Research Article

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Microbial indicators and environmental relationships in the Umhlangane River, Durban, South Africa

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Abstract: The use of rivers for recreational and domestic practices makes it imperative to scrutinize the water quality circulating within surrounding communities. The complexity of biological, physical and chemical constituents in water is constantly evolving. This study evaluated various microbial and physico-chemical parameters in a polluted river system over a 12-month period. Apart from an increase in chemical pollutants, elevated levels of *E. coli*, total (TC) and faecal (FC) coliforms, and *Shigella* species could be attributed to faecal contamination entering the catchment. Canonical correspondence analysis revealed a strong relationship between FC, TC and temperature whereas moderate interactions was seen between total dissolved solids, electrical conductivity, TC and FC populations. Furthermore, close relationships between the bacterial and phage communities were also observed. The complex interactions of these physico-chemical and microbial indicators could be due to anthropogenic activities, changing climatic conditions and the excreta of infected and non-infected individuals entering the river. Assessing the complexity of aquatic ecosystems can aid in the development of novel, customizable, inexpensive water purification tools.

Keywords: water quality; physico-chemical parameters; bacterial indicators; coliphage; canonical correspondence analysis

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1 Introduction

Water is pivotal to human health, dignity, and socio-economic improvement. In semi-arid to arid countries like South Africa, water is particularly precious since changing climatic conditions can lead to drought [1]. At present several countries are facing water scarcity issues, coinciding with extreme political pressures [2] and rapid population growth [3]. Additionally, economic challenges are responsible for the lack of microbiologically safe water in developed and developing countries [4]. Rivers contribute significantly toward agricultural, industrial and domestic practices [5] and are constantly subject to flow and quality fluctuations due to these anthropogenic practices [6]. These fluctuations occur over the course of flow for example, from a mountain to a stream [7]. Since rivers are frequent recipients of contaminants from their terrestrial surroundings, they establish water-land interactions. As a result, these interactions alter the health of both the landscape and river itself [6]. Apart from ecological properties, the biological aspects of river ecosystems also affect water quality [8]. Although microbial communities are naturally present in water, the introduction of pathogenic microorganisms originating from excreta is of most concern [9]. At present, the most widely used water quality indicators include *Escherichia coli* (*E. coli*), total and faecal coliforms, as well as intestinal *Enterococci* [10-12]. In addition to bacterial indicators, bacteriophages, specifically the somatic and F-specific RNA coliphages as well as several physico-chemical constituents are also included in water quality analyses [13, 14]. Coliphages are advantageous since they are dependent on their bacterial hosts for replication and resemble the survival characteristics of enteric viruses [15].

The Umhlangane River, located in the heart of Durban is surrounded by a plethora of both developed and undeveloped societies. The river spans approximately 15 km in length, draining directly into the Umgeni River making it a key component in KwaZulu-Natal's drinking water supply [16]. The river is constantly exposed to

changing conditions while simultaneously serving as a conduit for receiving wastewater, storm and urban water runoffs as well as animal faecal matter [16]. Therefore, the ever-changing dynamics between microbial and environmental factors (natural or introduced) can affect water treatment processes as well as public health outcomes. Understanding these dynamics can aid in innovative, technological advancements in water quality testing and treatment. Therefore, the aim of this study was to assess the relationship between microbial and physico-chemical parameters in a highly polluted river system at different time and sampling points.

2 Materials and methods

2.1 Sampling procedure

Five litres of river water was collected at 5 sampling points (designated P1 to P5) as described in Table 1. Each sampling point encompassed different landscapes to assess its impact on the catchment. Water samples were collected monthly (second week of each month) commencing in October 2013 and concluding in September 2014. Samples were collected in plastic containers previously disinfected with 70% (v/v) alcohol. The containers were rinsed with river water prior to being plunged approximately 0.3-0.5 m subsurface to circumvent the disinfectant effect of ultraviolet light [17]. All water samples were transported on ice and processed within 48 h of collection. As previously described [12, 23], several physico-chemical and microbial indicators utilized in water quality testing were assessed and are described below.

2.2 Physico-chemical assessment

Water sample temperature was measured *in situ* (°C) using a thermometer. Salinity, electrical conductivity (EC) and total dissolved solids (TDS) were measured using the HACH CDC401 probe. Turbidity and pH was measured using the portable 2100P turbidometer and pH meter, respectively. The biological oxygen demand (BOD) and dissolved oxygen (DO) levels was measured using the HACH HQ40d portable meter and LD101 DO probe. Finally, chemical oxygen demand (COD) was assessed using a thermoreactor and photometer (HACH). All physico-chemical analyses were conducted using standard methods [18].

2.3 Bacterial indicator enumeration

Eight bacterial indicators were enumerated using the membrane filtration technique according to standard methods [18]. Appropriate dilutions of each water sample were made before filtering 50 ml through a 0.45 µm membrane filter (PALL) into a previously autoclaved glass filtration unit (GLASCO). The membrane filters were transferred onto 65 mm petri plates of selective media and incubated at specific incubation conditions (Table 2). Growth was enumerated as colony forming units per 100 mL (CFU/100 mL). The faecal coliforms to faecal streptococci (FC/FS) ratio was used to partially determine the source of faecal pollution present in the Umhlangane River. Ratios were calculated and compared to the standard FC/FS ratio for human and animal based pollution [19].

2.4 Somatic and F-specific RNA coliphage determinations

Escherichia coli WG5 and *Salmonella typhimurium* WG49 was used as the somatic and F^{+}RNA hosts, respectively [20, 21]. Appropriate dilutions of the concentrated (0.22 µm filtered) water samples were made prior to the bacteriophage enumeration assay. The double overlay agar technique was used to enumerate the somatic and F-specific RNA coliphages [20, 21]. Briefly, 1 mL of host culture and sample dilutions was added to 8 ml soft agar.

Table 1. GPS coordinates and description of the five sampling points.

Sampling Points	GPS Coordinates		Site Description
	Latitude	Longitude	
P1	29° 42' 47''S	30° 59' 33''E	Phoenix industrial
P2	29° 43' 35''S	31° 00' 21''E	Upstream KwaMashu wastewater treatment plant
P3	29° 43' 35''S	30° 00' 21''E	Natural wetlands
P4	29° 45' 39''S	30° 01' 11''E	Riverhorse Valley business estate
P5	29° 46' 10''S	30° 00' 24''E	Springfield industrial

Table 2. Bacterial indicators with their appropriate incubation conditions and selective media [23].

Bacterial Indicators	Selective media	Incubation conditions
Total heterotrophs	Nutrient agar	24 h at 37°C
*Escherichia coli	Chromocult agar	24 h at 37°C
Total coliforms	m-Endo agar	24 h at 35°C
Faecal coliforms	m-FC agar	24 h at 44.5°C
Faecal streptococci	KF-streptococcus agar	48 h at 42°C
*Vibrio	Thiosulphate citrate bile salts sucrose agar	18–24 h at 37°C
*Salmonella	Salmonella-Shigella agar	24 h at 35°C
*Shigella	Salmonella-Shigella agar	24 h at 35°C

Note: * indicates presumptive enumeration

The mixture was vortexed and poured over the agar plates. After the agar had solidified, the plates were inverted and incubated for 24 h at 37°C. Plaques were enumerated as plaque-forming units per millilitre (PFU/mL) [22].

2.5 Statistical analysis

Correlation between the sampling months, points, physico-chemical parameters and microbial indicators was determined using the Pearson's correlation test (Student's *t*-test) in SPSS v.22 (SPSS Inc., Illinois). The level of significance was set at $p < 0.01$ and 0.05 [23]. Multivariate canonical correspondence analysis (CCA) was used to evaluate the relationship between the environmental and microbial indicators at every sampling point and month during the sampling period. Correlations were generated in an ordination bi-plot where the length of an arrow indicates a rate of change. Therefore, a longer arrow indicates a larger rate of change in the variable being investigated. A Monte Carlo permutation test of 499 random permutations was used to calculate the significance of the axes within the species data. Canoco v. 4.5 was used to determine the CCA statistical ordination plots [24].

Ethical approval: The conducted research is not related to either human or animals use.

3 Results

3.1 Physico-chemical analyses

Table 3 depicts the physico-chemical measurements at all sampling months and points along the Umhlangane River. Temperature varied throughout the sampling period ranging from 18°C (July and September 2014) to 28.5°C (January 2014). The pH of river water samples ranged from 6.00 to 9.04.

The BOD and COD content fluctuated throughout all sampling months and points with BOD ranging from 0.48 mg/L to 12.4 mg/L. A moderate correlation (r=-0.622; p<0.000) was observed between BOD and the sampling points. A COD value of <10 mg/L was recorded at sampling points P2 and P3 (February 2014), P3 (April 2014), P2–P5 (May 2014), P3 and P4 (June 2014) and at P2, P3 and P5 (September 2014). The highest COD measurement was recorded at P1 in May 2014 with a value of 269 mg/L. A significant difference (p<0.05) was observed between COD and the sampling points. The DO measurements ranged from 3.28 mg/L to 9.46 mg/L.

The TDS and EC values varied throughout the sampling period and points. The lowest and highest TDS values were observed at P5 in December 2013 (201 mg/L) and P2 in February 2014 (430 mg/L), respectively. The EC values ranged from 425 mS/m to 869 mS/m. Salinity fell within the range of 0.21% to 0.43% while turbidity ranged from 1.16 NTU to 62.4 NTU.

Table 3. Physico-chemical parameters recorded at all sampling points from October 2013 to September 2014.

Points	Months	Temp (°C)	pH	BOD (mg/L)	COD (mg/L)	DO (mg/L)	TDS (mg/L)	Turbidity (NTU)	EC (mS/m)	Salinity (%)
P1	October 2013	24.0	7.30	11.1	35.0	8.07	361	9.85	737	0.36
P2		23.5	7.32	4.48	19.0	8.10	263	6.04	542	0.26
P3		22.0	6.51	4.48	23.0	8.36	306	6.01	627	0.30
P4		23.0	6.98	3.06	22.0	7.99	331	5.08	677	0.33
P5		23.0	6.92	2.89	15.0	8.33	330	5.42	678	0.33
P1	November 2013	24.0	6.20	10.5	24.0	7.16	379	12.1	763	0.38
P2		24.0	7.00	8.07	18.0	8.03	240	11.9	630	0.25
P3		23.0	6.45	4.01	12.0	8.42	232	4.80	601	0.33
P4		21.0	6.04	3.20	14.0	7.79	328	5.72	648	0.31
P5		22.0	6.06	3.19	7.00	8.07	329	6.12	699	0.31
P1	December 2013	27.5	7.11	3.20	19.0	8.44	426	7.72	789	0.37
P2		27.0	7.59	3.99	16.0	8.14	374	7.73	671	0.23
P3		25.0	6.00	0.99	14.0	8.53	361	6.15	425	0.22
P4		26.0	6.04	1.42	7.00	9.02	218	3.30	598	0.24
P5		25.0	6.19	2.12	3.00	8.80	201	3.98	777	0.26
P1	January 2014	28.5	8.05	9.99	58.0	8.00	386	10.1	788	0.38
P2		28.0	8.00	6.14	47.0	7.97	278	9.40	572	0.28
P3		27.0	7.16	2.16	23.0	8.29	310	5.70	637	0.31
P4		27.0	7.09	1.41	31.0	8.07	316	5.64	650	0.32
P5		26.0	6.50	1.30	33.0	8.17	327	2.11	672	0.33
P1	February 2014	27.0	6.11	5.60	175	7.87	424	14.4	778	0.31
P2		25.0	6.20	4.31	<10	7.16	430	13.7	530	0.25
P3		25.0	6.01	0.97	<10	8.94	325	8.90	489	0.25
P4		24.5	6.56	1.40	18.0	8.61	311	7.22	579	0.21
P5		24.0	6.91	2.15	36.0	8.75	242	4.50	780	0.27
P1	March 2014	27.0	6.16	7.44	42.0	7.99	299	18.5	615	0.30
P2		26.5	6.27	6.98	1.00	8.50	230	12.1	477	0.23
P3		26.0	6.14	1.63	35.0	7.64	230	24.1	476	0.23
P4		23.0	6.09	2.11	68.0	3.28	278	17.3	578	0.28
P5		21.0	6.33	2.87	14.0	7.68	307	9.12	631	0.31
P1	April 2014	25.0	7.99	7.71	34.0	7.97	234	6.32	474	0.24
P2		25.0	7.18	7.65	26.0	6.42	225	5.41	466	0.22
P3		24.5	6.02	2.16	<10	7.35	273	6.21	562	0.27
P4		22.0	6.24	0.48	34.0	7.00	290	4.36	597	0.29
P5		22.0	6.23	1.91	30.0	7.29	295	1.16	606	0.29
P1	May 2014	25.0	8.92	2.40	269	7.79	360	11.9	737	0.36
P2		21.0	8.56	7.56	<10	8.15	275	9.48	566	0.27
P3		22.0	7.75	0.76	<10	8.47	293	6.79	602	0.29
P4		21.5	7.95	2.99	<10	8.51	302	7.89	621	0.30
P5		22.0	7.76	6.21	<10	8.83	311	8.10	639	0.31

Points	Months	Temp (°C)	pH	BOD (mg/L)	COD (mg/L)	DO (mg/L)	TDS (mg/L)	Turbidity (NTU)	EC (mS/m)	Salinity (%)
P1	June 2014	20.0	9.04	12.4	72.0	9.46	388	3.32	655	0.36
P2		20.0	8.87	6.74	6.00	8.77	301	3.68	650	0.29
P3		21.0	6.52	3.22	<10	6.09	269	5.05	425	0.26
P4		20.0	6.30	4.01	<10	7.76	284	5.80	672	0.31
P5		21.0	8.40	4.12	2.00	8.00	312	4.16	691	0.33
P1	July 2014	20.0	8.98	1.93	31.0	8.57	426	12.1	869	0.43
P2		19.0	8.05	8.41	19.0	5.66	267	6.18	552	0.27
P3		18.0	7.20	3.10	30.0	9.17	287	9.79	592	0.29
P4		19.0	7.14	3.18	32.0	8.19	297	7.96	611	0.30
P5		19.0	6.99	5.92	30.0	8.58	314	5.98	646	0.31
P1	August 2014	21.0	8.16	9.27	42.0	6.42	267	8.48	549	0.27
P2		21.0	8.22	5.47	37.0	4.84	233	8.71	484	0.23
P3		20.0	6.29	3.21	27.0	5.21	298	8.07	626	0.30
P4		19.0	6.11	2.92	28.0	6.88	290	7.23	596	0.29
P5		19.0	7.12	3.07	24.0	8.08	291	4.26	599	0.29
P1	September 2014	20.0	7.20	10.8	27.0	7.35	334	62.4	684	0.33
P2		21.0	7.01	10.3	<10	5.95	233	16.4	482	0.23
P3		19.5	6.92	4.62	<10	6.64	279	12.5	574	0.28
P4		18.0	6.68	3.04	71.0	6.89	301	12.1	619	0.30
P5		21.0	8.76	3.28	<10	6.76	316	8.57	650	0.32

Note: Temp, temperature; BOD, biological oxygen demand; COD, chemical oxygen demand; DO, dissolved oxygen; TDS, total dissolved

3.2 Bacterial indicator analysis

Total heterotrophic bacteria (THB) and presumptive *E. coli*, total coliforms (TC) and faecal coliforms (FC) fluctuated at all sampling points and months along the Umhlangane River (Figure 1a–d). The lowest and highest THB counts were detected at P3 (natural wetlands) and P1 (Phoenix industrial) with values ranging from 1.3 x 10^6 CFU/100 mL to 14.9 x 10^6 CFU/100 mL, respectively. The *E. coli* population ranged from 0.87 x 10^3 CFU/100 mL to 6.1 x 10^3 CFU/100 mL. The TC and FC counts ranged from 0.63 x 10^3 CFU/100 mL to 5.9 x 10^3 CFU/100 mL and 0.36 x 10^3 CFU/100 mL to 6.6 x 10^3 CFU/100 mL, respectively.

Presumptive faecal streptococci (FS), *Vibrio* spp. (VIB), *Salmonella* spp. (SAL) and *Shigella* spp. (SHIG) were enumerated at all sampling months and points (Figure 1e–h). FS counts ranged from 0.4 x 10^3 CFU/100 mL to 7.5 x 10^3 CFU/100 mL. The VIB counts depicted its lowest and highest values at P3 in March and February 2014 respectively, with values ranging from 0.5 x 10^3 CFU/100 mL to 7.2 x 10^3 CFU/100 mL. The SAL and SHIG counts ranged from 0.27 x 10^3 CFU/100 mL to 6.5 x 10^3 CFU/100 mL and 0.43 x 10^3 CFU/100 mL to 5.5 x 10^3 CFU/100 mL, respectively. Interestingly, a significant difference ($p<0.01$) was observed between every bacterial indicator and the sampling month but not with the sampling points.

While increased bacterial growth was observed during the warmer months (October–March) gradual decreases in colony counts was observed as the conditions became colder (May–August). Moreover, greater bacterial counts were observed at industrial sites P1, P2 and P5. Of note, the THB population depicted strong correlations with *E. coli* ($r=0.753$; $p<0.000$) and TC ($r=0.843$; $p<0.000$). Finally, the average FC/FS ratios, observed in Table 4 ranged from 0.41 and 1.19 in April and August 2014, respectively. Furthermore, October 2013 and April–May 2014 indicated strong evidence for animal pollution while the remaining months were predominately domestic waste in a mixed population (Table 4).

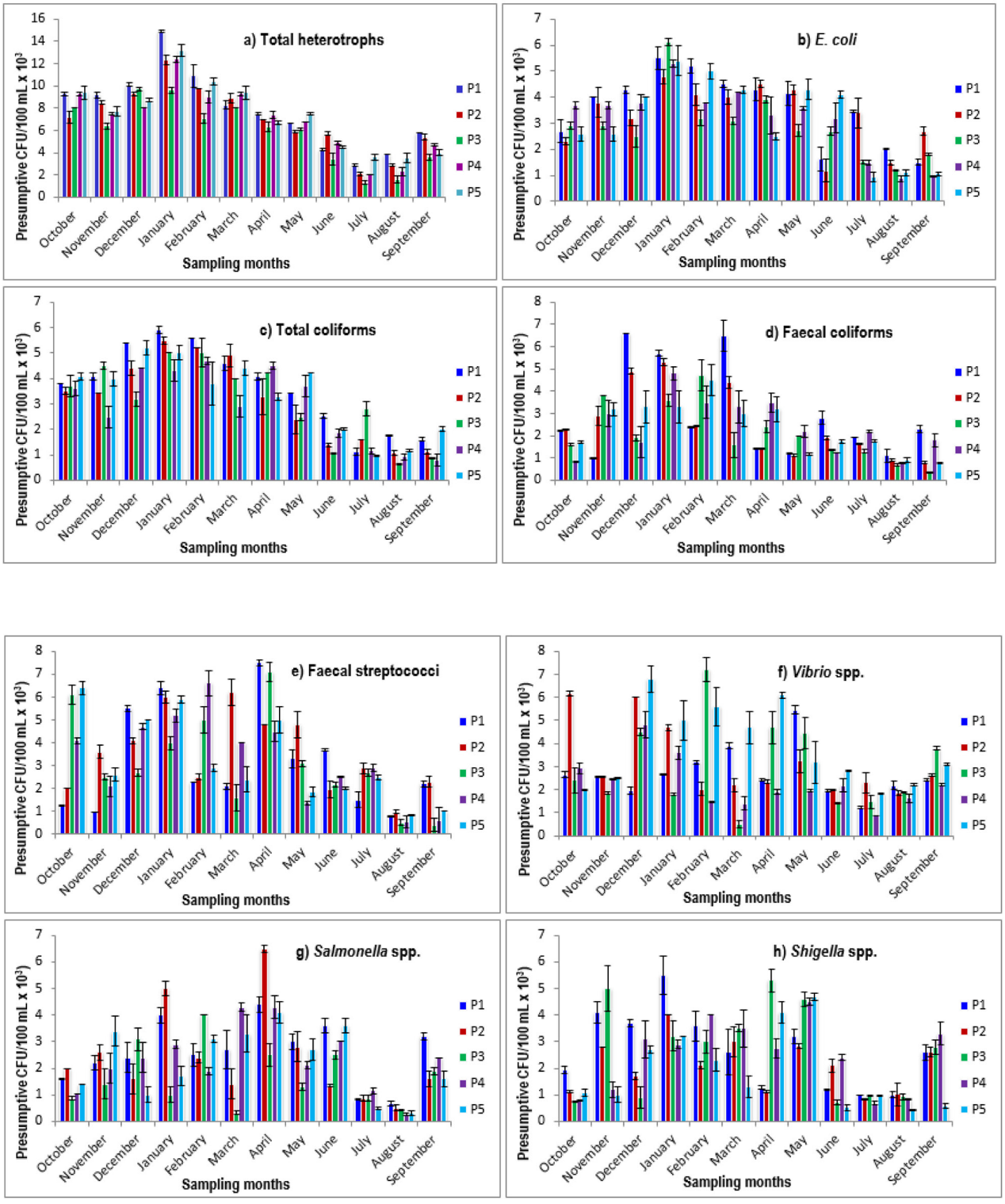

Figure 1 Presumptive (a) total heterotrophs, (b) *E. coli*, (c) total coliforms (d) faecal coliforms (e) faecal streptococci, (f) *Vibrio* spp., (g) *Salmonella* spp. and (h) *Shigella* spp. counts at the five sampling points along the Umhlangane River from October 2013 to September 2014. Bars indicate the averages (n=2) while standard deviation is depicted by the error bars.

Table 4. Average faecal coliform (FC) to faecal streptococci (FS) ratio from October 2013 to September 2014

Sampling months	FC/FS ratio	Sampling months	FC/FS ratio	Sampling months	FC/FS ratio
October	0,44[a]	February	0,91[b]	June	0,73[b]
November	1,18[b]	March	1,15[b]	July	0,70[a]
December	0,84[b]	April	0,41[a]	August	1,19[b]
January	0,83[b]	May	0,53[b]	September	0,93[b]

[a] Pollution is of animal origin

[b] Pollution is predominantly domestic wastes in mixed pollution

3.3 Somatic and F+RNA enumeration

The somatic and F⁺RNA coliphage counts are depicted in Figure 2. Moderate negative correlations were seen between the somatic (r=-0.653; p<0.000) and F⁺RNA (r=-0.643; p<0.000) coliphages and the time of sampling. Interestingly, both coliphages depicted the lowest counts at P3 (natural wetlands) in August 2013 and the highest at P1 (Phoenix industrial) in January 2014 ranging from 24.5 x 10^2 PFU/mL to 765 x 10^2 PFU/mL and 10 x 10^2 PFU/mL to 585 x 10^2 PFU/mL, respectively. The somatic and F⁺RNA coliphages displayed a similar trend to that of the bacterial indicators (Figure 1) wherein increased growth was observed during the warmer months in comparison to the colder sampling period. Lastly, a strong positive correlation (r=0.977; p<0.000) was seen between the somatic and F⁺RNA coliphage populations.

3.4 Canonical correspondence analysis

The ordination bi-plot for the physico-chemical and microbial indicators during the sampling months and points are depicted in Figure 3a–d. The bi-plot revealed a strong relationship between FC, TC and temperature while a moderate relationship was seen between TDS, EC, TC and FC populations. The THB populations correlated with salinity, TDS and EC. While an interaction was observed between turbidity, BOD and the SHIG population, pH and COD shared a strong relationship with *E. coli*. The bi-plot also revealed that the SAL, VIB and FS populations were not strictly associated with the physico-chemical parameters or other bacterial indicators. The variance of species data for CCA axis 1 was 8.6% with the species–environment relation equal to 72.2% (Figure 3a). This suggests strong variance between the physico-chemical and bacterial data compared to the species data alone.

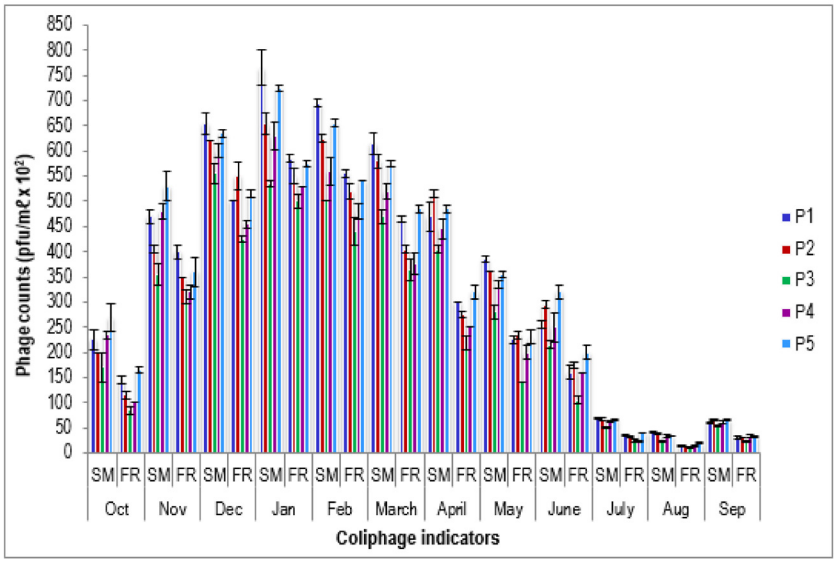

Figure 2 Somatic and F⁺RNA coliphage counts for the five sampling points along the Umhlangane River from October 2013 to September 2014. Bars indicate the averages (n=2) while standard deviation is depicted by the error bars. (SM: somatic phage; FR: F⁺RNA phage)

CCA also revealed that most sampling points from April to September (Winter–Spring; dry season) was near pH whereas temperature, salinity, TDS and EC indicated strong associations with most of the sampling points during the warmer months (January–March) (Figure 3b).

The CCA bi-plot revealed no direct relationship between the somatic and F⁺RNA coliphages and weak relations was observed between the physico-chemical and phage communities (Figure 3c). Bacteriophage populations during January and March were found to have a stronger association with turbidity, EC, temperature, DO and TDS. In the winter period phage populations were strongly associated with COD, salinity, pH and BOD. However, most points were found scattered suggesting that the sampling points at each month had a stronger relationship with the physico-chemical parameters rather than with the phage itself. Moreover, strong associations between the THB, TC, *E. coli*, SHIG and the somatic and F⁺RNA coliphages were observed (Figure 3d). This suggests that these bacterial communities contributed to the variance and prevalence of phage in the river water.

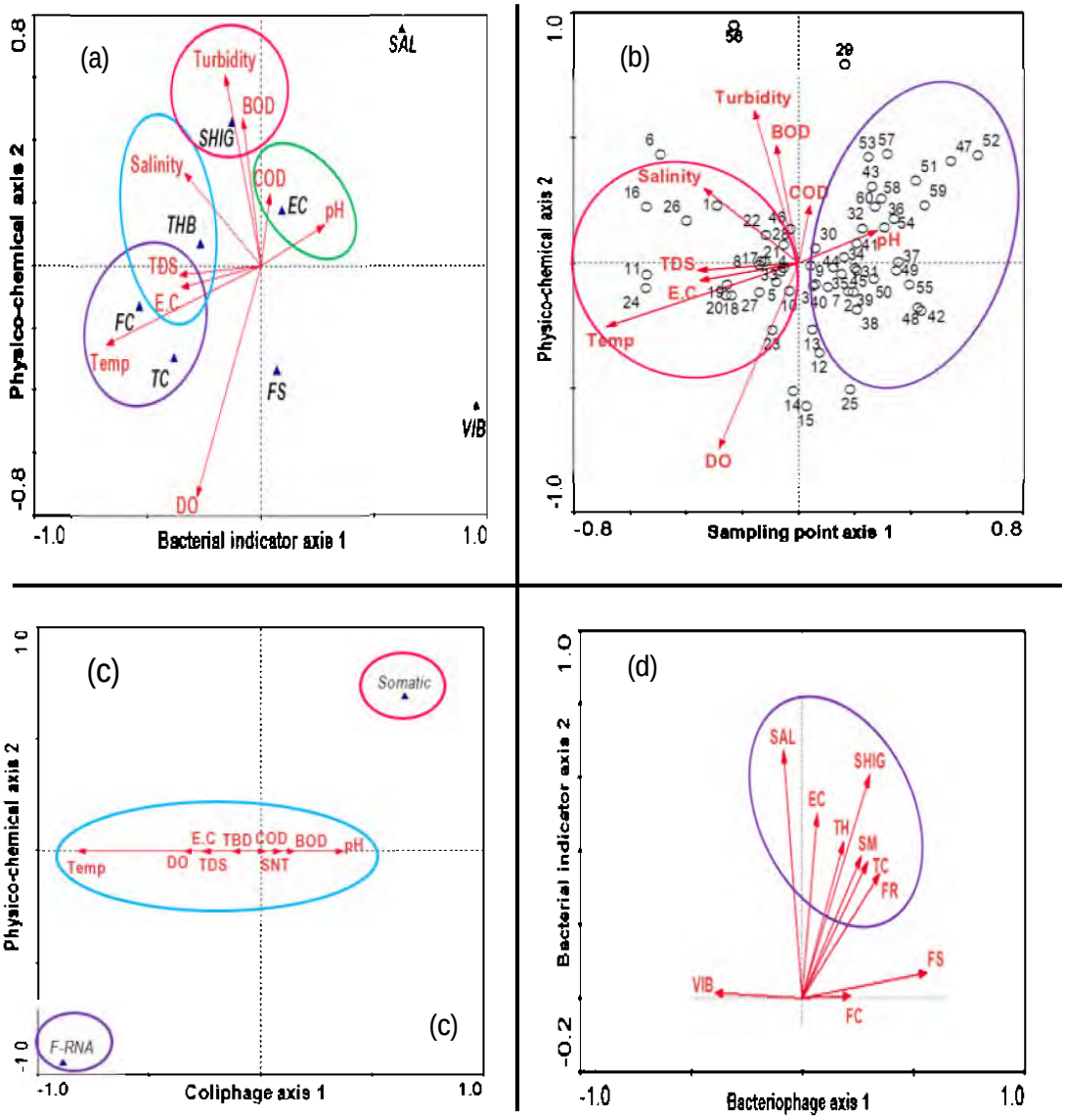

Figure 3 CCA bi-plots for the (a) physico-chemical and bacterial indicators, (b) physico-chemical, bacterial indicators and sampling points, (c) physico-chemical and coliphages and (d) coliphage and bacterial pollutions at the five sampling points from October 2013 to September 2014. Numbers 1–60 indicates sequential, continuous numbering of the sampling points at each month. (Temp: temperature; DO: dissolved oxygen, BOD: biological oxygen demand; COD: chemical oxygen demand; TDS: total dissolved solids; E.C: electrical conductivity; SNT: salinity; VIB: *Vibrio*; SHIG: *Shigella*; SAL: *Salmonella*; EC: *Escherichia coli*; TH: total heterotrophs; FC: faecal coliforms, TC: total coliforms; FS: faecal streptococci; SM: somatic phage; FR: F⁺RNA phage)

4 Discussion

This study evaluated the physico-chemical and biological properties of the Umhlangane River over a 12-month period at five different sampling points. High levels of BOD and COD have been shown to affect both the taste and odour of water sources [5]. The BOD and COD tests were used to quantify the degradation of organic and inorganic matter, respectively. No recommendations have been made regarding the maximum BOD limit for recreational or industrial use [13, 25]. However, <4 mg/L has been previously suggested as an acceptable range [26]. In this study, most BOD measurements did not fall within this limit. The COD content of the river water samples was found to exceed the recommended water quality limit of 0–10 mg/L for industrial use. While the natural lifecycle of many aquatic organisms contributes to the increased organic matter [27], agricultural, pasture, as well as urban and industrial waste further adds to these estimations [13]. Therefore, the differential landscapes may have contributed to higher COD levels in this instance. These observations were reiterated by the fluctuating DO content [28].

Since the flow of effluents and debris into rivers increases the turbidity during the wet periods [29], higher values were recorded during the rainy months (December–March). All TDS and EC values exceeded the permissible limit of 0–100 mg/L and 0–15 mS/m for industrial use, respectively [30]. However, the slight decrease in TDS and EC at P3 and P4 may be due to self-purification processes by natural wetlands along the river [5].

Faecal indicator bacteria are employed for the detection of pathogenic microorganisms, faecal pollution and the risk of transmissible waterborne infections [31]. The occurrence of these coliforms (TC, FC, *E. coli* and FS) suggests the entry of faecal contamination into the river [5]. The estimation of THB populations relates to poor water quality [13]. These contaminations are frequently manifested through diarrhoea and on occasion fever and other secondary complications [32]. According to the South African water quality guidelines for recreational use, the permissible limit for negligible risk to these bacteria were exceeded at all study points [25].

Vibrios have been associated with domestic sewage and can cause illness in both animals and humans if contaminated food and water has been consumed [33]. Since most *Vibrio* spp. enumerated are of animal origin [34], these estimations may be due to faecal matter from passing cattle along the low cost residential farmers in KwaMashu and Phoenix. However, since most *Vibrio* spp. exist in the viable but non-cultivable state (VBNC), their growth could be underestimated [35]. The presence of *Salmonella* spp. and *Shigella* spp. in the Umhlangane is a major health concern. Studies by [36] stated that 57% of all *Salmonella* occurrences in river water are due to pasture and agricultural runoffs as well as the inflow of animal and human faecal matter [36].

The intimate relationship found between the physico-chemical parameters, bacterial indicators and the sampling points and months indicates that microbial survivability is dependent on environmental and climatic conditions. Since the presence of bacteriophages are dependent on the survival of their respective bacterial hosts [37], similar replication trends were observed for both the coliphage and bacterial indicator populations. Although somatic coliphages usually outnumber F^{+}RNA coliphages by a factor of 5 [38], over-estimation is possible as these coliphages have been shown to replicate in river water [39]. The DWA (recreational use) recommended limit of 0–20 PFU/mL was exceeded by all tested river water samples [25]. Bacterial community structures have been affected by various factors such as light intensity [40], topographical environment [41], temperature [42], available nutrients [43] and pH [44]. The relationship between *E. coli*, COD and pH suggests that the proliferation of these coliforms is dependent on the amount of inorganic wastes and nutrients [45], whereas the prevalence of *Shigella* spp. was predominantly influenced by the lack of oxygen and suspended matter. The CCA bi-plot also revealed that salinity had an impact on the THB populations in the river water. Fluctuating anthropogenic activities may be one of the main drivers of the coliphage prevalence [46,47] in the Umhlangane River.

5 Conclusions

This study evaluated the Umhlangane River over a 12-month sampling period at five different points. The main findings of this study showed that elevated bacterial and phage populations were observed during the warmer months together with turbidity, TDS, E.C. and salinity. Furthermore, the change in phage and bacterial enumeration along the sampling points demonstrates that the complexity (mainly animal pollution) of the pollution at each land use zone played a pertinent role in microbial proliferation. Using this information novel, customizable and inexpensive water purification tools can be developed.

6 Study limitations

This study identified several microbial indicators commonly associated with polluted water systems. Although these data provided strong evidence of the complex relationships observed between microbial indicators and the physico-chemical environment, some limitations were noted. While bacteria belonging to the *Salmonella*, *Shigella* and *Vibrio* genus were identified, molecular testing is required to confirm its presence in the river. This is particularly important since these pathogens pose a serious public health risk. Additionally, future work characterizing these microbial pathogens using phylogenetic analyses can provide important information regarding the evolution and survival of these populations in this river. Moreover, to fully assess the impact of anthropogenic wastes on water quality dynamics, other trace elements such as lead, cadmium and zinc needs to be evaluated.

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