarias gariepinus*; *Oreochromis mossambicus*; *Pseudocrenilabrus philander* and *Tilapia sarrmanii*) were sampled at the five sampling sites in the Hex River during the 2017 to 2018 period (Table 8). The diversity of observed fish species was lower than expected at all of the sampling sites, indicating

<sup>9</sup> Recent literature (Yang *et al.*, 2015) recommend a name change of the genus '*Barbus*' to '*Enteromius*'. This was however contested and rejected by various authors (i.e. Schmidt and Bart, 2015) and requires further verification. Skelton (2016) supports the recommended name change and started implementing this in recent studies and literature.

lowered biotic integrity (when compared to natural expected conditions). Possible reasons for lowered species diversity are outlined in the paragraphs below, which deal with the Fish Assemblage Integrity Index (FAI) and Fish Response Assessment Index (FRAI) results.

### The Fish Assemblage Integrity Index (FAI) and Fish Response Assessment Index (FRAI)

For the purpose of this study, a simplified version of the FAI was used (presence / absence) to enable comparisons between each site (spatial analyses), while the FRAI was used to determine the estimated biotic integrity, based on fish, of the entire Hex River reach under investigation which would provide a valuable tool to provide an overall status of the reach under investigation and to determine long-term (temporal) changes.

Table 8: Fish species expected and observed during the last two surveys.

Species	Native/Exotic	Sites										
		Hex00		Hex01		Hex03		Hex04		Hex4B		
		Exp	Obs									
<i>Amphillius uranoscopus</i>	Native											
<i>Enteromius</i> <sup>#</sup> <i>paludinosus</i>	Native											
<i>Enteromius</i> <sup>#</sup> <i>trimaculatus</i>	Native											
<i>Enteromius</i> <sup>#</sup> <i>unitaeniatus</i>	Native											
<i>Chiloglanis pretoriae</i>	Native											
<i>Clarias gariepinus</i>	Native											
<i>Cyprinus carpio</i> <sup>*</sup>	Exotic											
<i>Labeobarbus marequensis</i>	Native											
<i>Labeo cylindricus</i>	Native											
<i>Labeo molybdinus</i>	Native											
<i>Mesobola brevianalis</i>	Native											
<i>Oreochromis mossambicus</i>	Native											
<i>Pseudocrenilabrus philander</i>	Native											
<i>Tilapia sparrmanii</i>	Native											
No. of naturally occurring species expected/present		9	4	10	3	10	5	13	4	13	3	
% expected / observed		44		30		50		31		23		

**Key:** sampled previous survey, sampled this survey, sampled last two surveys

\* Exotic species are by definition not expected to occur under natural conditions and therefore not taken into account for FAI calculations

# Previous genus name: Barbus

### Fish Assemblage Integrity Index (FAI)

Based on morphological characteristics and the limited number of sites, each sampling site was classified as a separate fish habitat segment. Therefore, the "frequency of occurrence of fish within segments" was omitted from FAI for separate monitoring sites. Comparison of relative FAI scores for different sites would firstly give a perspective on the relative condition of the fish community at different sites and secondly indicate the impact of various anthropogenic activities up- and downstream of the different sites. Scores should however be treated with circumspection because the *frequency of occurrence* criterion was not considered, and the FAI scores are therefore less accurate. The list of fish species expected to occur at the sites under investigation is based on information from Skelton (1993) and Le Roux & Steyn (1968), as well as experience from previous surveys (this biomonitoring programme as well as various other mining related biomonitoring programmes, research and Department of Water Affairs' reserve determination studies). The expected species list is also updated with the knowledge gained from this biomonitoring programme. The species intolerance ratings used in the calculation of the FAI were taken from Kleynhans (2002) and were based on specialisation of preferences towards habitat, food, flowing water and water quality.

The composition of the fish community and the relative FAIL (Fish Assemblage Integrity Index) are based on the last two surveys. This is done to increase the accuracy of the results and to avoid the incidental omission of a particular species at a particular site. Furthermore, fish generally take longer to react to stressors (compared with macro-invertebrates) and are therefore more applicable as an indicator over a period of time (as opposed to a snapshot at any given time).

The biotic integrity (as reflected by the fish assemblage integrity index) increased slightly from site Hex00 (23%) to Hex01 (27%) (Table 9 & Appendix tables; Figure 12). This is an indication that the biotic integrity (based on the fish communities) was not recently deteriorated due to by impacts in the area between these two sites. This is a similar trend as observed with the macro-invertebrate results, which indicated stable biotic conditions between these sites.

Table 9: Relative FAIL scores calculated at different sampling sites (2017 to 2018).

Locality	Relative FAIL (%)
Hex00	23
Hex01	27
Hex03	46
Hex04	22
Hex4B	22

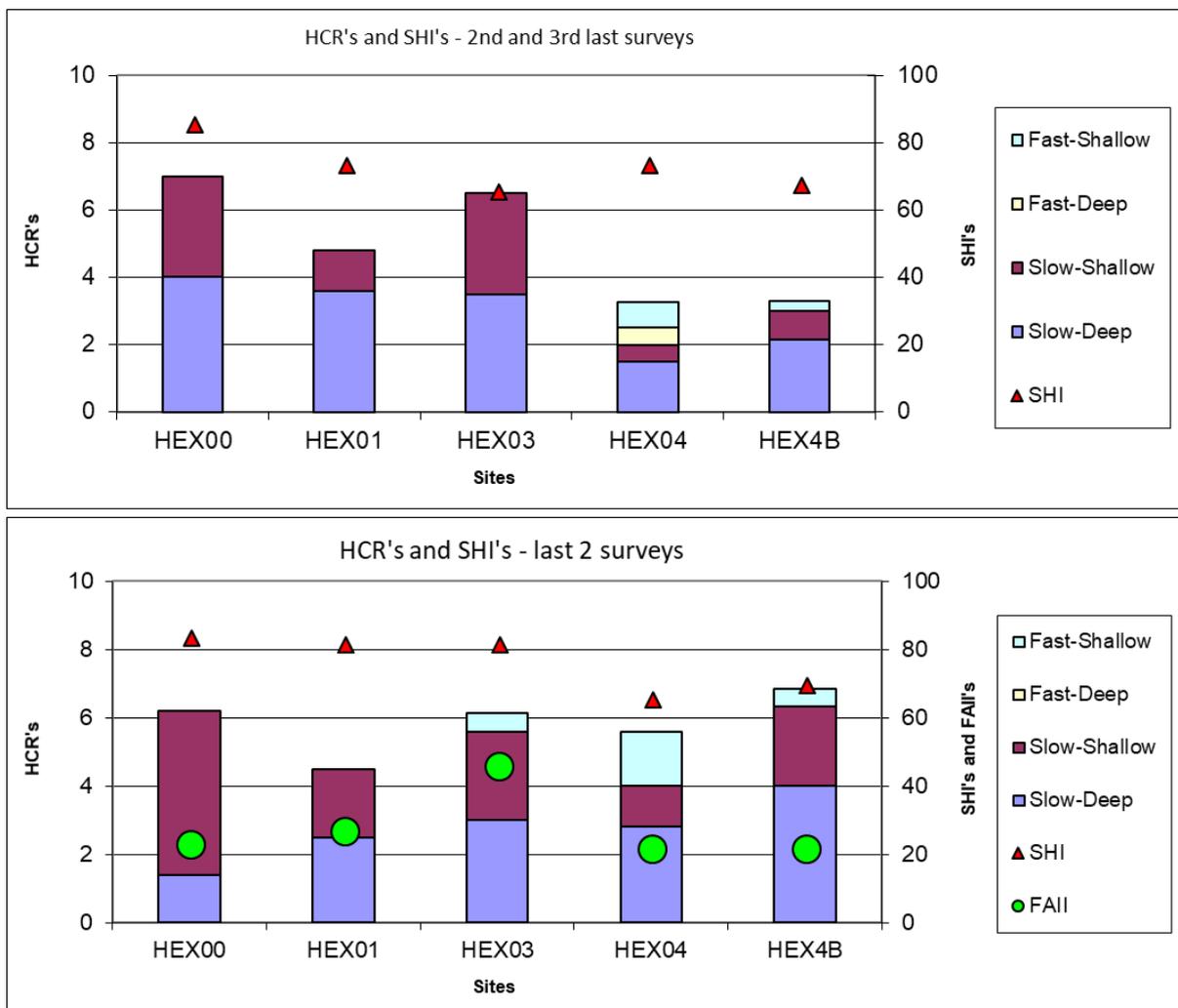


Figure 12: Relative FAIL scores, HCR's and SHI at the different biomonitoring sites.

A spatial improvement was observed from site Hex01 to Hex03, as shown by the FAIL scores increasing from 27% to 46% (Table 9; Figure 12). The spatial improvement was mainly attributed to the presence of *Enteromius trimaculatus* and *Enteromius unitaeniatus* at site Hex03. Both species are tolerant to moderately intolerant to water quality changes (Table 10) and their absence from site Hex01 is therefore not likely to be water quality related. Its absence from site Hex 00 during the 2017 to 2018 period is likely to be a response to lower habitat diversity and availability at this site. Based on these results, it appears that biotic integrity (based on fish) was probably not reduced by deteriorating water quality originating from the Klipfonteinspruit (possibly RPM-related) and/or from the sewage plant (non-RPM-related). This deduction is similar to the macro-invertebrate based deduction between these sites.

Table 10: The relative tolerance of each species towards changes in the environment.

SPECIES NAME	Common name	Trophic specialisation	Habitat specialisation	Flow dependance	Requirement for high water quality	Total intolerance ratings
<i>Amphilius uranoscopus</i>	Stargazer	4.7	4.9	4.8	4.8	4.8
<i>Chiloglanis pretoriae</i>	Shortspine suckermouth	4.4	4.8	4.8	4.5	4.6
<i>Labeo molybdinus</i>	Leaden labeo	3.3	3.0	3.3	3.2	3.2
<i>Labeo cylindricus</i>	Redeye labeo	3.3	3.0	3.1	3.1	3.1
<i>Labeobarbus marequensis</i>	Largescale yellowfish	2.4	2.8	3.2	2.1	2.6
<i>Mesobola brevianalis</i>	River sardine	3.1	2.2	1.1	2.8	2.3
<i>Enteromius trimaculatus</i>	Threespot barb	3.1	1.4	2.7	1.8	2.2
<i>Enteromius paludinosus</i>	Straightfin barb	1.6	1.4	2.3	1.8	1.8
<i>Enteromius unitaeniatus</i>	Longbeard barb	1.1	1.3	2.3	2.2	1.7
<i>Oreochromis mossambicus</i>	Mazambique tilapia	1.2	1.9	0.9	1.3	1.3
<i>Pseudocrenilabrus philander</i>	Southern mouthbrooder	1.3	1.4	1.0	1.4	1.3
<i>Tilapia sparrmanii</i>	Banded tilapia	1.6	1.4	0.9	1.4	1.3
<i>Clarias gariepinus</i>	Sharptooth catfish	1.0	1.2	1.7	1.0	1.2

**Key:**

Intolerance ratings are colour shaded on a scale from green to red, with green being least intolerant and red being most intolerant

Species are sorted in descending order from most intolerant (total intolerance rating) to least intolerant

1-2 = Tolerant 2-3 = Moderate tolerant 3-4 = Moderately intolerant 4-5 = Intolerant

As also observed previously, the FAIL scores were largely reduced from site Hex03 towards site Hex04 (Table 9). The potential impact of water quality on the biotic integrity of the Hex River (as measured by the FAIL at site Hex04) should therefore not be disregarded as both macro-invertebrates (during 2016 and 2018) and fish (last 4 years) are now indicating as such. Potential sources of reduced water quality between sites Hex03 and Hex04 include the Klipgatspruit and the Dorpspruit (see also previous discussions regarding potential Dorpspruit impacts).

The biotic integrity (based on fish) was similar between site Hex04 to site Hex4B (Table 9 & Appendix tables; Figure 12), being very poor at both sites. The same poor conditions (albeit spatially increased) was indicated by the macro-invertebrate assessment for these sites.

### Fish Response Assessment Index (FRAI)

As mentioned earlier, the Fish Response Assessment Index was adopted to assist in the classification of the Ecological Status, based on fish, of the entire Hex River reach under investigation. The results are therefore pooled for all sites. The resulting classification is therefore not a reflection of RPM mining impacts, but rather a reflection of the overall cumulative impact/s derived from the catchment.

The section below shows the individual metric driver results (Velocity-Depth, Cover, Flow, Physico-chemical, Migration and Introduced species), as well as the overall FRAI categories and category descriptions for the Hex River (Table 11).

Table 11: Fish Response Assessment Index (FRAI) results for the Hex River reach (all sites) (2017/8 results).

METRIC GROUP	METRIC	*RATING (CHANGE)	METRIC GROUP WEIGHT (%)
VELOCITY-DEPTH CLASSES METRICS	Response of species with high to very high preference for FAST-DEEP conditions	-5	97
	Response of species with high to very high preference for FAST-SHALLOW conditions	-5	
	Response of species with high to very high preference for SLOW-DEEP conditions	-2.5	
	Response of species with high to very high preference for SLOW-SHALLOW conditions	-2	
COVER METRICS	Response of species with a very high to high preference for overhanging vegetation	-1.5	100
	Response of species with a very high to high preference for undercut banks and root wads	-0.5	
	Response of species with a high to very high preference for a particular substrate type	-5	
	Response of species with a high to very high preference for instream vegetation	-0.5	
	Response of species with a very high to high preference for the water column	-3	
FLOW DEPENDANCE METRICS	Response of species intolerant of no-flow conditions	-5	94
	Response of species moderately intolerant of no-flow conditions	-5	
	Response of species moderately tolerant of no-flow conditions	-2	
	Response of species tolerant of no-flow conditions	-1.5	
PHYSICO-CHEMICAL METRICS	Response of species intolerant of modified physico-chemical conditions	-5	64
	Response of species moderately intolerant of modified physico-chemical conditions	-5	
	Response of species moderately tolerant of modified physico-chemical conditions	-4	
	Response of species tolerant of modified physico-chemical conditions	-1	
MIGRATION METRICS	Response in terms of distribution/abundance of spp with catchment scale movements	0	61
	Response in terms of distribution/abundance of spp with requirement for movement between reaches or fish habitat segments	4	
	Response in terms of distribution/abundance of spp with requirement for movement within reach or fish habitat segment	2	
INTRODUCED SPECIES METRICS	The impact/potential impact of introduced competing/predaceous spp?	0	45
	How widespread (frequency of occurrence) are introduced competing/predaceous spp?	0	
	The impact/potential impact of introduced habitat modifying spp?	2	
	How widespread (frequency of occurrence) are habitat modifying spp?	1	
FRAI SCORE (%)		32.3	
FRAI CATEGORY		E	
FRAI CATEGORY DESCRIPTION		Seriously modified	

- Reduced flows and altered flooding regime of the river.
- Cover metrics: Seriously deterioration in substrate as cover, most probably associated with extensive algal growth (as described earlier in this report), flow modification (decreased riffle/rapid habitats) and sedimentation.
- Flow dependence metrics: Serious modification of fish species intolerant to moderately intolerant to no-flow conditions, again indicating on altered hydrological regime (altered flows and floods).
- Physico-chemical metrics: Seriously modified conditions indicated by fish species that are intolerant to moderately intolerant of modified water quality, indicating on seriously deteriorated water quality prevailing in this river reach.

- Migration metrics: Indicating seriously modified migratory impacts, associated with various physical and potentially also chemical migration barriers within this reach.
- Introduced species metrics: Slight impacts associated with the presence of the habitat modifying alien Common carp (*Cyprinus carpio*).

Table 12: Descriptive categories used to describe the present ecological status (PES) of biotic components (adapted from Kleynhans, 1999).

CATEGORY	BIOTIC INTEGRITY	DESCRIPTION OF GENERALLY EXPECTED CONDITIONS
<b>A</b>	Excellent	Unmodified, or approximates natural conditions closely. The biotic assemblages compares to that expected under natural, unperturbed conditions.
<b>B</b>	Good	Largely natural with few modifications. A change in community characteristics may have taken place but species richness and presence of intolerant species indicate little modifications. Most aspects of the biotic assemblage as expected under natural unperturbed conditions.
<b>C</b>	Fair	Moderately modified. A lower than expected species richness and presence of most intolerant species. Most of the characteristics of the biotic assemblages have been moderately modified from its naturally expected condition. Some impairment of health may be evident at the lower end of this class.
<b>D</b>	Poor	Largely modified. A clearly lower than expected species richness and absence or much lowered presence of intolerant and moderately intolerant species. Most characteristics of the biotic assemblages have been largely modified from its naturally expected condition. Impairment of health may become evident at the lower end of this class.
<b>E</b>	Very Poor	Seriously modified. A strikingly lower than expected species richness and general absence of intolerant and moderately tolerant species. Most of the characteristics of the biotic assemblages have been seriously modified from its naturally expected condition. Impairment of health may become very evident.
<b>F</b>	Critical	Critically modified. Extremely lowered species richness and an absence of intolerant and moderately tolerant species. Only intolerant species may be present with complete loss of species at the lower end of the class. Most of the characteristics of the biotic assemblages have been critically modified from its naturally expected conditions. Impairment of health generally very evident.

## 4 CONCLUSIONS AND RECOMMENDATIONS

The following conclusions are based on the aquatic macroinvertebrate assessments performed during June 2019. Reference is not made to fish-based conclusions since the new scope of work (study area) has invalidated spatial and temporal findings, which will be refined when fish assessments are once again performed (scheduled once per annum). Fish sampling was scheduled for the June 2019 survey, but could not be conducted due to safety risks and will again be attempted during the next scheduled survey.

The most important **spatial** conclusions are as follows:

- Biotic conditions, based on the total SASS5 and ASPT scores, **remained stable from site H-US-KF to site H-DS-KF** (in contrast to the downstream deterioration observed during the October 2018 survey). This is in line with most previous surveys when no spatial deterioration was observed. The most similar biotopes (GSM and Vegetation) showed contrasting results, and *in-situ* water quality measures indicated downstream improvement in dissolved oxygen levels, but deterioration in salinity. Overall the inflow from the Klipfonteinspruit did not appear to impact on the macroinvertebrate-based biotic integrity of the Hex River at the time of sampling.
- Biotic conditions, based on the total SASS5 scores and ASPT values, **deteriorated from site H-DS-KF to Hex03**. Lowered habitat availability and suitability at site Hex03 likely contributed to this deterioration, however comparison of the most similar biotope indicate that reduced water quality also played a role. Low dissolved oxygen levels measured at site Hex03 further supported reductions in water quality. It must be noted that organic enrichment and solid waste disposal appears extensively at this site and will likely affect biotic integrity if not mitigated. It is again noted that the reason for lowered dissolved oxygen levels are unlikely to be related to APPD activities because levels were within the guideline at site H-DS-KF and no further APPD activities take place towards site Hex03.
- Biotic conditions, based on the total SASS5 scores and ASPT values, **were stable to slightly improved from site Hex03 to Hex03-B**. Better habitat availability and suitability at Hex03B likely played a part, but *in-situ* measures also showed considerable improvement in water quality towards site Hex03B. None of the biotopes were directly comparable but all showed indicated downstream improvement. Site Hex03-B was included for the first time during the October 2018 survey. This was done to gauge the point-source effect, on the spatial integrity of the Hex River taking into consideration the Klipgatspruit. APPD is a potential contributor to pollution of the Klipgatspruit and continued monitoring (comparison of sites Hex03 and Hex03-B) will be essential to verify any possible impact and the severity thereof.

The most important **temporal (long- and medium-term)** conclusions regarding the biotic integrity of the Hex River are as follows:

- Long-term trends indicated **fair but stable (to slightly improving)** biotic conditions at sites H-US-KF and H-DS-KF, with site H-US-KF generally displaying slightly better conditions. Medium-term analyses confirm initially lower biotic conditions at site H-DS-KF (downstream of potential APPD impacts) but, encouragingly, **biotic conditions have improved** to such an extent that biotic integrity is now better at site H-DS-KF than at site H-US-KF. The inflow of the Klipfonteinspruit (and potential associated APPD impacts), therefore do not appear to have affected the macroinvertebrate-based biotic integrity of this reach of the Hex River over the medium to long term.
- Long- and medium-term trends at site Hex03 show a **steady deterioration** in biotic integrity. The recently observed organic pollution is almost certainly the cause, being **unrelated** to APPD activities.
- Continued monitoring will be essential to amass a database at the newly adopted downstream site (Hex03-B). This will serve to gauge the temporal effect of water users (including APPD) within the Klipgatspruit catchment, on the receiving environment (Hex River).

## General conclusions and recommendations

In conclusion, it can be stated that various sections of the Hex River within the study area show clear signs of reduced biotic integrity, based on macroinvertebrates. This was especially evident with the previous extended study area (now reduced due to Sibanye Stillwater sale and the complete scope no longer tasked to Clean Stream Biological Services). As such, a steady deterioration in biotic integrity in a downstream direction has consistently been recorded. However, the biotic integrity of the Hex River currently does improve on a spatial scale at certain sites and appears to be more stable within the recently adopted reduction of the study area.

Future biomonitoring should be maintained on at least a biannual interval to gauge the trend of deterioration/improvement. This would facilitate the identification of possible impacts by APPD (and others) to this aquatic ecosystem. Early identification of impacts to the biota should prompt the identification of contaminants and the implementation of mitigation measures to reduce or prevent continued risk to the aquatic ecosystem.

It is strongly recommended that definitive toxicity testing be continued for the PCDs that regularly display toxicity levels of Class III or higher. Definitive toxicity testing will allow for the calculation of safe dilution ratios and will allow for the process of risk assessment. The risk assessment involves predicting the amount of a substrate that may enter the environment and comparing this with definitive toxicity results.

Calculated dilution ratios will be essential for environmental managers to predict whether the toxicity of polluted water will be negated if released or accidentally spilled into the receiving environment. Definitive testing will furthermore assist with scheduling planned licenced releases (*i.e.* whether water could be released during the dry season and, if not, whether sufficient dilution is likely to be achieved during the wet season/times of high river flow). All discharges should fall within the ambit of an approved water use licence, with biomonitoring and toxicity data being essential for the licensing process. In addition, increasing the frequency of testing of the pollution control facilities to at least twice a year should be considered. The confidence of results is relatively low if testing is only performed once a year, especially since toxicity hazards could conceivably change on a daily basis. More regular testing will therefore increase the confidence of results and lead to more informed management decisions.

It is recommended to continue including both site KF and KFD (in the Klipfonteinspruit) for toxicity testing (in addition to the Klipgatspruit; site KGT). The effect of different sources of pollution can then be distinguished more accurately.

## 5 REFERENCES

- ABOATOX Oy. 2012. BO1243-500 BioTox™ Kit. Instructions for use. Savikuja 2. FIN-21250, Masku Finland. [www.aboatox.com](http://www.aboatox.com)
- BARBOUR MT and STRIBLING JB (1994) A technique for assessing stream habitat structure. Pp. 156-178, In: Proceedings of the conference "Riparian Ecosystems of the Humid U.S. Management, functions, and Values". National Association of Conservation Districts. Washington, DC
- DEPARTMENT OF WATER AFFAIRS AND FORESTRY. 1996. South African Water Quality Guidelines (second edition). Volume 6: Agricultural water use: Aquaculture.
- DEPARTMENT OF WATER AFFAIRS AND FORESTRY, 2003. The Management of Complex Industrial Waste Water Discharges. Introducing the Direct Estimation of Ecological Effect Potential (DEEEP) approach, a discussion document. Institute of Water Quality Studies, Pretoria.
- DICKENS C and GRAHAM M (2001) South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers. River Health Programme Web Page.
- EUROPEAN Standard, 1998. "Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) – Part 3 for the method using freeze-dried bacteria", EN ISO 11348-3. European Committee for Standardization, Brussels.
- KEMPSTER, P. L. HATTINGH, W. H. J. & VAN VLIET, H. R. 1982. Summarised water quality criteria. Technical report NR. Tr 108. Department of Environmental Affairs.
- KILIAN V. 1996. Fish Pathology (FHAI): A biomonitoring procedure for rapid evaluation of fish health and condition. Report used during Field biosurveys and Integrated ecological assessment course, Institute of Water Quality Studies, DWAF.
- KLEYNHANS, C. J. 1997. *An exploratory investigation of the Instream Biological Integrity of the Crocodile River, Mpumalanga, as based on the Assessment of Fish Communities*. Draft Report, Department of Water Affairs and Forestry, Institute for Water Quality Studies. 61 pp.
- KLEYNHANS, C. J. 1997. *An exploratory investigation of the Instream Biological Integrity of the Crocodile River, Mpumalanga, as based on the Assessment of Fish Communities*. Draft Report, Department of Water Affairs and Forestry, Institute for Water Quality Studies. 61 pp.
- KLEYNHANS, C.J. 2002. Fish Intolerance ratings. Personal electronic communication of proceedings resulting from the national fish workshop held at the WRC during 2001.
- LE ROUX, P & STEYN, S. 1968. *Visse van Transvaal*. Kaap & Transvaal Drukkers Beperk, Kaapstad. 108pp.
- McMILLAN, P. H. 1998. *An Integrated Habitat Assessment System (IHAS v2), for the Rapid Biological Assessment of Rivers and Streams*. A CSIR research project. Number ENV-P-I 98132 for the Water Resources Management Programme. CSIR. ii + 44 pp.
- MICROBIOTEST INC. 2012. DAPHTOXKIT F™ MAGNA. Crustacean Toxicity Screening Test for freshwater. Standard Operational Procedure. Kleimoer 15, 9030 Mariakerke (Gent), Belgium. [www.microbiotest.be](http://www.microbiotest.be).
- ODUM EP (1971). *Fundamentals of Ecology*. Third Edition. W. B. Saunders Co. London.
- PERSOONE G, BLAHOŠLAV M, BLINOVA I, TÖRÖKNE A, ZARINA T, MANUSADZIANAS L, NALECZ-JAWECKI G, TOFAN L, STEPANOVA L, TOTHOVA L, KOLAR B. A practical and user-friendly toxicity classification system with Microbiotests for natural waters and wastewaters (personal communication).
- ROUX DJ (1999). Incorporating technologies for the monitoring and assessment of biological indicators into a holistic resource-based water quality management approach- conceptual models and some case studies. Ph.D Thesis. Rand Afrikaans University, JHB, SA.
- SKELTON P. H., 1993. *A complete guide to freshwater fishes of Southern Africa*. Southern Book Publishers (Pty) Ltd., Halfway House. 388pp.
- THIRION, C. A.; MOCKE, A & WOEST, R. 1995. *Biological Monitoring of Streams and Rivers using SASS4: A User Manual*. Final Report, No. N 000/00/REQ/1195. Institute of Water Quality Studies, Department of Water Affairs and Forestry.
- UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (US EPA), 1996. *Ecological effects test guidelines. Fish acute toxicity test – Freshwater and marine*. OPPTS 850.1075. Report number EPA-712-c-96-118.
- UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (US EPA), 1993. *Method for measuring the acute toxicity of effluent and receiving waters to freshwater and marine organisms*. EPA/600/4-90/027F, 4<sup>th</sup> edition. Office of Research and Development, Washington.
- YANG L, SADO T, HIRT MV, PASCO-VEIL E, ARUNACHALAM M, Li J, WANG X, FREYHOF J, SAOTOH K, SIMINS AM, MIYA M, He S, MAYDEN RL. 2015. Phylogeny and polyploidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). *Mol. Phylogenet. Evol.* 85, 97 – 116.

## Appendix 1: Methodology applied during this biomonitoring assessment.

### 1. *In-situ* water quality

The following surface water quality variables were measured on site: pH, Conductivity, water temperature, dissolved oxygen and oxygen saturation (Hach HQ40d Multimeter; Serial Number: 130300086148).

### 2. Habitat assessment

An evaluation of habitat quality and availability to biota is critical to any assessment of ecological integrity and should be conducted at each site at the time of biological sampling. On site habitat assessments were conducted by using existing habitat evaluation indices.

#### 2.1 Habitat condition

The United States Environment Protection Agency Habitat Assessment Index (HAI) index was used to determine the general physical habitat condition at each site. Habitat parameters used by this index in this assessment of habitat integrity include the following: Epifaunal substrate/Available cover, Pool substrate characteristics, Pool variability, Channel alteration, Sediment deposition, Channel sinuosity, Channel flow status, Bank vegetative protection, Bank stability and Riparian vegetative zone width. Each of the above mentioned criteria was assessed and according to its condition, rated in one of the following classes, namely: Optimal/Excellent, Sub optimal/Good, Marginal/Fair or Poor. For each criterion, a score was given within the selected class. The sum of these scores gives a final score for this Index, and can be used in comparison to other sites or, if possible, to the baseline or reference condition to indicate its physical integrity (Barbour *et al.*, 1999).

#### 2.2 Fish Habitat Assessment

This assessment is aimed at the determination of the potential of a site to provide habitat for fish (Fish habitat cover ratings) and to identify the potential human impact on the fish habitat (Site fish habitat integrity) (Kleynhans, 1997).

##### *Fish Habitat Cover Rating (HCR)*

This approach was developed to assess habitats according to different attributes that are surmised to satisfy the habitat requirements of various fish species (Kleynhans, 1997). At each site, the following depth-flow (df) classes are identified, namely:

Slow (<0.3m/s), shallow (<0.5m) - Shallow pools and backwaters.

Slow, deep (>0.5m) - Deep pools and backwaters.

Fast (>0.3m/s), shallow - Riffles, rapids and runs.

Fast, deep - Usually rapids and runs.

The relative contribution of each of the above mentioned classes at a site was estimated and indicated as:

0 = Absent

1 = Rare (<5%)

2 = Sparse (5-25%)

3 = Moderate (25-75%)

4 = Extensive (>75%)

For each depth-flow class, the following cover features (cf), considered to provide fish with the necessary cover to utilise a particular flow and depth class, were investigated:

- Overhanging vegetation

- Undercut banks and root wads

- Stream substrate

- Aquatic macrophytes

The amount of cover present at each of these cover features (cf) was noted as:

0 = absent

1 = Rare/very poor (<5%)

2 = Sparse/poor (5-25%)

3 = Moderate/good (25-75%)

4 = Extensive/excellent (>75%)

The fish habitat cover rating (HCR) was calculated as follows:

- The contribution of each depth-flow class at the site was calculated ( $df/\Sigma df$ ).

- For each depth-flow class, the fish cover features (cf) were summed ( $\Sigma cf$ ).

$HCR = df/\Sigma df \times \Sigma cf$ .

##### *Site fish habitat integrity (SHI)*

This approach is based on the assessment of physical habitat disturbance and is directed towards the indirect qualitative evaluation of fish habitat integrity, compared to the expected natural condition (Kleynhans, 1997). The following impacts (cause for fish habitat integrity degradation) is investigated, namely: Water abstraction, flow modification, bed modification, channel modification, inundation, exotic macrophytes, solid waste disposal, indigenous vegetation removal, exotic vegetation encroachment and bank erosion. Estimation of the impact of each of these modifications on the fish habitat integrity at a site is scored as follows:

No Impact = 0

Small impact = 1

Moderate Impact = 3

Large impact = 5

### 3. Aquatic invertebrate assessment: South African Scoring System, Version 5.

Benthic macro-invertebrate communities of the selected sites were investigated according to the South African Scoring System, version 5 (SASS5) approach (Dickens & Graham, 2001). This method is based on the British Biological Monitoring Working Party (BMWP) method and has been adapted for South

African conditions by Dr. F. M. Chutter (Thirion *et al.*, 1995). The SASS method is a rapid, simple and cost effective method, which has progressed through four different upgrades/versions. The current upgrade is Version 5, which is specifically designed to comply with international accreditation protocols.

#### **Sample Collection**

An invertebrate net (30 x 30cm square with 1mm mesh netting) was used for the collection of the organisms. The available biotopes at each site were identified on arrival. Each of the biotopes was sampled by different methods explained later (samples should not be collected when the river is in flood).

The biotopes were combined into three different groups, which were sampled and assessed separately:

#### **a) Stone (S) Biotopes:**

**Stones in current (SIC) or any solid object:** *Movable stones of at least cobble size (3 cm diameter) to approximately 20 cm in diameter, within the fast and slow flowing sections of the river.* Kicksampling is used to collect organisms in this biotope. This is done by putting the net on the bottom of the river, just downstream of the stones to be kicked, in a position where the current will carry the dislodged organisms into the net. The stones are then kicked over and against each other to dislodge the invertebrates (kicksampling) for  $\pm 2$  minutes.

**Stones out of current (SOOC):** *Where the river is still, such as behind a sandbank or ridge of stones or in backwaters.* Collection is again done by the method of kicksampling, but in this case the net is swept across the area sampled to catch the dislodged biota. Approximately 1 m<sup>2</sup> is sampled in this way.

**Bedrock or other solid substrate:** Bedrock includes stones greater than 30cm, which are generally immovable, including large sheets of rock, waterfalls and chutes. The surfaces are scraped with a boot or hand and the dislodged organisms collected. Sampling effort is included under SIC and SOOC above.

#### **b) Vegetation (VG) Biotopes:**

**Marginal vegetation (MV):** *This is the overhanging grasses, bushes, twigs and reeds growing on the edge of the stream, often emergent, both in current (MvegIC) and out of current (MvegOOC).* Sampling is done by holding the net perpendicular to the vegetation (half in and half out of the water) and sweeping back and forth in the vegetation ( $\pm 2$ m of vegetation).

**Submerged vegetation (AQV):** *This vegetation is totally submerged and includes Filamentous algae and the roots of floating aquatics such as water hyacinth.* Sampled by pushing the net (under the water) against and amongst the vegetation in an area of approximately one square meter.

#### **c) Gravel, Sand and Mud (GSM) biotopes:**

**Sand:** *This includes sandbanks within the river, small patches of sand in hollows at the side of the river or sand between the stones at the side of the river.* This biotope is sampled by stirring the substrate by shuffling or scraping of the feet, which is done for half a minute, whilst the net is continuously swept over the disturbed area.

**Gravel:** *Gravel typically consists of smaller stones (2-3 mm up to 3 cm).* Sampling similar to that of sand.

**Mud:** *It consists of very fine particles, usually as dark-collared sediment.* Mud usually settles to the bottom in still or slow flowing areas of the river. Sampling similar to that of sand.

#### **d) Hand picking and visual observation:**

Before and after disturbing the site, approximately 1 minute of "hand-picking" for specimens that may have been missed by the sampling procedures was carried out.

#### **Sample preparation**

The organisms sampled in each biotope group were identified and their relative abundance also noted on the SASS5 datasheet.

#### **SASS-Habitat Assessment**

A SASS-habitat assessment index, according to the habitats sampled, was performed due to the fact that changes in habitat can be responsible for changes in SASS5 scores. This was done by the application of Integrated Habitat Assessment System (IHAS version 2) (McMillan, 1998).

#### **4. Fish Assemblage Integrity Index (FAII) (Kleynhans, 1997)**

Due to the difficulty of applying the generally used Index of Biotic Integrity (IBI) in rivers of South Africa, Kleynhans (1997) developed an alternative approach. The following procedures were used in the application of the FAII:

##### *Species tolerance ratings*

The species intolerance ratings used in the calculation of the FAII were taken from Kleynhans (2002). Four components are taken into account in estimating the intolerance of the relevant fish species, namely habitat preferences and specialisation (HS), food preference and specialisation (TS), requirements for flowing water during different life-stages (FW) and water quality requirements (WQ). Each of these aspects are scored for a species according to low requirement/specialisation (rating=1), moderate requirement/ specialisation (rating=3) and high requirement/specialisation (rating=5). The total intolerance (IT) of a fish species is estimated as follows:

$$IT = (HS+TS+FW+WQ)/4$$

##### *Health*

The percentage of fish with externally evident disease or other anomalies are used to score this metric. The following procedure is used to score the health of individual species:

Frequency of affected fish >5%, score = 1

Frequency of affected fish 2 - 5%, score = 3

Frequency of affected fish <2%, score = 5

The expected health for a species living under unperturbed conditions is assumed to be unimpaired and would score 5.

##### **The FAII is calculated as follows:**

The *expected index score* [FAII (exp.)] per segment:

$$FAII (exp.) = \sum(T \times H)$$

where: T = Tolerance rating for individual species

H = Expected health rating for individual species.

The *observed index score* [FAII (obs)] is calculated on a similar basis but is based on the information collected during the survey:

$$FAII (obs) = \sum(T \times H)$$

The observed fish assemblage index score for a segment is expressed as a percentage of the expected total FAII score to arrive at a relative FAII rating:

$$FAII (obs) / FAII (exp.) \times 100$$

**Fish Response Assessment Index (FRAI)**

The determination and description of the present ecological status (PES) of the aquatic ecosystems in the study area, in terms of fish, was done according to the methodology described for River EcoClassification during Reserve Determinations (Kleynhans & Louw, 2008) using the Fish Response Assessment Index (FRAI) (Kleynhans, 2008). The results were then used to classify the present state of the fish assemblage into a specific descriptive category (A to F) (Table A1).

The FRAI is not in its conventional form designed for the application per site, but rather to a reach with a few sites. Metrics are therefore based on spatial frequency of occurrence of a species within the reach.

Table A1: Descriptive categories used to describe the present ecological status (PES) of biotic components (adapted from Kleynhans, 1999).

CATEGORY	BIOTIC INTEGRITY	DESCRIPTION OF GENERALLY EXPECTED CONDITIONS
A	Excellent	Unmodified, or approximates natural conditions closely. The biotic assemblages compares to that expected under natural, unperturbed conditions.
B	Good	Largely natural with few modifications. A change in community characteristics may have taken place but species richness and presence of intolerant species indicate little modifications. Most aspects of the biotic assemblage as expected under natural unperturbed conditions.
C	Fair	Moderately modified. A lower than expected species richness and presence of most intolerant species. Most of the characteristics of the biotic assemblages have been moderately modified from its naturally expected condition. Some impairment of health may be evident at the lower end of this class.
D	Poor	Largely modified. A clearly lower than expected species richness and absence or much lowered presence of intolerant and moderately intolerant species. Most characteristics of the biotic assemblages have been largely modified from its naturally expected condition. Impairment of health may become evident at the lower end of this class.
E	Very Poor	Seriously modified. A strikingly lower than expected species richness and general absence of intolerant and moderately tolerant species. Most of the characteristics of the biotic assemblages have been seriously modified from its naturally expected condition. Impairment of health may become very evident.
F	Critical	Critically modified. Extremely lowered species richness and an absence of intolerant and moderately tolerant species. Only intolerant species may be present with complete loss of species at the lower end of the class. Most of the characteristics of the biotic assemblages have been critically modified from its naturally expected conditions. Impairment of health generally very evident.

It must be emphasized that the A→F scale represents a continuum, and that the boundaries between categories are notional, artificially-defined points along the continuum (as presented below). This situation falls within the concept of a fuzzy boundary, where a particular entity may potentially have membership of both classes (Robertson *et al.* 2004). For practical purposes, these situations are referred to as boundary categories and are denoted as B/C, C/D, and so on.



**Appendix 2: Site photos of biomonitoring sites (last two surveys)**



Plate 1: Upstream view of H-US-KF (2019-06)



Plate 2: Downstream view of H-US-KF (2019-06)



Plate 3: Upstream view of H-US-KF (2018-10)



Plate 4: Downstream view of H-US-KF (2018-10)



Plate 5: Upstream view of KF (2019-06)



Plate 6: Downstream view KF (2019-06)



Plate 7: Upstream view of kF (2018-10)



Plate 8: Downstream view KF (2018-10)



Plate 9: Upstream view of KFD (2019-06)



Plate 10: Downstream view of KFD (2019-06)

Not included in survey

Plate 11: Upstream view of KFD (2018-10)

Plate 12: Downstream view of KFD (2018-10)



Plate 13: Upstream view of H-DS-KF (2019-06)

Photo not available

Plate 14: Downstream view of H-DS-KF (2019-06)



Plate 15: Upstream view of H-DS-KF (2018-10)



Plate 16: Downstream view of site H-DS-KF (2018-10)



Plate 16: Upstream view of Hex03 (2019-06)



Plate 17: Downstream view of Hex03 (2019-06)



Plate 18: Upstream view of Hex03 (2018-10)



Plate 19: Downstream view of site Hex03 (2018-10)



Plate 20: Upstream view of KGT (2019-06)



Plate 21: Downstream view of KGT (2019-06)



Plate 22: Upstream view of KGT (2018-10)



Plate 23: Downstream view of site KGT (2018-10)



Plate 24: Upstream view of Hex03B (2019-06)



Plate 25: Downstream view of Hex03B (2019-06)



Plate 26: Upstream view of Hex03B (2018-10)



Plate 27: Downstream view of site Hex03B (2018-10)

Appendix 3: Tables

**Table A1:** SASS5 analysis including macro-invertebrate families sampled and habitat suitability scores calculated for the various sites (June 2019).

Taxon	Common name	H-US-KF				H-DS-KF				HEX03				HEX3B			
		Stones	Veg	GSM	Total	Stones	Veg	GSM	Total	Stones	Veg	GSM	Total	Stones	Veg	GSM	Total
Oligochaeta	Aquatic earthworms	B	A	A	B	A	-	-	A	-	-	A	A	-	-	-	-
Leeches	Leeches	A	B	B	B	-	-	1	1	-	B	-	B	-	-	-	-
Potamonautidae*	Crabs	1	-	-	1	-	-	-	-	-	-	-	-	-	-	1	1
Baetidae 1 sp.	Small minnow flies	A	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-
Coenagrionidae	Damselflies	-	-	-	-	-	A	-	A	-	-	-	-	-	B	-	B
Belostomatidae*	Giant water bug	1	A	-	A	-	-	-	-	-	A	-	A	-	A	-	A
Corixidae*	Water boatmen	A	A	A	B	-	A	1	A	-	-	-	-	-	-	-	-
Gerridae*	Pond skater	-	-	-	-	-	A	-	A	-	-	-	-	-	-	-	-
Naucoridae*	Creeping water bugs	A	A	-	A	A	A	1	A	-	-	-	-	-	1	-	1
Nepidae*	Water scorpions	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
Veliidae*	Broad-shouldered water strid	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Ecnomidae	Caseless caddisflies	-	-	-	-	A	-	-	A	-	-	-	-	-	-	-	-
Hydropsychidae 1sp.	Caseless caddisflies	-	-	-	-	B	-	-	B	-	-	-	-	-	-	-	-
Dytiscidae (adults*)	Predacious diving beetles	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-
Hydraenidae (adults*)	Minute moss beetles	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Ceratopogonidae	Biting midges	-	A	-	A	-	-	-	-	-	-	-	-	-	-	-	-
Chironomidae	Midges	B	B	-	B	B	A	A	B	-	A	B	B	C	B	C	C
Culicidae*	Mosquitoes	-	-	B	B	-	-	-	-	-	-	-	-	-	A	-	A
Muscidae	House flies	-	-	-	-	1	1	-	A	-	-	-	-	-	-	-	-
Simuliidae	Black flies	A	B	A	B	B	A	1	B	-	A	-	A	-	-	-	-
Physidae*	Pouch snails	-	-	-	-	-	A	-	A	-	A	-	A	-	B	A	B
Total SASS5 score		31	42	13	50	28	30	20	46	0	21	3	22	2	23	8	26
No. of families		9	10	5	13	7	8	5	12	0	6	2	7	1	7	3	8
ASPT		3.44	4.20	2.60	3.85	4.00	3.75	4.00	3.83	#DIV/0!	3.50	1.50	3.14	2.00	3.29	2.67	3.25
Total IHAS					71				65				44				62
IHAS - Habs sampled					42				36				19				33
IHAS - Stream condition					29				29				25				29
Suitability score		3	4	3	10	5	4	3	12	0	3	1	4	1	4	2	7

Key: High requirement for unmodified water quality Veg=Vegetation

Moderate requirement for unmodified water quality

Low requirement for unmodified water quality

Very low requirement for unmodified water quality

A = 1-10 individuals; B = 11-100 individuals; C = 101-1000 individuals; ASPT = Average score per taxon.

**Table A3:** Fish Assemblage Integrity Index (FAII) scores calculated for the various sampling sites (2017-2018).

SPECIES	Intolerance rating					Health rating					SCORE				
	HEX00	HEX01	HEX03	HEX04	HEX4B	HEX00	HEX01	HEX03	HEX04	HEX4B	HEX00	HEX01	HEX03	HEX04	HEX4B
<i>Amphillius uranoscopus</i>	4.8	4.8	4.8	4.8	4.8	5	5	5	5	5	24.0	24.0	24.0	24.0	24.0
<i>Barbus paludinosus</i>	1.8	1.8	1.8	1.8	1.8	5	5	5	5	5	9.0	9.0	9.0	9.0	9.0
<i>Barbus trimaculatus</i>	2.2	2.2	2.2	2.2	2.2	5	5	5	5	5	11.0	11.0	11.0	11.0	11.0
<i>Barbus unitaeniatus</i>		1.7	1.7	1.7	1.7		5	5	5	5	0.0	8.5	8.5	8.5	8.5
<i>Chiloglanis pretoriae</i>				4.6	4.6				5	5	0.0	0.0	0.0	23.0	23.0
<i>Clarias gariepinus</i>	1.2	1.2	1.2	1.2	1.2	5	5	5	5	5	6.0	6.0	6.0	6.0	6.0
<i>Labeobarbus marequensis</i>	2.6	2.6	2.6	2.6	2.6	5	5	5	5	5	13.0	13.0	13.0	13.0	13.0
<i>Labeo cylindricus</i>				3.1	3.1				5	5	0.0	0.0	0.0	15.5	15.5
<i>Labeo molybdinus</i>				3.2	3.2				5	5	0.0	0.0	0.0	16.0	16.0
<i>Mesobola brevianalis</i>	2.3	2.3	2.3	2.3	2.3	5	5	5	5	5	11.5	11.5	11.5	11.5	11.5
<i>Oreochromis mossambicus</i>	1.3	1.3	1.3	1.3	1.3	5	5	5	5	5	6.5	6.5	6.5	6.5	6.5
<i>Pseudocrenilabrus philander</i>	1.3	1.3	1.3	1.3	1.3	5	5	5	5	5	6.5	6.5	6.5	6.5	6.5
<i>Tilapia sparrmanii</i>	1.3	1.3	1.3	1.3	1.3	5	5	5	5	5	6.5	6.5	6.5	6.5	6.5
	<b>Total Expected</b>										<b>94.0</b>	<b>102.5</b>	<b>102.5</b>	<b>157.0</b>	<b>157.0</b>
<i>Amphillius uranoscopus</i>											0.0	0.0	0.0	0.0	0.0
<i>Barbus paludinosus</i>	1.8	1.8	1.8	1.8	1.8	5	5	5	5	5	9.0	9.0	9.0	9.0	9.0
<i>Barbus trimaculatus</i>			2.2					5			0.0	0.0	11.0	0.0	0.0
<i>Barbus unitaeniatus</i>			1.7					5			0.0	0.0	8.5	0.0	0.0
<i>Chiloglanis pretoriae</i>											0.0	0.0	0.0	0.0	0.0
<i>Clarias gariepinus</i>		1.2	1.2	1.2	1.2		5	5	5	5	0.0	6.0	6.0	6.0	6.0
<i>Labeobarbus marequensis</i>											0.0	0.0	0.0	0.0	0.0
<i>Labeo cylindricus</i>											0.0	0.0	0.0	0.0	0.0
<i>Labeo molybdinus</i>											0.0	0.0	0.0	0.0	0.0
<i>Mesobola brevianalis</i>											0.0	0.0	0.0	0.0	0.0
<i>Oreochromis mossambicus</i>			1.3	1.3	1.3			5	5	5	0.0	0.0	6.5	6.5	6.5
<i>Pseudocrenilabrus philander</i>	1.3	1.3	1.3	1.3	1.3	5	5	5	5	5	6.5	6.5	6.5	6.5	6.5
<i>Tilapia sparrmanii</i>	1.3	1.3		1.3	1.3	5	5		5	5	6.5	6.5	0.0	6.5	6.5
	<b>Total Observed</b>										<b>22.0</b>	<b>28.0</b>	<b>47.5</b>	<b>34.5</b>	<b>34.5</b>
	<b>Relative FAII (%)</b>										<b>23</b>	<b>27</b>	<b>46</b>	<b>22</b>	<b>22</b>

**END OF REPORT**

**Addendum 1: Toxicity test report/s (Biotox Laboratory Services)**

Submitted as separate PDF document/s

# Appendix C

---

## *Toxicity test report*

Anglo American Platinum – Rustenburg Operations  
Annual integrated water monitoring report  
Volume I



## TOXICITY TEST REPORT

**For:**

Aquatico Scientific (Pty) Ltd

89 Regency Drive, Route 21 Corporate Park, Irene  
PO Box 905008, Garsfontein, 0042

**Survey:**  
2018-10

**Report reference:**  
RPM-B-18\_TOX

**Revision:**  
0

**Project:**  
Anglo Rustenburg (RPM)

**Samples:**  
KF, KGT (Klipgat)

Tests performed by: Marrilize Bylsma (Technical Manager); Marlise Brown (Junior Analyst)  
Inputs and results verified by: Marrilize Bylsma (Technical Manager)  
Classification (DEEEP) performed by: Lizet Moore (Quality Manager)

Report authorized by: Technical Signatory

Lizet Moore

Marrilize Bylsma

27 November 2018  
Report Date

## Table of contents

1. Analyses requested and sample description.....	3
2. Methodology .....	3
2.1 Sampling and sample handling .....	3
2.2 Bio-toxicity assessments .....	3
2.3 Toxicity test results classification system .....	5
3. Results and discussion .....	6
3.1 2018-10 survey - water .....	6
4. Literature references .....	7
END OF REPORT.....	7

## List of Tables

<b>Table 1:</b> Analyses requested and description for the different samples, including sampling and delivery dates.....	3
<b>Table 2:</b> Test results and risk classification for water samples during October 2018.....	6

## 1. Analyses requested and sample description

**Table 1:** Analyses requested and description for the different samples, including sampling and delivery dates.

Sample name	Sampling date	Sampled by	Delivery date	Delivered by	Additional comments (sample description or deviations)	Tests requested - Marked with X							
						Screening	Definitive	Water				Sediment	
								<i>Daphnia magna</i>	<i>Poecilia reticulata</i>	<i>Vibrio fischeri</i>	<i>Selenastrum capricornutum</i>	Phyto seeds	Ostracod
KF	2018/10/25	CSBS	2018/10/25	CSBS	None	X		X	X	X			
KGT	DRY												

Screening = 100% (undiluted) sample tested only

Definitive = Series of sample dilutions tested to enhance classification accuracy and to determine safe dilution

CSBS - Clean Stream Biological Services

## 2. Methodology

### 2.1 Sampling and sample handling

Refer to Technical Standard Operating procedures 05 & 06 (SOP05 & SOP06). These documents are available on request.

### 2.2 Bio-toxicity assessments

Acute (and short-chronic) toxicity testing (as applied for this assessment) is applied by exposing biota to water sources in order to determine the potential risk of such waters to the biota/biological integrity of the receiving water bodies. A risk category is determined based on the percentage of mortalities (or inhibition-stimulation) of the exposed biota. It is important to note that the hazard classification is based on the standardised battery of selected test biota and therefore represents the risk/hazard towards similar biota in the receiving aquatic environment. The **toxicity** hazard is therefore in terms of the aquatic biotic integrity and does in no way represent **toxicology** towards humans or other mammals.

Standard, internationally accepted methods and materials were applied in order to conduct acute and short-chronic toxicity testing and hazard classification based on 3 trophic levels (3 taxonomic groups, namely *Vibrio fischeri* (bacteria), *Daphnia magna* (crustaceans) and *Poecilia reticulata* (fish)) at each of the selected sites/samples, as specifically requested by Aquatico.

All tests were conducted in environmental controlled rooms using the following internationally standardized methods:

#### 2.2.1 *Vibrio fischeri* bioluminescent test (A)

Standard method: SANS 11348-3:2013

Deviation from standard method: None

Test species: *Vibrio fischeri* (NRRL B-11177)

Exposure period: 15 and 30 minutes

Test sample volume: 500 µl

Number of replicates: 2

Measurement equipment: Luminoscan TL, Hygiene Monitoring System

Test endpoint: Screening test - % growth inhibition or stimulation relative to control; Definitive test - EC20 and EC50 - values

Statistical method used: Biotox software (from supplier)/Manual plotting – Normalized regression of relevant data points

Batch numbers/expiry dates: VF 180328 / 2020-10; RD 180328 / 2020-10; SD 171214 / 2020-7

The percentage uncertainty for this test is 11.07% at a coverage factor of 2.36 and a level of confidence of 95%

Correction factor (validity of test): 0.79 (valid if between 0,6 & 1,8)

### 2.2.2 *Daphnia magna* acute toxicity test (A)

Standard method: SANS 6341:2015

Deviation from standard method: None

Test species: *Daphnia magna*

Test species age: Less than 24h old

Exposure period: 24 and 48h

Test sample volume: 25 ml

Number of test organisms per well: 5

Replicate number of wells per sample: 4

Test temperature: 21 ± 2°C

Test endpoint: Screening test - % mortality. Definitive test – LC<sub>10</sub> and LC<sub>50</sub> values

Statistical method used: Trimmed Spearman Karber (TSK)/ Graphical interpolation calculated by linear regression of relevant data points, EXCEL spread sheet

Batch numbers: Ehippia - 310518; ISO control medium – 080618

The percentage uncertainty for this test is 17.61% at a coverage factor of 2.05 and a level of confidence of 95%

Control mortality/immobility rate (validity of test): 0% (valid if ≤10%)

### 2.2.3 *Poecilia reticulata* acute toxicity test (A)

Standard method: OECD guide 203

Deviation from standard method: None

Test species: *Poecilia reticulata*

Test species age: Less than 21 days

Exposure period: 96h

Test sample volume: 200 ml

Number of test organisms per beaker: 6

Replicate number beakers per sample: 2

Test temperature: 21±2°C

Test endpoint: Screening test - %mortality; Definitive test – LC<sub>10</sub> and LC<sub>50</sub> values

Statistical method used: Trimmed Spearman Karber (TSK)/ Graphical interpolation calculated by linear regression of relevant data points, EXCEL spread sheet

Batch numbers: Control medium –080618

The percentage uncertainty for this test is 28.28% at a coverage factor of 2.36 and a level of confidence of 95%

Test validation: 0% control mortalities (valid if ≤10%)

### 2.2.4 pH (A)

Biotox Method 05

Test temperature: 25°C±3°C

Instrument used: HQ440D multimeter

The percentage uncertainty for this test is 0.01%(pH 2), 0.03% (pH 4), 0.01% (pH 7) and 0.10% (pH 10) at a coverage factor of 2 and a level of confidence of 95%

Batch numbers of buffers: pH4 – A7214

pH7 – A7222

pH10 – A7234

### 2.2.5 *Electrical conductivity* (A)

Biotox Method 06

Test temperature: 25°C±3°C

Instrument used: HQ440D multimeter

The percentage uncertainty for this test is 3.3% (1413µS/cm) and 0.23% (147µS/cm) at a coverage factor of 2 and a level of confidence of 95%

Batch numbers of buffers: 1413µS/cm - A8109

Quality assurance

The following quality assurance information would be made available on request:

- In-house reference toxicant test data and control charts.
- Additional lot, batch numbers and raw test data.
- Participation in proficiency testing scheme (SABS, NLA)

**2.3 Toxicity test results classification system**

A risk/hazard category was determined by application of the DEEEP<sup>1</sup> DWA recommended protocols and hazard classification. This risk category equates to the level of acute/chronic risk posed by the selected potential pollution source (water sample).

After the determination of the percentage effect<sup>1</sup> (EP), obtained with each of the battery of toxicity tests performed, the sample is ranked into one of the following five classes, based on screening testing protocols:

**Hazard classification system for screening tests**

<b>Class I</b>	<b>No acute/short-chronic environmental toxicity hazard</b> - none of the tests shows a toxic effect
<b>Class II</b>	<b>Slight acute/short-chronic environmental toxicity hazard</b> - a statistically significant percentage effect is reached in at least one test, but the effect level is below 50%
<b>Class III</b>	<b>Acute/short-chronic environmental toxicity hazard</b> - the percentage effect level is reached or exceeded in at least one test, but the effect level is below 100%
<b>Class IV</b>	<b>High acute/short-chronic environmental toxicity hazard</b> - the 100% percentage effect is reached in at least one test
<b>Class V</b>	<b>Very high acute/short-chronic environmental toxicity hazard</b> - the 100% percentage effect is reached in all the tests

**Weighting:** Each sample is furthermore weighted according to its relative toxicity levels (out of 100%). Higher values indicate that more of the individual tests indicated toxicity within a specific class.

<sup>1</sup> DEEEP = Direct Estimation of Ecological Effect Potential. This is a battery of tests that can measure toxicity of complex mixtures based on a set of parameters stemming from the results of effects, even if all constituents are not known. A hazard class is determined based on the resulting parameters of the battery of tests

<sup>1</sup> EP (Percentage effect) = an effect measured either as a mortality rate or inhibition rate (depending on the type of test). A 10% effect is regarded as slight acute toxicity for *Daphnia* and *Poecilia*, while a 20% effect is regarded as slight short-chronic toxicity for *Vibrio*. A 50% effect is regarded as an acute/short-chronic toxicity for all of the tests (*Daphnia*, *Poecilia* and *Vibrio*)

### 3. Results and discussion

#### 3.1 2018-10 survey - water

Refer to table 2 and table 3 below for individual test results and overall hazard classification of the different samples.

**Table 2:** Test results and risk classification for water samples during October 2018.

	Results	KF
woWater quality	pH @ 25°C (A)	7,7
	EC (Electrical conductivity) (mS/m) @ 25°C (A)	501,0
	Dissolved oxygen (mg/l) (NA)	7,6
V. fischeri (bacteria) (A)	Test started on yy/mm/dd	18/11/01
	%30min inhibition (-) / stimulation (+) (%)	44
	EC/LC20 (30 mins)	*
	EC/LC50 (30 mins)	*
	Toxicity unit (TU) / Description	no short-chronic hazard
D. magna (waterflea) (A)	Test started on yy/mm/dd	18/10/29
	%48hour mortality rate (-%)	0
	EC/LC10 (48hours)	*
	EC/LC50 (48hours)	*
	Toxicity unit (TU) / Description	no acute hazard
P. reticulata (guppy) (A)	Test started on yy/mm/dd	18/10/29
	%96hour mortality rate (-%)	0
	EC/LC10 (96hours)	*
	EC/LC50 (96hours)	*
	Toxicity unit (TU) / Description	no acute hazard
<b>Overall classification - Hazard class***</b>		Class I - No acute/short-chronic hazard
Weight (%)		0

**Key:**

\* = EC/LC values not determined, definitive testing required if a hazard was observed and persists over subsequent sampling runs.

\*\*\* = The overall hazard classification takes into account the full battery of tests and is not based on a single test result. Note that the overall hazard classification is expressed as acute/chronic level of toxicity, due to the fact that the *S. capricornutum* (micro-algae) and the *V. fischeri* tests are regarded as short-chronic levels of toxicity tests and the overall classification therefore contains a degree of chronic toxicity assessment.

**Weight (%)** = relative toxicity levels (out of 100%), higher values indicate that more of the individual tests indicated toxicity within a specific class.

site/sample name shaded in purple = screening test

site/sample name shaded in orange = definitive test

---

#### 4. Literature references

- ABOATOX Oy. 2012. BO1243-500 BioTox™ Kit. Instructions for use. Savikuja 2. FIN-21250, Masku Finland. [www.aboatox.com](http://www.aboatox.com)
- DEPARTMENT OF WATER AFFAIRS AND FORESTRY, 2003. The Management of Complex Industrial Waste Water Discharges. Introducing the Direct Estimation of Ecological Effect Potential (DEEEP) approach, a discussion document. Institute of Water Quality Studies, Pretoria.
- EUROPEAN Standard, 1998. "Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) – Part 3 for the method using freeze-dried bacteria", EN ISO 11348-3. European Committee for Standardization, Brussels.
- MICROBIOTEST INC. 2012. DAPHTOXKIT F™ MAGNA. Crustacean Toxicity Screening Test for freshwater. Standard Operational Procedure. Kleimoer 15, 9030 Mariakerke (Gent), Belgium. [www.microbiotest.be](http://www.microbiotest.be).
- PERSOONE G, BLAHOSLAV M, BLINOVA I, TörökNE A, ZARINA T, MANUSADZIANAS L, NALECZ-JAWECKI G, TOFAN L, STEPANOVA L, TOTHOVA L, KOLAR B. A practical and user-friendly toxicity classification system with Microbiotests for natural waters and wastewaters (personal communication).
- UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (US EPA), 1996. Ecological effects test guidelines. Fish acute toxicity test – Freshwater and marine. OPPTS 850.1075. Report number EPA-712-c-96-118.
- UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (US EPA), 1993. Method for measuring the acute toxicity of effluent and receiving waters to freshwater and marine organisms. EPA/600/4-90/027F, 4<sup>th</sup> edition. Office of Research and Development, Washington.

---

**END OF REPORT**

## TOXICITY TEST REPORT

**For:**  
Aquatico Scientific Services

Route 21, Corporate Park, 89 Regency Drive, Irene  
PO Box 905008, Garsfontein, 0042

**Survey:**  
2019-05

**Report reference:**  
RPM-A-19\_TOX

**Revision:**  
0

**Project:**  
Anglo Rustenburg Process

**Samples:**  
K035 (Klipgat Dam), K098 (ACP Dam),  
K160 (RBMR Dam 3A), K161 (RBMR Dam 3B),  
K162 (RBMR Triangular Dam), K209 (PMR Dam 2),  
K210 (PMR Dam 3A), K211 (PMR Dam 3B),  
K212 (PMR Dam 4+5), K213 (PMR Dam 6E)

Tests performed by: Marrilize Bylsma (Technical Manager), Marlise Brown (Senior Analyst)  
Inputs and results verified by: Marrilize Bylsma (Technical Manager), Marlise Brown (Senior Analyst)  
Classification (DEEEP) performed by: Marrilize Bylsma (Technical Manager)

Report authorized by: Technical Signatory

Lizet Moore

Marrilize Bylsma

12 June 2019  
Report Date

## Table of contents

1. Analyses requested and sample description .....	3
2. Methodology .....	3
2.1 Sampling and sample handling .....	3
2.2 Bio-toxicity assessments .....	3
2.3 Toxicity test results classification system .....	6
3. Results and discussion .....	7
3.1 2019-05 survey - water .....	7
4. Literature references .....	8
END OF REPORT .....	8

## List of Tables

<b>Table 1:</b> Analyses requested and description for the different samples, including sampling and delivery dates.....	3
<b>Table 2:</b> Test results and risk classification for water samples during May 2019. ....	7

## 1. Analyses requested and sample description

**Table 1:** Analyses requested and description for the different samples, including sampling and delivery dates.

Sample nameB2:015	Sampling date	Sampled by	Delivery date	Delivered by	Additional comments (sample description or deviations)	Tests requested - Marked with X								
						Screening	Definitive	Water					Sediment	
								<i>Daphnia magna</i>	<i>Poecilia reticulata</i>	<i>Allivibrio fischeri</i>	<i>Selenastrum capricornutum</i>	Phyto seeds	Ostracod	
K035(Klipgat Dam)	2019/05/23	Aquatico	2019/05/24	Aquatico	None	X	X	X	X					
K098 (ACP Dam)	2019/05/23	Aquatico	2019/05/24	Aquatico	None	X	X	X	X					
K160 (RBMR Dam3A)	2019/05/23	Aquatico	2019/05/24	Aquatico	None	X	X	X	X					
K161 (RBMR Dam 3B)	2019/05/23	Aquatico	2019/05/24	Aquatico	None	X	X	X	X					
K162 (RBMR Triangular Dam)	2019/05/23	Aquatico	2019/05/24	Aquatico	None	X	X	X	X					
K209 (PMR Dam2)	2019/05/23	Aquatico	2019/05/24	Aquatico	None	X	X	X	X					
K210 (PMR Dam 3A)	2019/05/23	Aquatico	2019/05/24	Aquatico	None	X	X	X	X					
K211 (PMR Dam 3B)	2019/05/23	Aquatico	2019/05/24	Aquatico	None	X	X	X	X					
K212 (PMR Dam 4+5)	2019/05/23	Aquatico	2019/05/24	Aquatico	None	X	X	X	X					
K213 (PMR Dam 6E)	2019/05/23	Aquatico	2019/05/24	Aquatico	None	X	X	X	X					

## 2. Methodology

### 2.1 Sampling and sample handling

Refer to Technical Standard Operating procedures 05 & 06 (SOP05 & SOP06). These documents are available on request.

### 2.2 Bio-toxicity assessments

Acute (and short-chronic) toxicity testing (as applied for this assessment) is applied by exposing biota to water sources in order to determine the potential risk of such waters to the biota/biological integrity of the receiving water bodies. A risk category is determined based on the percentage of mortalities (or inhibition-stimulation) of the exposed biota. It is important to note that the hazard classification is based on the standardised battery of selected test biota and therefore represents the risk/hazard towards similar biota in the receiving aquatic environment. The **toxicity** hazard is therefore in terms of the aquatic biotic integrity and does in no way represent **toxicology** towards humans or other mammals.

Standard, internationally accepted methods and materials were applied in order to conduct acute and short-chronic toxicity testing and hazard classification based on 3 trophic levels (3 taxonomic groups, namely *Allivibrio fischeri* (bacteria), *Daphnia magna* (crustaceans) and *Poecilia reticulata* (fish)) at each of the selected sites/samples specifically requested by Aquatico.

All tests were conducted in environmental controlled rooms using the following internationally standardized methods:

### 2.2.1 *Allivibrio fischeri* bioluminescent test (A)

Standard method: SANS 11348-3:2013

Deviation from standard method: None

Exposure period: 15 and 30 minutes

Test sample volume: 500 µl

Number of replicates: 2

Measurement equipment: Luminoscan TL, Hygiena Monitoring System

Test endpoint: Screening test - % growth inhibition or stimulation relative to control; Definitive test - EC20 and EC50 - values

Statistical method used: Biotox software (from supplier)/Manual plotting – Normalized regression of relevant data points

Batch numbers/expiry dates: VF 181212 / 2021-04; RD 181212 / 2021-04; SD 181212 / 2021-01

The percentage uncertainty for this test is 11.07% at a coverage factor of 2.36 and a level of confidence of 95%

Correction factor (validity of test): 1.3/1.6/1.2/1.1/1.5/0.9/1.7 (valid if between 0,6 & 1,8)

### 2.2.2 *Daphnia magna* acute toxicity test (A)

Standard method: SANS 6341:2015

Deviation from standard method: None

Test species: *Daphnia magna*

Test species age: Less than 24h old

Exposure period: 24 and 48h

Test sample volume: 25 ml

Number of test organisms per well: 5

Replicate number of wells per sample: 4

Test temperature: 21 ± 2°C

Test endpoint: Screening test - % mortality. Definitive test – LC<sub>10</sub> and LC<sub>50</sub> values

Statistical method used: Trimmed Spearman Karber (TSK)/ Graphical interpolation calculated by linear regression of relevant data points, EXCEL spread sheet

Batch numbers: Ehippia - 131218; ISO control medium – 080119

The percentage uncertainty for this test is 17.61% at a coverage factor of 2.05 and a level of confidence of 95%

Control mortality/immobility rate (validity of test): 0% (valid if ≤10%)

### 2.2.3 *Poecilia reticulata* acute toxicity test (A)

Standard method: OECD guide 203

Deviation from standard method: None

Test species: *Poecilia reticulata*

Test species age: Less than 21 days

Exposure period: 96h

Test sample volume: 200 ml

Number of test organisms per beaker: 6

Replicate number beakers per sample: 2

Test temperature: 21±2°C

Test endpoint: Screening test - %mortality; Definitive test – LC<sub>10</sub> and LC<sub>50</sub> values

Statistical method used: Trimmed Spearman Karber (TSK)/ Graphical interpolation calculated by linear regression of relevant data points, EXCEL spread sheet

Batch numbers: Control medium –080119

The percentage uncertainty for this test is 28.28% at a coverage factor of 2.36 and a level of confidence of 95%

Test validation: 0% control mortalities (valid if ≤10%)

#### 2.2.4 pH (A)

Biotox Method 05

Test temperature: 25°C±3°C

Instrument used: HQ440D multimeter

The percentage uncertainty for this test is 0.01% (pH 2), 0.03% (pH 4), 0.01% (pH 7) and 0.10% (pH 10) at a coverage factor of 2 and a level of confidence of 95%

Batch numbers of buffers: pH4 – A6124

pH7 – A8087

pH10 – A8317

#### 2.2.5 Electrical conductivity (A)

Biotox Method 06

Test temperature: 25°C±3°C

Instrument used: HQ440D multimeter

The percentage uncertainty for this test is 3.3% (1413µS/cm) and 0.23% (147µS/cm) at a coverage factor of 2 and a level of confidence of 95%

Batch numbers of buffers: 1413µS/cm - A8275

#### Quality assurance

The following quality assurance information would be made available on request:

- In-house reference toxicant test data and control charts.
- Additional lot, batch numbers and raw test data.
- Participation in proficiency testing scheme (SABS, NLA)

### 2.3 Toxicity test results classification system

A risk/hazard category was determined by application of the DEEEP<sup>1</sup> DWA recommended protocols and is broadly based on the hazard classification system of Persoone *et.al.* (2003). This risk category equates to the level of acute/chronic risk posed by the selected potential pollution source (water sample).

After the determination of the percentage effect<sup>1</sup> (EP), obtained with each of the battery of toxicity tests performed, the sample is ranked into one of the following five classes, based on definitive testing protocols:

#### Hazard classification system for definitive tests

<b>Class I</b>	<b>No acute/short-chronic environmental toxicity hazard</b> - none of the tests shows a toxic effect (i.e. an effect value significantly higher than that in the control)
<b>Class II</b>	<b>Slight acute/short-chronic environmental toxicity hazard</b> - the percentage effect observed in at least one toxicity test is significantly higher than in the control, but the effect level is below 50% (TU is <1)
<b>Class III</b>	<b>Acute/short-chronic environmental toxicity hazard</b> - the L(E)C50 is reached or exceeded in at least one test, but in the 10 fold dilution of the sample the effect level is less than 50% ( $1 \leq TU \leq 9,99$ )
<b>Class IV</b>	<b>High acute/short-chronic environmental toxicity hazard</b> - the L(E)C50 is reached in the 10 fold dilution for at least one test, but not in the 100 fold dilution ( $10 \leq TU \leq 99,99$ )
<b>Class V</b>	<b>Very high acute/short-chronic environmental toxicity hazard</b> - the L(E)C50 is reached in the 100 fold dilution for at least one test (TU is $\geq 100$ )

**Note:**  
The samples are classified into one of the above five classes on the basis of the highest toxicity unit (TU) found in the battery of toxicity definitive tests performed

**Weighting:** Each sample is furthermore weighted according to its relative toxicity levels (out of 100%). Higher values indicate that more of the individual tests indicated toxicity within a specific class.

<sup>1</sup> DEEEP = Direct Estimation of Ecological Effect Potential. This is a battery of tests that can measure toxicity of complex mixtures based on a set of parameters stemming from the results of effects, even if all constituents are not known. A hazard class is determined based on the resulting parameters of the battery of tests

<sup>1</sup> EP (Percentage effect) = an effect measured either as a mortality rate or inhibition rate (depending on the type of test). A >10% effect is regarded as slight acute toxicity for *Daphnia* and *Poecilia*, while a >20% effect is regarded as slight short-chronic toxicity for *Allivibrio*. A 50% effect is regarded as an acute/short-chronic toxicity for all of the tests (*Daphnia*, *Poecilia* and *Allivibrio*)

### 3. Results and discussion

#### 3.1 2019-05 survey - water

Refer to table 2 below for individual test results and overall hazard classification of the different samples.

**Table 2:** Test results and risk classification for water samples during May 2019.

Results		K035 (Klipgat Dam)	K098 (ACP Dam)	K160 (RBMR Dam 3A)	K161 (RBMR Dam 3B)	K162 (RBMR Triangular Dam)	K209 (PMR Dam 2)	K210 (PMR Dam 3A)	K211 (PMR Dam 3B)	K212 (PMR Dam 4+5)	K213 (PMR Dam 6E)
Water quality	pH @ 25°C (A)	7.3	2.7	3.2	5.2	10.5	1.2	9.8	9.4	5.5	5.6
	EC (Electrical conductivity) (mS/m) @ 25°C (A)	539.0	186.9	6340.0	3880.0	6090.0	10650.0	42.4	63.7	18940.0	24000.0
	Dissolved oxygen (mg/l) (NA)	9.4	7.5	7.4	7.5	7.3	7.6	8.6	7.8	7.6	7.5
A. fischeri (bacteria) (A)	Test started on yy/mm/dd	19/05/29	19/05/29	19/05/29	19/05/29	19/05/30	19/05/30	19/06/05	19/06/05	19/06/05	19/06/06
	%30min inhibition (-) / stimulation (+) (%)	55	-100	-100	-100	-100	-100	-97	-31	-100	-100
	EC/LC20 (30 mins)	n.r	2	n.c	n.c	2	n.c	36	89	n.c	n.c
	EC/LC50 (30 mins)	n.r	9	n.c	0.5	3	n.c	45	n.r.	4	3
	Toxicity unit (TU) / Description	<1	11.8	>100	25.6	29.5	>100	2.2	<1	27.1	30.6
D. magna (waterflea) (A)	Test started on yy/mm/dd	19/05/27	19/05/27	19/05/27	19/05/27	19/05/27	19/05/27	19/05/27	19/05/27	19/05/27	19/05/27
	%48hour mortality rate (-%)	-5	-100	-100	-100	-100	-100	-5	0	-100	-100
	EC/LC10 (48hours)	n.r	10	n.c	n.c	2	n.c	n.r	n.r	n.c	n.c
	EC/LC50 (48hours)	n.r	19	n.c	n.c	8	0.3	n.r	n.r	4	3
	Toxicity unit (TU) / Description	<1	5.4	>100	>100	12.9	>100	<1	<1	28.0	32.5
P. reticulata (guppy) (A)	Test started on yy/mm/dd	19/05/30	19/05/30	19/05/30	19/05/30	19/05/30	19/05/30	19/05/30	19/05/30	19/05/30	19/05/30
	%96hour mortality rate (-%)	0	-100	-100	-100	-100	-100	-25	0	-100	-100
	EC/LC10 (96hours)	n.r	12	n.c	n.c	10	2	70	n.r	5	2
	EC/LC50 (96hours)	n.r	18	n.c	0.3	17	6	n.r.	n.r	7	6
	Toxicity unit (TU) / Description	<1	5.7	>100	>100	5.9	18.2	<1	<1	14.3	18.2
Estimated safe dilution factor (%) [for definitive testing only]	None required	2	<1	<1	2	<1	36	89	<1	<1	
Overall classification - Hazard class***	Class I - No acute/short-chronic hazard	Class IV - High acute/short-chronic hazard	Class V - Very high acute/short-chronic hazard	Class V - Very high acute/short-chronic hazard	Class IV - High acute/short-chronic hazard	Class V - Very high acute/short-chronic hazard	Class III - Acute/short-chronic hazard	Class II - Slight short-chronic hazard	Class IV - High acute/short-chronic hazard	Class IV - High acute/short-chronic hazard	
Weight (%)	0	78	100	100	89	100	67	33	100	100	

**Key:**  
 % = for definitive testing, only the 100% concentration (undiluted) sample mortality/inhibition/stimulation is reflected by this summary table. The dilution series results are considered for EC/LC values and Toxicity unit determinations  
 n.r. = not relevant, i.e. the 100% concentration caused less than 10/20/50% (effective concentration) mortalities or inhibition  
 n.c. = not calculable, although the 100% concentration led to more than 10/20/50% mortalities/inhibition, the 10/20/50% mortality/inhibition rate was exceeded throughout the test  
 \*\*\* = The overall hazard classification takes into account the full battery of tests and is not based on a single test result. Note that the overall hazard classification is expressed as acute/short-chronic level of toxicity, due to the fact that the *A. fischeri* test is regarded as short-chronic level of toxicity test and the overall classification therefore contains a degree of short-chronic toxicity assessment.  
 Weight (%) = relative toxicity levels (out of 100%), higher values indicate that more of the individual tests indicated toxicity within a specific class  
 site/sample name shaded in purple = screening test  
 site/sample name shaded in orange = definitive test

Sample **K035 (Klipgat Dam)** showed “no acute/short-chronic environmental toxicity hazard” (Class I). Sample **K211 (PMR Dam 3B)** showed a “slight short-chronic environmental toxicity hazard” (Class II) based on the 31% bacterial light emission inhibition effect noted during testing (highest toxicity unit <1). Sample **K210 (PMR Dam 3A)** showed an “acute/short-chronic environmental toxicity hazard” (Class III) based on the highest toxicity unit (2.2) calculated on a bacterial level of testing. Samples **K098 (ACP Dam)**, **K162 (RBMR Triangular Dam)**, **K212 (PMR Dam 4+5)** and **K213 (PMR Dam 6E)** showed a “high acute/short-chronic environmental toxicity hazard” (Class IV) based on the 100% effect reached in at least one test for each of the samples with toxicity units ranging from 5.4 - >100. Samples **K160 (RBMR Dam 3A)**, **K161 (RBMR Dam 3B)** and **K209 (PMR Dam 2)** showed a “very high acute/short-chronic environmental toxicity hazard” (Class V) based on the toxicity units >100 calculated for these samples during testing at all 3 trophic levels. It should also be noted that the toxicity effects noted for K160 was so severe, that neither the LC/EC50 or the LC10/EC20 values could be calculated (thus the toxicity effect could not be diluted out up to a very low dilution concentration (0.195% of the original sample) . Refer to section 2.3 for details on hazard classification.

Very low safe dilution factors (<1) were calculated for samples K160, K161, K209, K212 and K213 and therefore water from these facilities should not be allowed to reach the natural environment. Safe dilution factors ranging between 2% and 89% were calculated for K035, K162, K210 and K211 (e.g. 2 parts of K098 water diluted with 98 parts ‘unpolluted’ water should be sufficient to negate toxicity effects at these trophic levels should these waters reach the natural environment).

**IMPORTANT:** Although sample K035(Klipgat Dam) was classified as Class I using the normal range of dilutions at a macro-invertebrate level, for dilutions 100% to 1% (dilutions from original sample) a normal decreasing toxicity effect trend was noted (resulting in the Class I classification). However from a 0.1% dilution level, significant mortalities was noted at this level of testing (100%). It is suggested that the facility investigates probable causes e.g. nano-materials affecting organisms more severely at lower concentrations. This can also be achieved by performing toxicity investigation evaluations (TIE). Even though K098 (ACP Dam) showed mortalities from the highest concentration (100% effect) and also following a normal decreasing trend up to 1% dilution (50% effect), the same significant increasing mortality effect was noted as for sample K035 (Klipgat Dam) at the 0.1% dilution level.

#### 4. Literature references

DEPARTMENT OF WATER AFFAIRS AND FORESTRY, 2003. The Management of Complex Industrial Waste Water Discharges. Introducing the Direct Estimation of Ecological Effect Potential (DEEEP) approach, a discussion document. Institute of Water Quality Studies, Pretoria.

OECD GUIDELINE FOR TESTING OF CHEMICALS (1992). Fish Acute toxicity test.

PERSOONE G, BLAHOSLAV M, BLINOVA I, TÖRÖKNE A, ZARINA T, MANUSADZIANAS L, NALECZ-JAWECKI G, TOFAN L, STEPANOVA L, TOTHOVA L, KOLAR B (2003). A practical and user-friendly toxicity classification system with Microbiotests for natural waters and wastewaters (personal communication).

SOUTH AFRICAN NATIONAL STANDARD, 2015. “Water quality – Determination of the inhibition of the mobility of *Daphnia magna* Straus (*Cladocera*, *Crustacea*) – Acute toxicity test.

SOUTH AFRICAN NATIONAL STANDARD, 2013. “Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test). Part 3: Method using freeze-dried bacteria

---

**END OF REPORT**

## TOXICITY TEST REPORT

**For:**

Aquatico Scientific (Pty) Ltd

89 Regency Drive, Route 21 Corporate Park, Irene  
PO Box 905008, Garsfontein, 0042

**Survey:**  
2019-06

**Report reference:**  
RPM-B-19\_TOX

**Revision:**  
0

**Project:**  
Anglo Platinum Process Division (RPM)

**Samples:**  
KF, KFD, KGT

Tests performed by: Marlise Brown (Senior Analyst), Praise Manyenga (Junior Analyst)  
Inputs and results verified by: Marrilize Bylsma (Technical Manager), Marlise Brown (Senior Analyst)  
Classification (DEEEP) performed by: Marrilize Bylsma (Technical Manager)

Report authorized by: Technical Signatory

Lizet Moore

Marrilize Bylsma

08 July 2019  
Report Date

## Table of contents

1. Analyses requested and sample description .....	3
2. Methodology .....	3
2.1 Sampling and sample handling .....	3
2.2 Bio-toxicity assessments .....	3
2.3 Toxicity test results classification system .....	5
3. Results and discussion .....	6
3.1 2019-06 survey - water .....	6
4. Literature references .....	7
END OF REPORT .....	7

## List of Tables

<b>Table 1:</b> Analyses requested and description for the different samples, including sampling and delivery dates.....	3
<b>Table 2:</b> Test results and risk classification for water samples during June 2019. ....	6

## 1. Analyses requested and sample description

**Table 1:** Analyses requested and description for the different samples, including sampling and delivery dates.

Sample name	Sampling date	Sampled by	Delivery date	Delivered by	Additional comments (sample description or deviations)	Tests requested - Marked with X							
						Screening	Definitive	Water				Sediment	
								<i>Daphnia magna</i>	<i>Poecilia reticulata</i>	<i>Allivibrio fischeri</i>	<i>Selenastrum capricornutum</i>	Phyto seeds	Ostracod
KF	2019/06/18	CSBS	2019/06/20	CSBS	None	X		X	X	X			
KFD	2019/06/18	CSBS	2019/06/20	CSBS	None	X		X	X	X			
KGT	DRY												

**Key:**

Screening = 100% (undiluted) sample tested only

Definitive = Series of sample dilutions tested to enhance classification accuracy and to determine safe dilution

## 2. Methodology

### 2.1 Sampling and sample handling

Samples were analysed as received from the Client. Refer to QM7.3/SOP-09.

### 2.2 Bio-toxicity assessments

Acute (and short-chronic) toxicity testing (as applied for this assessment) is applied by exposing biota to water sources in order to determine the potential risk of such waters to the biota/biological integrity of the receiving water bodies. A risk category is determined based on the percentage of mortalities (or inhibition-stimulation) of the exposed biota. It is important to note that the hazard classification is based on the standardised battery of selected test biota and therefore represents the risk/hazard towards similar biota in the receiving aquatic environment. The **toxicity** hazard is therefore in terms of the aquatic biotic integrity and does in no way represent **toxicology** towards humans or other mammals.

Standard, internationally accepted methods and materials were applied in order to conduct acute and short-chronic toxicity testing and hazard classification based on 3 trophic levels (3 taxonomic groups, namely *Allivibrio fischeri* (bacteria), *Daphnia magna* (crustaceans) and *Poecilia reticulata* (fish)) at each of the selected sites/samples as specifically requested by Clean Stream.

All tests were conducted in environmental controlled rooms using the following internationally standardized methods:

#### 2.2.1 *Allivibrio fischeri* bioluminescent test (A)

Standard method: SANS 11348-3:2013

Deviation from standard method: None

Exposure period: 15 and 30 minutes

Test sample volume: 500 µl

Number of replicates: 2

Measurement equipment: Luminoscan TL, Hygiene Monitoring System

Test endpoint: Screening test - % growth inhibition or stimulation relative to control; Definitive test - EC20 and EC50 - values

Statistical method used: Biotox software (from supplier)/Manual plotting – Normalized regression of relevant data points

Batch numbers/expiry dates: VF 181212 / 2021-04; RD 181212 / 2021-04; SD 181212 / 2021-01

The percentage uncertainty for this test is 11.07% at a coverage factor of 2.36 and a level of confidence of 95%

Correction factor (validity of test): 1.1 (valid if between 0,6 & 1,8)

### 2.2.2 *Daphnia magna* acute toxicity test (A)

Standard method: SANS 6341:2015  
 Deviation from standard method: None  
 Test species: *Daphnia magna*  
 Test species age: Less than 24h old  
 Exposure period: 24 and 48h  
 Test sample volume: 25 ml  
 Number of test organisms per well: 5  
 Replicate number of wells per sample: 4  
 Test temperature: 21 ± 2°C  
 Test endpoint: Screening test - % mortality. Definitive test – LC<sub>10</sub> and LC<sub>50</sub> values  
 Statistical method used: Trimmed Spearman Karber (TSK)/ Graphical interpolation calculated by linear regression of relevant data points, EXCEL spread sheet  
 Batch numbers: Ehippia - 280219; ISO control medium – 070319  
 The percentage uncertainty for this test is 17.61% at a coverage factor of 2.05 and a level of confidence of 95%  
 Control mortality/immobility rate (validity of test): 5% (valid if ≤10%)

### 2.2.3 *Poecilia reticulata* acute toxicity test (A)

Standard method: OECD guide 203  
 Deviation from standard method: None  
 Test species: *Poecilia reticulata*  
 Test species age: Less than 21 days  
 Exposure period: 96h  
 Test sample volume: 200 ml  
 Number of test organisms per beaker: 6  
 Replicate number beakers per sample: 2  
 Test temperature: 21±2°C  
 Test endpoint: Screening test - %mortality; Definitive test – LC<sub>10</sub> and LC<sub>50</sub> values  
 Statistical method used: Trimmed Spearman Karber (TSK)/ Graphical interpolation calculated by linear regression of relevant data points, EXCEL spread sheet  
 Batch numbers: Control medium –070319  
 The percentage uncertainty for this test is 28.28% at a coverage factor of 2.36 and a level of confidence of 95%  
 Test validation: 0% control mortalities (valid if ≤10%)

### 2.2.4 pH (A)

Biotox Method 05  
 Test temperature: 25°C±3°C  
 Instrument used: HQ440D multimeter  
 The percentage uncertainty for this test is 0.01%(pH 2), 0.03% (pH 4), 0.01% (pH 7) and 0.10% (pH 10) at a coverage factor of 2 and a level of confidence of 95%  
 Batch numbers of buffers: pH4 – A6124                      pH7 – A8219A                      pH10 – A8150

### 2.2.5 *Electrical conductivity* (A)

Biotox Method 06  
 Test temperature: 25°C±3°C  
 Instrument used: HQ440D multimeter  
 The percentage uncertainty for this test is 3.3% (1413µS/cm) and 0.23% (147µS/cm) at a coverage factor of 2 and a level of confidence of 95%  
 Batch numbers of buffers: 1413µS/cm - A8261

Quality assurance

The following quality assurance information would be made available on request:

- In-house reference toxicant test data and control charts.
- Additional lot, batch numbers and raw test data.
- Participation in proficiency testing scheme (SABS, NLA)

**2.3 Toxicity test results classification system**

A risk/hazard category was determined by application of the DEEEP<sup>1</sup> DWA recommended protocols and is broadly based on the hazard classification system of Persoone *et.al.* (2003). This risk category equates to the level of acute/chronic risk posed by the selected potential pollution source (water sample).

After the determination of the percentage effect<sup>1</sup> (EP), obtained with each of the battery of toxicity tests performed, the sample is ranked into one of the following five classes, based on screening testing protocols:

**Hazard classification system for screening tests**

<b>Class I</b>	<b>No acute/short-chronic environmental toxicity hazard</b> - none of the tests shows a toxic effect (i.e. an effect value significantly higher than that in the control)
<b>Class II</b>	<b>Slight acute/short-chronic environmental toxicity hazard</b> - a statistically significant (P<0,05) percentage effect is reached in at least one test, but the effect level is below 50%
<b>Class III</b>	<b>Acute/short-chronic environmental toxicity hazard</b> - the percentage effect level is reached or exceeded in at least one test, but the effect level is 50-99%
<b>Class IV</b>	<b>High acute/short-chronic environmental toxicity hazard</b> - the 100% percentage effect is reached in at least one test
<b>Class V</b>	<b>Very high acute/short-chronic environmental toxicity hazard</b> - the 100% percentage effect is reached in all the tests

**Weighting:** Each sample is furthermore weighted according to its relative toxicity levels (out of 100%). Higher values indicate that more of the individual tests indicated toxicity within a specific class.

<sup>1</sup> DEEEP = Direct Estimation of Ecological Effect Potential. This is a battery of tests that can measure toxicity of complex mixtures based on a set of parameters stemming from the results of effects, even if all constituents are not known. A hazard class is determined based on the resulting parameters of the battery of tests

<sup>1</sup> EP (Percentage effect) = an effect measured either as a mortality rate or inhibition rate (depending on the type of test). A >10% effect is regarded as slight acute toxicity for *Daphnia* and *Poecilia*, while a >20% effect is regarded as slight short-chronic toxicity for *Allivibrio*. A 50% effect is regarded as an acute/short-chronic toxicity for all of the tests (*Daphnia*, *Poecilia* and *Allivibrio*)

### 3. Results and discussion

#### 3.1 2019-06 survey - water

Refer to table 2 below for individual test results and overall hazard classification of the different samples.

**Table 2:** Test results and risk classification for water samples during June 2019.

	Results	KF	KFD
w <sub>0</sub> Water quality	pH @ 25°C (A)	8.4	8.4
	EC (Electrical conductivity) (mS/m) @ 25°C (A)	565.0	765.0
	Dissolved oxygen (mg/l) (NA)	7.8	8.1
A. fischeri (bacteria) (A)	Test started on yy/mm/dd	19/06/28	19/06/28
	%30min inhibition (-) / stimulation (+) (%)	21	33
	EC/LC20 (30 mins)	*	*
	EC/LC50 (30 mins)	*	*
	Toxicity unit (TU) / Description	no short-chronic hazard	no short-chronic hazard
D. magna (waterflea) (A)	Test started on yy/mm/dd	19/07/01	19/07/01
	%48hour mortality rate (-%)	-5	0
	EC/LC10 (48hours)	*	*
	EC/LC50 (48hours)	*	*
	Toxicity unit (TU) / Description	no acute hazard	no acute hazard
P. reticulata (guppy) (A)	Test started on yy/mm/dd	19/06/24	19/06/24
	%96hour mortality rate (-%)	0	0
	EC/LC10 (96hours)	*	*
	EC/LC50 (96hours)	*	*
	Toxicity unit (TU) / Description	no acute hazard	no acute hazard
<b>Overall classification - Hazard class***</b>	Class I - No acute/short-chronic hazard	Class I - No acute/short-chronic hazard	
Weight (%)	0	0	

**Key:**

\* = EC/LC values not determined, definitive testing required if a hazard was observed and persists over subsequent sampling runs  
 \*\*\* = The overall hazard classification takes into account the full battery of tests and is not based on a single test result. Note that the overall hazard classification is expressed as acute/short-chronic level of toxicity, due to the fact that the *A. fischeri* test is regarded as a short-chronic level of toxicity test and the overall classification therefore contains a degree of short-chronic toxicity assessment.  
 Weight (%) = relative toxicity levels (out of 100%), higher values indicate that more of the individual tests indicated toxicity within a specific class  
 site/sample name shaded in purple = screening test  
 site/sample name shaded in orange = definitive test

Samples **KF** and **KFD** showed “no acute/short-chronic environmental toxicity hazard” (Class I).

---

#### 4. Literature references

DEPARTMENT OF WATER AFFAIRS AND FORESTRY, 2003. The Management of Complex Industrial Waste Water Discharges. Introducing the Direct Estimation of Ecological Effect Potential (DEEEP) approach, a discussion document. Institute of Water Quality Studies, Pretoria.

OECD GUIDELINE FOR TESTING OF CHEMICALS (1992). Fish Acute toxicity test.

PERSOONE G, BLAHOSLAV M, BLINOVA I, TÖRÖKNE A, ZARINA T, MANUSADZIANAS L, NALECZ-JAWECKI G, TOFAN L, STEPANOVA L, TOTHOVA L, KOLAR B (2003). A practical and user-friendly toxicity classification system with Microbiotests for natural waters and wastewaters (personal communication).

SOUTH AFRICAN NATIONAL STANDARD, 2015. "Water quality – Determination of the inhibition of the mobility of *Daphnia magna* Straus (*Cladocera*, *Crustacea*) – Acute toxicity test.

SOUTH AFRICAN NATIONAL STANDARD, 2013. "Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test). Part 3: Method using freeze-dried bacteria

---

**END OF REPORT**