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Physicochemical and FT-IR analyses of some freshwater and inflow wastewater-treated effluents in the Eastern Cape Province of South Africa

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Abstract

Monitoring changes occurring in water bodies is a tool to make a sound decision on water quality management. This work investigates water quality parameters and metabolites in the Bloukrans, Buffalo, Swartkops and Tyhume Rivers in the Eastern Cape Province of South Africa. The water physicochemical parameters assessed include the dissolved oxygen, oxidation-reduction potentials, temperature, Hydrogen ion concentration, salinity, conductivity, turbidity and total dissolved solids measured with a pre-calibrated Hanna Multiparameter instrument on-site. Chemical oxygen demand, phosphate, chloride, ammonium, sulphate and nitrate ions were measured in the laboratory with a UVmini spectrophotometer. FT-IR analysis of extracted metabolites was done on a Perkin Elmer FT-IR spectrometer. The result shows that upstream river samples have lower COD values and below the detection level in some. The concentrations of phosphate, chloride, ammonium, sulphate and nitrate ions were above the recommended values in the wastewater effluents, midstream and downstream river samples. FT-IR peaks similar to vinylidene, saturated aliphatic aldehydes, aryl aldehydes, primary amines, hydantoin amides, halogen, and organofluorides were common in the wastewater effluent samples and the receiving rivers. Grahamstown treated wastewater effluents and its receiving Bloukrans River were distinguished with absorption peaks of urethane and aromatic aldehydes. Absorption peaks of sulphonyl halides were present only on the spectra of treated wastewater effluents of Alice and the receiving downstream Tyhume River. Multivariate analysis shows correlations between the water samples from different geographical locations and treated wastewater effluents. It is concluded that the quality of the rivers was very poor and more efforts are needed to make the treated wastewater conform to the acceptable standard for a sustainable aquatic ecosystem.

Keywords: Freshwater; Spectroscopy; Pollution; Metabolites; Multivariate analysis

1. Introduction

The surface waters are currently in crisis globally as recipients of human-generated wastes (Cobbing and de Witt, 2018). Even when these wastes are released into the air or soil, they are eventually washed into water bodies where they contribute to water pollution, which has become a global problem. Human activities and population increase have led to more waste (World Bank, 2019). The United Nations Organisation's (UNO) Sustainable Development Goals (SDGs) article 6 is to "ensure availability and sustainable management of water and sanitation for all" (UNDP 2019). Water quality around the world has been in danger due to the increasing population, which is threatening human health, food security and biodiversity (Rafi et al., 2019; World Bank, 2019). Nutrient enrichment due to run-off from agricultural inputs such as pesticides and other agrochemicals, mining wastes, and untreated and poorly treated municipal wastewater are other primary sources of surface water pollution. United Nations Environmental Programme (UNEP) estimates that 90% of the global municipal wastewater released to receiving water bodies is untreated (UNEP, 2019). Wastewater may be classified as domestic (with excreta, urine and faecal sludge), institutional wastewater (from establishments, institutions and hospitals), industrial and urban run-off and agricultural wastewater. Poorly treated

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wastewaters alter the characteristics of the receiving water bodies thereby causing a shift in the natural balance onto which aquatic organisms already adjusted (Jin et al., 2017; Edokpayi et al., 2017).

Water quality parameter takes into consideration the physicochemical characteristics among others. Chemical oxygen demand (COD) is the amount of oxygen available for consumption in oxidation reactions in water bodies (Geerdink et al., 2017). In water research, COD is an indirect measure of the amount of organic compounds or oxidisable pollutants in water (Dhanjai et al., 2019). Dissolved oxygen (DO) is the amount of molecular oxygen available in the water for the respiration of aquatic organisms, it is an index of the life-supporting ability of the water. Most aquatic organisms, except some mud-dwellers, require high oxygen concentrations for survival (Pearce and Schumann, 2003). Anoxic conditions will lead to the death of aerobic organisms in the water. The oxidation-reduction potential (ORP) is the ability of a water body to purify itself or break down contaminants inside it (Al-Samawi and Al-Hussaini, 2016). The higher the ORP value, the healthier the water body because higher values encourage decomposing bacteria to thrive since oxygen will be made available to them (Horne and Goldman, 1994). Temperature is the primary determinant in water chemistry, biogeochemical processes, and the physiology of the organisms inside the water (Dallas and Ross-Gillespie, 2015). Temperature also affects the solubility of oxygen in water and determines the susceptibility of organisms to diseases and parasites (Bhateria and Jai, 2016). The temperature of a freshwater body depends on seasons, solar radiation, hyporheic exchange, turbidity and flow rate (Chikita, 2018). A change in the optimum temperature will affect the enzyme systems of organisms in the aquatic ecosystem. Extreme temperatures may inactivate or denature the enzymes leading to a modification of behaviour, metabolisms and growth rates (Naresh and Rehana, 2017). The presence of strong ionising compounds such as NaCl (Na+ and Cl-) increases the electrical conductivity (EC) of water. In water quality assessment, EC is an indirect measurement of dissolved salts. Conductivity is related to total dissolved solids (TDS) since it is a measure of the total inorganic salts (ions) in the solution (Niekerk et al., 2014). Conductivity measures the activity of the ions with their electrical charge, but TDS is a measure of the amount of dissolved salts. EC must be low in rivers to support plant and animal life. Freshwater is low in EC (0 - 1,500 μS/cm) but seas and oceans may reach 50,000 μS/cm. Living things are sensitive to hydrogen ion concentrations (pH), and extremes of it can damage their internal and external structures. The pH of water determines the distribution of bacteria in wastewater (Tyson et al., 2004). The solubility and convertibility of some compounds in water are also pH-dependent. Metals are more soluble in water and mobilised from the sediments at lower or acidic pH (Xie et al., 2018; Potysz et al., 2017), while ammonium ions will be converted to ammonia (toxic) at pH above 8.5 (Leoni et al., 2018). When mobilised because of the acidity of the water, metals can accumulate on the gills of young fishes and cause deformities and death in young fishes (Oram, 2019). Under anaerobic conditions, especially in river sediments, sulphates may split to form hydrogen sulphides. Hydrogen sulphides are respiratory toxicants that create extreme environmental conditions and produce obnoxious odour in water bodies. Water with hydrogen sulphides will be avoided by humans and fishes (Tobler et al., 2018). A high concentration of sulphate in water may affect the taste and have laxative effects when combined with magnesium or sodium (Kumar and Kumar, 2018). Chloride concentration may reach up to 20 g/L in seas and oceans but less than 50 mg/L in freshwater, a range necessary for the normal functioning of the ecosystem (Hunt et al., 2012). Increased chloride concentration in freshwater may be a result of runoff or pollution from wastewater. The interest in ammonium is because it can exert oxygen demand on the aquatic ecosystem, transformed into nitrite ions, which in turn form ammonia (Du et al., 2017). Ammonia is toxic to fishes and other aquatic animals, even at low concentrations (lethal dose 0.2 – 2.0 mg/L) (Du et al., 2017). In the event of ammonia pollution in water, fishes will experience loss of equilibrium, high respiratory activities, increased oxygen intake, convulsion, coma and death (Oram, 2014).

About 77% of the needed water resources in South Africa are from surface water, which is under threat due to relatively low rainfall (about 450 mm annually) with little runoffs to boost the surface water (Binns et al., 2001). The country is developing with an increasing population, and so is the demand for use and access to water resources. Expansion of industrial and agricultural activities and the population pressure had impacts on the water quality, availability and the state of aquatic ecosystems (Maree et al., 2016). Reports showed that 50,000 litres of untreated wastewater flow into South African rivers every second because most municipalities are unable to meet the cost of maintenance of the WWTPs (Kings, 2017). A report claimed that only 60 out of 824 WWTPs in South Africa release adequately treated effluents to the environment; the rest release partially or untreated wastewater to the receiving rivers (Khumalo, 2017). This study aimed to investigate the physicochemical parameters of wastewater-treated effluents and receiving surface water and use FT-IR spectra analysis combined with multivariate statistics to investigate the similarities of metabolites in wastewater-treated effluents and the receiving rivers.

2. Materials and Methods

2.1. Study sites

Four major rivers sampled in Eastern Cape Province include Bloukrans in Makana Municipality, Tyhume in Nkonkobe Municipality, Buffalo in Buffalo City Municipality and Swartikops in Nelson Mandela Bay Municipality. Three sampling points were established in each river: upstream, midstream and downstream. The midstream samples were collected immediately after the rivers passed through their respective major towns but before receiving wastewater effluents. Samples of treated wastewater effluents released into these rivers were obtained from Grahamstown, Alice, King Williams Town and Uitenhage WWTPs. Coordinates of the sample sites (Table 1) were obtained with the aid of eTrex Vista HCx (Garmin Ltd, Kansas City, USA).

Table 1 Coordinates and altitude of the sampling sites

River		Coordinate/Altitude (metres above sea level)		
		Upstream	midstream	downstream
Bloukrans	Location	33° 19' 0.07" S; 26° 31' 20.9" E	33° 18'51.4"S, 26° 33'11.5" E	33° 19' 07.1" S, 26° 34' 05.7" E
	Altitude	480.7m	428.5m	421.4m
Buffalo	Location	32° 4 7' 23.74" S, 27° 22' 10.56" E	32° 53' 49.14" S, 27° 23' 34.08" E	32° 56' 3.6" S; 27° 26' 25.18" E
	Altitude	372.2m	272.6m	256.2m
Swartkops	Location	33° 42' 59.64" S 25° 17' 16.43" E	33° 47' 31.08" S, 25° 24' 26.96" E	33° 47' 31.92" S; 25° 29'26.26" E;
	Altitude	8.6m	5.9m	4.3m
Tyhume	Location	32° 36' 38.72" S; 26° 54' 34.15" E	32° 47' 42.95" S, 26° 50' 88" E	32° 50' 15" S, 26° 53' 31.27" E
	Altitude	782.5m	479.8m	452m

2.2. Materials

Sample bottles and chemicals were purchased from Sigma Aldrich (Johannesburg, South Africa), and eTrex Vista HCx from Garmin Ltd, Kansas City, USA. Hanna HI9829 Multiparameter was purchased from Hanna Instruments, Woonsocket USA. Spectroquant test kits were from Merck (Germany) and the spectrophotometer UVmini-1240 was from Shimadzu Corporation, Japan.

2.3. Procedure

Each water sample for physicochemical analysis was collected into 1L Schott's bottle already prepared by washing with a phosphate-free detergent, dried and rinsed with acetone, rinsed again with deionised water and allowed to dry. Sample water was used to rinse the bottles three times at the point of collection to condition the bottles (UNEP/WHO, 1996). The bottles were filled with the sample water, leaving no space between the sample and the cover to exclude air that may aid oxidation of materials of interest in the samples. The bottles were tightly covered, labelled and preserved in an icebox for further analyses in the laboratory.

The organic contents of the samples were extracted by freeze-drying in liquid Nitrogen and dissolved in methanol. The supernatant was extracted into a vial and allowed to dry in an oven at 35 °C. Extracted samples were dried and analysed on a Perkin Elmer 400 FT-IR spectrometer operated with Spectrum Quant (version 10.5.4) software mounted on Microsoft Windows. FT-IR absorption peaks of the samples were recorded at mid-IR radiation, between 4000 and 650 cm^{-1}. The IR spectra obtained were smoothened (smooth factor 2.00), subjected to baseline corrections and the peaks automatically labelled. Spectra were analysed with BioRad Knowitall (2024 Edition) software. Infrared Spectrum Tables were obtained from Sigma-Aldrich (2018), BioRad software with Sadler's note (Bartels, 1978), Talari et al. (2017), Coates (2000), Socrates (2004) and Larkin (2011) to determine the functional group absorption peaks. Data collected

on the spectrometer were saved as ASCII files for statistical analysis. Saved spectra were imported into Metaboanalyst 6.0 for multivariate analyses.

2.4. Sample Analyses

Some physicochemical characteristics measured at the point of the collection with a pre-calibrated Hanna HI9829 Multiparameter instrument include pH, oxidation-reduction potentials (ORP), dissolved oxygen (DO), conductivity, total dissolved solids (TDS), salinity and temperature. All readings were taken in triplicates at different points within each sampling site. Physicochemical parameters measured in the laboratory include chemical oxygen demand (COD), phosphate, chloride, ammonium, sulphate, and nitrate ions. Concentrations of these ions were analysed with Spectroquant (Merck, Germany) test kits and assayed on a spectrophotometer (UVmini-1240, Shimadzu Corporation, Japan). Light waves were filtered on a spectrophotometer as recommended by the kit manufacturer; COD solutions were filtered at 610nm wavelength, phosphate ion 660nm, chloride ion 450nm, ammonium ion 660nm, and sulphate ion 420nm. The optical densities (OD) of the solutions were recorded, and standard curves were plotted to determine the concentration of each solution. Nitrate and nitrite contents were measured with Spectroquant (Merck, Germany) test dip-stick. All solutions were prepared in triplicates and analysed accordingly.

For FT-IR analysis, binned FT-IR spectra data were arranged in rows and the features in columns before uploading to MetaboAnalyst 6.0 designed by McGill University, Parasitology Building, 21111 Lakeshore Road Ste. Anne de Bellevue, QC, Canada (http://www.metaboanalyst.ca) for multivariate analyses. All the peak intensities loaded have positive values. The samples were not filtered, and there were no missing values in the loaded data. Sample normalisation was according to the method of Dieterle et al. (2006). The procedure for normalisation was row-wise, by sample median. Generalised log transformation (glog 2) was adopted for a better comparison of the features. Pareto (mean-centred and divided by the square root of the standard deviation of each variable) was used for data scaling (Dieterle et al., 2006).

3. Results and Discussions

The standard curves plotted for the optical density (OD) measurement of the samples on the spectrophotometer yield excellent linearity ($R^2 > 0.95$). The results were pulled together according to the sampling sites (upstream, midstream and downstream) of the rivers. Table 2 shows the result for some physicochemical parameters observed. Upstream river samples (R1U, R2U, R3U and R4U) have low COD values, below the detection level. Generally, the midstream samples show higher COD values than others with the Bloukrans River (R1M) having the highest mean COD with 331.31 mg/L followed by Buffalo River (R2M) with 256.5 mg/L COD. The high values of COD of the midstream samples may be due to contributions from runoff. The lower values of COD of downstream samples might be due to the natural cleansing capacity of the rivers. However, no COD was detected for King Williams Town effluents (E2), while effluents E3 and E4, had mean COD above the recommended value of 30 mg/L (DWA Act No 991- 18 May 1984). Apart from the upstream samples, all other freshwater samples have oxygen demand above the recommended value for a healthy aquatic ecosystem. The COD of the wastewater-treated effluents did not appear to influence the COD of the receiving water in this study. Agoro et al. (2018) observed a COD range of 17-394 mg/L in their study of three WWTPs in the Eastern Cape Province of South Africa. In another study, COD values between 14 and 20 mg/L were observed for effluents in the study of three wastewater treatment plants (WWTPs) in the Eastern Cape while the recipient rivers had values between 7 and 15 mg/L (Osode, 2007). Edokpayi et al. (2015), in a study of peri-urban WWTPs, recorded COD of effluents ranging from 50 – 105 mg/L. Igbinosa and Okoh (2009) recorded a COD range between 36 and 238 mg/L for effluents in their studies of a WWTP in the Eastern Cape Province. The observations in this study favourably compare with the cited works.

Table 2 Concentrations of some physicochemical parameters of the samples (values are means ± standard deviation; ND = not detected)

Sample	COD (mg/L)	DO (mg/L)	ORP (mV)	Temp (°C)	pH	Salinity (PSU)	Conductivity (µS/cm)	Turbidity (NTU)	TDS (mg /L)
R1U	6.30 ± 8.40	4.36 ± 1.66	88.41 ± 46.88	14.56 ± 2.68	7.39 ± 0.42	0.15 ± 0.03	305.22 ± 62.96	2.25 ± 3.00	152.56 ± 31.41
R1M	331.31 ± 16.34	3.97 ± 1.02	70.20 ± 16.45	15.26 ± 2.69	8.06 ± 0.27	0.79 ± 0.06	1566.44 ± 109.92	214.24 ± 125.40	782.89 ± 55.19
R1D	141.81 ± 47.60	3.93 ± 1.30	66.13 ± 25.58	14.71 ± 3.28	7.61 ± 0.54	0.70 ± 0.07	1386.56 ± 127.26	40.18 ± 9.44	693.22 ± 63.70
E1	3.11 ± 2.07	6.35 ± 1.18	87.14 ± 29.04	17.45 ± 2.9	7.65 ± 0.22	17.45 ± 2.90	661.33 ± 436.89	31.16 ± 4.75	330.56 ± 218.37
R2U	ND	2.63 ± 0.72	69.77 ± 7.31	14.08 ± 2.93	7.91 ± 0.75	0.26 ± 0.10	540.89 ± 193.85	2.84 ± 3.79	275.89 ± 93.41
R2M	256.5 ± 28.8	3.89 ± 0.93	77.32 ± 8.75	14.84 ± 3.92	9.03 ± 0.79	0.245 ± 0.025	503.17 ± 48.5	22.49 ± 17.22	251.32 ± 24.34
R2D	96.98 ± 24.66	3.92 ± 0.75	68.70 ± 11.93	14.90 ± 2.51	7.67 ± 0.60	0.31 ± 0.03	757.11 ± 116.37	289.55 ± 340.30	319.56 ± 33.63
E2	ND	3.55 ± 0.47	121.00 ± 50.47	16.1 ± 1.31	8.16 ± 0.82	16.10 ± 1.35	483.50 ± 5.50	10.79 ± 10.79	241.84 ± 2.83
R3U	17.12 ± 11.42	3.23 ± 2.01	123.65 ± 34.58	16.11 ± 2.58	8.73 ± 1.75	0.09 ± 0.00	198.67 ± 3.56	0.00	99.67 ± 1.78
R3M	169.86 ± 37.86	3.59 ± 0.51	99.20 ± 14.73	17.59 ± 1.81	9.65 ± 0.97	1.50 ± 0.46	2857.50 ± 830.83	11.34 ± 11.34	1428.67 ± 415.34
R3D	94.17 ± 32.78	3.81 ± 1.00	85.10 ± 11.69	19.95 ± 2.26	8.92 ± 1.20	1.46 ± 0.12	2806.56 ± 212.59	23.98 ± 0.32	1402.55 ± 106.15
E3	70.31 ± 10.31	4.04 ± 0.40	105.19 ± 16.22	18.86 ± 0.63	9.25 ± 0.99	18.86 ± 0.63	1093.11 ± 176.57	28.00 ± 4.24	956.50 ± 128.17
R4U	19.90 ± 26.53	3.87 ± 2.48	92.00 ± 21.62	9.56 ± 2.18	8.70 ± 1.33	0.03 ± 0.00	63.33 ± 3.11	22.59 ± 15.06	32.00 ± 1.33
R4M	122.36 ± 41.87	5.48 ± 1.11	80.84 ± 17.07	12.04 ± 0.05	9.35 ± 1.03	0.17 ± 0.02	350.67 ± 31.34	222.84 ± 222.84	175.50 ± 15.50
R4D	47.48 ± 19.84	6.73 ± 2.21	87.26 ± 5.70	14.80 ± 1.46	8.13 ± 0.37	0.18 ± 0.07	375.44 ± 133.92	125.00 ± 133.78	188.11 ± 67.26
E4	125.99 ± 21.99	5.48 ± 0.52	129.88 ± 41.15	14.25 ± 0.60	8.05 ± 0.22	14.25 ± 0.60	474.67 ± 3.34	13.79 ± 13.79	234.17 ± 4.83

Bloukrans River, upstream (R1U), midstream (R1M), downstream(R1D); Buffalo River, upstream (R2U), midstream (R2M), downstream (R2D); Swartkops River, upstream (R3U), midstream (R3M), downstream (R3D); Tyhume River, upstream (R4U), midstream (R4M), downstream (R4D); Grahamstown wastewater treated effluent (E1), King Williams Town wastewater effluents (E2), Uitenhage wastewater effluents (E3), Alice wastewater effluents (E4).

All the river samples show positive values for oxidation-reduction potentials (ORP) (Table 2) with the highest values observed in the upstream sites. Positive values indicate the oxidation potentials or the ability of the water to cleanse itself (Al-Samawi and Al-Hussaini, 2016). The effluents had positive ORP values, indicating the removal or reduction of oxygen-demanding compounds from the wastewater during treatment. The mean range of dissolved oxygen (DO) observed for the rivers in this study was 2.63 mg/L to 6.73 mg/L. The midstream and some downstream samples have higher DO values than the upstream samples. Oxygen concentration reduces with altitude (Huang et al., 2017), and this might be the reason the upstream reaches did not have the highest concentrations of dissolved oxygen. The altitudes of the sampling sites are reflected in Table 1. Low levels of DO in rivers will cause severe stress in the aquatic ecosystem with reduced physiological activities of fishes, and increased mortality when the DO falls below 9.5 mg/L (Sharma and Gupta, 2016; Huang et al., 2017). Igbinosa and Okoh (2009) observed DO values ranging from 4.15-11.22 mg/L in a study of Eastern Cape Rivers, Omole et al. (2016) recorded a DO range from 5.42-6.98 mg/L, Fatoki et al. (2003) reported 2.70 – 3.60 mg/L and Mathebula (2015) observed a range of 7.26-12.21 mg/L. The results of DO in freshwater in this study compare favourably with those reported by these workers.

The temperature of the rivers in this study ranges from 7.11 to 19.99 °C. The upstream reach of the Tyhume River has the lowest mean temperature, this may be due to its altitude and plant canopy (Table 2). Upstream Tyhume is located on Hogsback Mountain, hence the low mean temperature observed. Temperature determines the water chemistry, and the physiology of the living things inside the water (Dallas and Ross-Gillespie, 2015), it also affects the solubility of oxygen and some compounds in water and determines the susceptibility of organisms to diseases and parasites (Bhateria and Jai, 2016). The extreme temperature may lead to migration or death of organisms since they prefer optimum conditions for survival. The temperature of the water may be influenced by the prevailing weather and the time of sampling. The water temperature will be higher in the afternoon than morning and in summer than other seasons. Department of Water Affairs and Forestry, DWAF (1996) recommends a maximum temperature of 25 °C for effluent discharge to the environment. The temperatures recorded in this research are within the permissible limit for effluent discharge into water bodies.

Hydrogen ion concentration (pH) of the rivers ranged from 7.39 – 9.65 with midstream Swartkops River (R3M) having the highest value. pH was generally higher at midstream than at other sites. Some of the pH values were above the recommended values of 6.5 - 8.5 for aquatic life by DWAF (1996). The salinity observed for the rivers, in this study was near zero as characteristic of freshwater. The midstream and downstream reaches of the Swartkops River had values above 1 mg/L. A salinity value above 0.5 mg/L in freshwater is an indication of pollution and salinisation of the river (Lee et al., 2013). Uitenhage wastewater effluent had a salinity above 0.5 mg/L. Salinisation of rivers has been attracting attention as a global problem stemming from irrigation, mining activities, de-icing salts and so on (Cañedo-Argüelles et al., 2013). Salinisation will lead to loss of species diversity and compromise the ecosystem. Electrical conductivity (EC) is a function of the dissolved salts; hence both parameters are related. EC was low in Tyhume River compared to others. Swartkops River had the highest EC, followed by Bloukrans. Upstream reaches of the rivers showed lower values of conductivity compared to other sites. EC of treated wastewater effluents did not appear to raise that of the receiving water in this study. Matshakeni (2016) cited conductivity ranging from 0-4000 µS/cm in a review of some water quality parameters in South African rivers. Igbinosa and Okoh (2009) recorded an EC range of 225.53 – 490.80 µS/cm for freshwater and 268.33 – 298.50 µS/cm for treated effluents. Edokpayi et al. (2015) recorded an EC range of 340 – 1250.3 µS/cm for effluents. DWAF recommended a maximum of 250 µS/cm EC for treated effluents before discharge to water bodies. The result obtained in this study shows higher values than the recommended level.

Turbidity values observed for upstream reaches of the rivers were closer to zero except for the R4U with a mean of 22.59 NTU. R2D had the highest mean turbidity of 289.55 NTU, followed by R4M with 222.84 NTU. The turbidity values of midstream and downstream reaches of the rivers were higher than 50 NTU recommended for agricultural and recreational purposes (DWAF, 1996). The turbidity observed by Fatoki et al. (2003) ranges from 14.9 – 90 NTU and Edokpayi et al. (2015) observed 4.3 - 14.6 NTU for treated effluents. Dissolved and suspended materials, including microorganisms contribute to water turbidity and may intercept or absorb light waves. Turbidity determines light penetration into the water. Light penetration is vital in water bodies because it is the principal input in primary productivity. Absorbed light energy will raise the water temperature above normal. Reducing the turbidity of wastewater before discharge is an essential aspect of water quality management.

The midstream sample of Swartkops River (R3M) had the highest mean concentration of dissolved solids (TDS) with 1428.67 mg/L, followed by its downstream samples (R3D) with 1402.55 mg/L. The upstream samples of Tyhume River (R4U) had the least amount of dissolved solids, with a mean of 32.0 mg /L. The wastewater effluent from Uitenhage (E3) has a TDS of 956.50 mg/L. High values of the physicochemical parameters in E3 may be related to the industrial activities in the town and the inability of the WWTP to properly handle the wastewater. The high values of TDS in R3

samples correlate with other parameters measured in this study. Dissolved solids are naturally present in environmental waters and may comprise organic materials, minerals from rocks and mining activities, and toxic metals (Weber-Scannell, 2007). Water bodies must have dissolved solids moderately because a very low TDS may discourage some aquatic macrofauna (Olson and Hawkins, 2017) and, when high, poses a threat to aquatic ecosystem health (Cañedo-Argüelles et al., 2016). The DWAF recommended a maximum of 450 mg/L TDS for surface waters.

Table 3 shows the concentrations of some chemical components of the water samples. The downstream sample of the Swartkops River (R3D) has the highest mean concentration of phosphate ions, with 19.27 mg/L. Phosphates were generally low in the river samples and below the limit of detection in upstream samples. The levels of phosphate in the midstream samples suggested other environmental sources. High concentrations of phosphates in treated wastewater effluent E2 and E3 might have influenced R2D and R3D respectively. The concentrations of phosphate ions observed for wastewater effluents in this research were higher than the recommended value of 5.0 mg/L (DWAF, 1996). Matshakeni (2016) reported a phosphate range of 0.0 – 200 mg/L from an eight-year study of the Eerste River. Osode (2007) in a study of rivers in Buffalo City and Nkonkobe municipalities of Eastern Cape, reported phosphate concentrations ranging from 3.70 to 11.58 mg/L; and 0.07 to 4.81 mg/L were reported by Igbinosa and Okoh (2009). Edokpayi et al. (2015) reported a range of 1.572 – 32.554 mg/L phosphate concentration in effluent samples. Not all the phosphates in the wastewater influents were removed at the WWTPs before releasing the effluents to the receiving rivers. An elevated phosphate level in the rivers will promote eutrophication (Matthews, 2014).

Table 3 Concentrations of some chemical parameters in the water samples (values are means ± standard deviation; ND = not detected)

Sample	PO_4^- (Mg/L)	Cl^- (Mg /L)	NH_4^+ (Mg /L)	SO_4^- (Mg /L)	NO_3^- (Mg /L)
R1U	ND	27.13 ± 6.31	20.38 ± 1.43	ND	ND
R1M	13.39 ± 7.47	215.49 ± 248.87	95.02 ± 23.23	174.71 ± 77.91	4.44 ± 1.85
R1D	9.33 ± 2.93	196.68 ± 227.12	88.17 ± 42.07	171.02 ± 134.74	5.0 ± 1.11
E1	20.53 ± 3.29	201.19 ± 255.32	67.06 ± 15.06	48.69 ± 47.02	8.33 ± 4.44
R2U	ND	15.02 ± 20.03	18.07 ± 2.45	ND	1.67 ± 2.22
R2M	ND	351.36± 10.04	18.69 ± 2.77	ND	10.83 ± 4.17
R2D	7.75 ± 2.24	380.85 ± 55.43	17.07 ± 1.86	ND	2.78 ± 2.59
E2	16.18 ± 0.43	354.21 ± 28.44	21.69 ± 7.07	2.17 ± 0.69	4.17 ± 4.17
R3U	0.08 ± 0.06	40.00 ± 53.34	28.39 ± 5.19	ND	1.11 ± 1.48
R3M	5.28 ± 2.88	274.23 ± 210.10	48.71 ± 15.58	36.45 ± 36.45	4.17 ± 2.5
R3D	19.27 ± 5.72	179.03 ± 204.66	38.05 ± 3.82	ND	5.56 ± 1.85
E3	25.83 ± 1.19	254.79 ± 224.78	94.72 ± 50.82	9.65 ± 7.06	5.83 ± 0.83
R4U	ND	39.07 ± 44.69	22.87 ± 3.02	ND	ND
R4M	1.90 ± 1.00	137.57 ± 84.51	32.71 ± 11.37	3.56 ± 3.56	3.33 ± 1.67
R4D	0.86 ± 0.57	146.81 ± 80.98	49.66 ± 29.96	57.88 ± 77.18	5.56 ± 1.85
E4	12.43 ± 2.43	148.22 ± 100.15	51.85 ± 25.34	6.82 ± 6.57	30.83 ± 9.17

Bloukrans River, upstream (R1U), midstream (R1M), downstream(R1D); Buffalo River, upstream (R2U), midstream (R2M), downstream (R2D); Swartkops River, upstream (R3U), midstream (R3M), downstream (R3D); Tyhume River, upstream (R4U), midstream (R4M), downstream (R4D); Grahamstown wastewater influent (E1), King Williams Town wastewater effluents (E2), Uitenhage wastewater effluents(E3), Alice wastewater effluents (E4).

Chloride ions concentrations in the river samples were highest in downstream Buffalo River (R2D) with a mean of 380.85 mg/L, followed by its midstream sample (R2M) with 351.36 mg/L. Chloride concentrations were low in the upstream samples (Table 3). The levels of chloride in the midstream samples were indicators that non-point sources contributed to its concentrations. Sample E2 has a mean concentration of 354.21 mg/L while E1 has 301.79 mg/L, indicating that WWTPs did not remove all the chloride ions from the wastewater, thereby contributing to their concentrations in downstream samples. Fatoki et al. (2003) reported 25.7 mg/L chloride concentration in the

Keiskamma River. Edokpayi et al. (2015) reported a range of 15.293 – 56.524 mg/L chloride concentration in wastewater effluents. The result shows that chloride concentrations recorded in this study were higher than those reported by the above workers. The limit set by DWAF (1996) for chloride in wastewater effluents is 100 mg/L, but the observation from this study shows that the limit was exceeded in most samples. Huizenga (2011) observed that chloride and sulphate contamination are the main factors that characterise South African surface water chemistry. Uncontrolled levels of chloride in freshwater will promote the mobilisation of toxic metals from the soils and sediments. In high concentrations, chlorides form a soluble complex with heavy metals, which is useful in extracting heavy metals from residues, known as brine leaching (Stec et al., 2020). Chloride ions will also cause reproduction impairment and mortality of aquatic organisms, alteration of the steady state of the aquatic ecosystem, corrosion of pipes and taste problems (Muralikrishna and Manickam, 2017).

Ammonium ions were present in all the water samples, but the concentrations in the upstream samples of the rivers were lesser (Table 3). The highest mean concentration of ammonium was recorded in sample R1M with 95.02 mg/L, followed by R1D with 88.17 mg/L. Mathebula (2015), in a 12-year study, observed an increasing trend of ammonium in rivers from 2 mg/ L to 10 mg/L over the years. Matshakeni (2016) observed ammonium concentration in the rivers in the range of 0-55 mg/L. The observed levels of ammonium in this study show higher values than those recorded by earlier workers. Ammonium is not toxic at a pH near neutral but may convert to poisonous ammonia at a pH above 11 (DWAF, 1996). Ammonium can exert oxygen demand on the aquatic ecosystem and transform it into nitrile ions, which in turn form ammonia (Du et al., 2017). Ammonia is toxic to fish and other aquatic animals, even at very low concentrations of 0.2 – 2.0 mg/L (Gupta et al., 2015; Du et al., 2017). In the event of ammonia pollution, fishes will lose equilibrium, increase in respiratory activities and oxygen intake, convulsions, coma and death (Oram, 2014).

Sulphate ions were below the detection limit in the upstream samples of the rivers (Table 3). The Bloukrans River had a high concentration of sulphate with a mean value of 174.71 mg/L in its midstream sample (R1M) and 171.02 mg/L in the downstream (R1D). The Bloukrans River passes through the centre of Grahamstown and Belmont Valley with many farm settlements. Wastes were indiscriminately dumped into the Bloukrans River which made it stinks. Sulphate ions were below the detection limit in the Buffalo River samples. The safe limit of sulphate recommended for a healthy aquatic ecosystem is 100 mg/L (DWAF, 1996). In river sediments, sulphate may split under anaerobic conditions to form hydrogen sulphides, producing an obnoxious odour, and rendering the water useless to man and fish (Kumar and Kumar, 2018).

The midstream Buffalo River sample (R2M) had the highest mean nitrate concentration of 10.83 mg/L. The higher amounts of nitrates in the effluents E1 and E2 might have resulted from improperly treated effluents or contamination of the waste stabilisation ponds with plant materials and dung from grazing animals around the pond. Nitrate ions were not detected in the upstream samples. Matshakeni (2016) reported a range of 0-9 mg/L of nitrate ions in a project; Igbinosa and Okoh (2009) reported a range of 1.82 – 13.24 mg/L while Edokpayi et al. (2015) reported 7.454 -19.413 mg/L for treated effluents. DWA (1996) recommends 15.0 mg/L of nitrates in effluents before releasing to the environment. No nitrite ion was detected in the samples.

Table 4 shows the correlation statistics (r) of the samples, showing the similarities in the physicochemical components. All the samples show positive correlation values with each other but at different degrees. Correlation values above 0.5 were strong, while below that were weak. Samples R1M and R1D had a correlation value above 0.9, while upstream samples R1U and R3U were above 0.8. R1U and R4U were both upstream samples of Bloukrans and Tyhume Rivers respectively, but with a correlation just above 0.5.

Table 4 Correlations coefficient of the sites (Marked correlations are significant at p < .05000)

Variable	R1U	R1M	R1D	R2U	R2M	R2D	R3U	R3M	R3D	R4U	R4M	R4D	E1	E2	E3	E4
R1U	1.00000															
R1M	0.93309	1.000000														
R1D	0.94361	0.986189	1.00000													
R2U	0.98797	0.954842	0.96383	1.00000												
R2M	0.77309	0.872636	0.84321	0.76056	1.00000											
R2D	0.83205	0.925093	0.88656	0.83671	0.90251	1.00000										
R3U	0.87669	0.798351	0.81101	0.80985	0.85666	0.81947	1.00000									
R3M	0.96885	0.980288	0.98485	0.99115	0.81393	0.87228	0.79981	1.00000								
R3D	0.96899	0.978134	0.98104	0.99154	0.81279	0.87484	0.79970	0.99964	1.00000							
R4U	0.54011	0.475530	0.42860	0.42624	0.68099	0.56349	0.79832	0.41112	0.40899	1.00000						
R4M	0.78625	0.879927	0.80965	0.78408	0.81879	0.95671	0.74468	0.80623	0.80858	0.61338	1.00000					
R4D	0.84170	0.921282	0.92646	0.84226	0.79961	0.89152	0.78553	0.86729	0.85905	0.51602	0.86307	1.00000				
E1	0.92425	0.941465	0.95916	0.92236	0.88580	0.92534	0.90079	0.94192	0.94193	0.51548	0.81673	0.89593	1.00000			
E2	0.84278	0.847369	0.86538	0.81696	0.90357	0.90185	0.93737	0.83979	0.84137	0.60695	0.77208	0.82524	0.96699	1.00000		
E3	0.92887	0.942173	0.94315	0.94588	0.82308	0.84198	0.80540	0.95605	0.95638	0.44632	0.78807	0.82844	0.92290	0.84367	1.00000	
E4	0.87659	0.921500	0.886822	0.862228	0.935856	0.854657	0.850385	0.886969	0.884568	0.700142	0.826306	0.809278	0.860909	0.815652	0.870983	1.00000

Bloukrans River, upstream (R1U), midstream (R1M), downstream(R1D); Buffalo River, upstream (R2U), midstream (R2M), downstream (R2D); Swartkops River, upstream (R3U), midstream (R3M), downstream (R3D); Tyhume River, upstream (R4U), midstream (R4M), downstream (R4D); Grahamstown wastewater influent (E1), King Williams Town wastewater effluents (E2), Uitenhage wastewater effluents(E3), Alice wastewater effluents (E4).

3.1. FT-IR Spectroscopy

Figure 1 shows the FT-IR spectra bands of the water samples. The functional group region (4000-1500 cm^{-1}) of the spectra was similar except for the differences in the intensity between 1800 and 1500 cm^{-1}. Characteristically all the spectra show uniqueness at the fingerprint region (1500-650 cm^{-1}). All the samples exhibited absorption peaks similar to alkane group bonds. C-H vibrations in cyclohexyl (strong peak intensity at 3000-2900 cm^{-1} and medium-strong intensity at 920-880 cm^{-1}), C-H vibrations in normal alkanes (-CH2-CH2- CH2- CH2-C-) with strong peak intensity between 2936-2916 cm^{-1} with or without variable intensity from 2936-2916 cm^{-1}, variable intensities from 1490-1430 cm^{-1} (C-H vibrations in R-CH3) are common to all samples. Samples R1M and E2 had medium peak intensities at 1258-1200 cm^{-1} (skeletal vibrations of C-C in branched alkanes) while E1 and E2 had medium peaks at 1048-1000 cm^{-1} (ring deformation vibration in cyclopropyl alkanes). Alkane bonds are prevalent among organic compounds and therefore are not very useful in determining structures in IR spectroscopy (Bartels, 1978). The spectra of cyclic alkanes of five or more ring carbons show ring CH$_2$ stretching frequencies, which overlap those of CH$_3$ and CH$_2$ groups of their alkyl substituents (Bartels, 1978). These frequencies also overlap those of the CH$_3$ and CH$_2$ stretching of acyclic alkanes. Numerous authors noted that the spectral region of 2800-2600 cm^{-1} confirms the presence of saturated simple ring structures (Talari et al., 2017; Coates, 2000; Larkin, 2011). Absorption at this region consists of a weak band or bands whose patterns and locations help confirm or indicate the presence of these rings. Although such absorption features have a limited diagnostic value, it is most reliable when the absorption occurs in the spectra of simple saturated aliphatic hydrocarbons. The presence of alkanes in the water samples is due to organic sources such as microbial products, decayed organic matter, aquatic plant products, industrial and household wastes, oils, fossil fuels, and natural gas, among others (Guo and Fang, 2012). Natural waters may not be free of alkanes due to the influence of runoff.
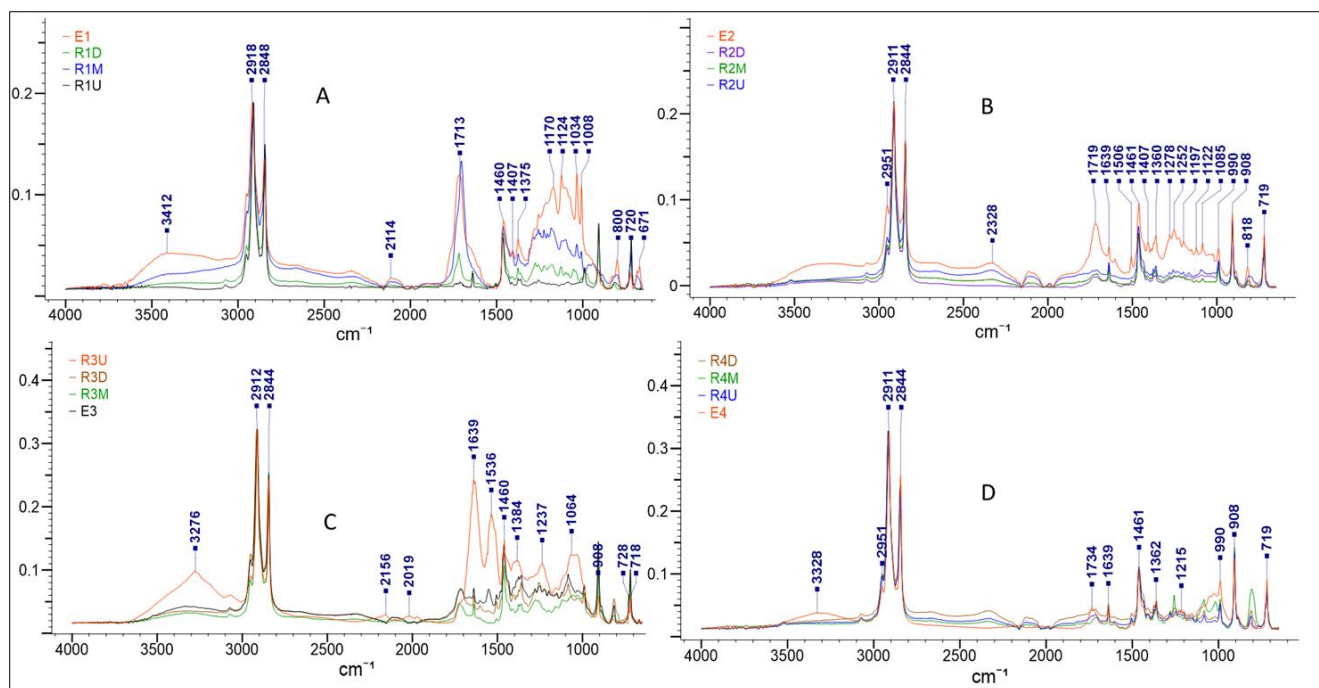

Figure 1 Spectral bands of the samples. Bloukrans River (A): upstream (R1U), midstream (R1M), downstream(R1D), Grahamstown wastewater influent (E1); Buffalo River (B): upstream (R2U), midstream (R2M), downstream (R2D), King Williams Town wastewater effluents (E2); Swartkops River (C): upstream (R3U), midstream (R3M), downstream (R3D), Uitenhage wastewater effluents (E3); Tyhume River (D): upstream (R4U), midstream (R4M), downstream (R4D), Alice wastewater effluents (E4).

Alkene group absorption peaks in the samples, 1662-1631 cm^{-1}, showed weak C=C bond stretching vibrations in asymmetrically substituted alkenes (vinylidene). These peaks were limited to wastewater effluents E2, E3 and E4 with rare presence in midstream, and downstream samples of the rivers. Alkenes in water originated from hydrocarbon pollutants, mostly from plastic products and plastic industries. Some algae, especially chrysophytes and diatoms, can produce certain alkenes in the aquatic environment by enzymatic breakdown of polyunsaturated fatty acids (Satchwill et al., 2007). Alkenes are toxic and carcinogenic pollutants, which may enter the food through packaging materials. Acute or chronic exposure to some alkenes can cause significant damage to organs and systems (Zhang et al., 2016). The vinylidene peaks in the samples might be due to pollutants, and biotic, and abiotic degradation of polyvinylidene

products in the industrial and municipal wastewaters (Benson, 2003). Their presence in treated effluents shows that they were not effectively removed from wastewater during treatment; this will harm the organisms in the receiving water bodies.

Alkyne group peaks observed on the spectra were limited to downstream river and wastewater effluent samples. Peak 2165-2110 cm^{-1} is a weak C≡C stretching vibration in monosubstituted alkynes (RC≡CH), observed in samples E2 and E3. Peak 1740-1720 cm^{-1}, weak-medium stretching vibration C=O in saturated aliphatic aldehydes occurred in E2 and E4. The strong peak at 1715-1685 cm^{-1}, C=O stretching vibrations in aryl aldehydes (Ph-CHO), occurred in E1, E2 and E4. Stretching vibration of the C-C bond in aromatic aldehydes (medium peaks 1210-1150 cm^{-1}) also occurred in samples R1M and R1D. Few alkyne peaks found in this study may be due to symmetrical substitution of the alkyne, and if the internal alkyne is symmetrical, the peaks may be absent (Bryan, 1999). Vibrations of monosubstituted alkynes were observed mainly in effluent samples. Their absence in the upstream samples of the rivers might be an indication that they are associated with pollution. Alkynes are present in some drugs such as Efavirenz (an antiretroviral), calicheamicin (antitumour), and antifungal terbinafine, and they are also an essential component of both the natural and synthetic hormone oestradiol (Stevenson et al., 2019). Most oral contraceptives contain synthetic oestradiol, which may be passed to household wastewater through urines. The presence of this functional group in the treated effluent samples might be an indication of inadequate treatment. Since this functional group is associated with emerging contaminants in water, they will affect the physiological processes of organisms that depend on these rivers, especially at midstream and downstream.

Aldehydes, ketone, ester, carboxylic acids and amides are characterised by C=O bond. Carbonyl stretching has a strong absorption on IR and is very useful in structure determination (Bartels, 1978). The commonest of the aldehydes identified is aryl. Weak-medium Peaks at 1740-1720 cm^{-1} is the C=O stretching vibration in saturated aliphatic aldehydes, present mainly in E2, E4, R2D and R3D. Samples E1, E2 and E3 show a strong stretching vibration of C=O (1715-1685 cm^{-1}) in Aryl aldehydes (Ph-CHO). Medium stretching vibrations of the C-C bond in aromatic aldehydes (1210-1150 cm^{-1}) were observed in samples R1M, E1 and R1D. Aryl compounds are present in some products such as dyes in textile industries, cosmetics and various drugs such as antibacterial, antiviral and antifungal pharmaceuticals (Ali et al., 2018). Their presence in water is due to various wastes. Saturated aliphatic aldehydes are present in wastewater-treated effluents. The aliphatic aldehydes with C8 – C10 are common in natural products such as fruits, honey of different floral origin, oils, mushrooms, coffee, wines and other products (Cullere et al., 2011; López-Galilea et al., 2006). Their sources in the water samples are from industrial, household and agricultural wastes. Aryl aldehydes peaks were present in wastewater effluents, an indication of partial or non-removal, and observed in the downstream samples of the receiving water. Aliphatic aldehydes were present mainly in the midstream and downstream reaches of the rivers but not in treated effluents, indicating sources other than wastewater effluents.

Most of the peaks characterising alcohol vibrations were not common in the spectra of upstream river samples. Alcohol spectra are present in all treated wastewater effluent samples (E1, E2, E3 and E4). Peaks 1390-1330 cm^{-1} (OH deformation) appeared limited to samples from the midstream of the rivers. Peaks 1260-1180 cm^{-1} occurred only in river samples. Their presence in the treated effluent samples may be due to the disinfectants used in water treatment or an indication of improper treatment of wastewater.

Ketone peaks are typical of carbonyl groups, especially aldehydes, but in IR absorption of a single compound, aldehyde has a broad impurity absorption between 2700 cm^{-1} and 2800 cm^{-1} but not ketone (Smith, 2017). Alcohols have broad absorption between 4000 cm^{-1} and 3000 cm^{-1} but do not have carbonyl double bond peaks. Since the samples in this work are from environmental sources, they may contain ketones. The peaks from 1725-1705 cm^{-1} (saturated aliphatic open-chain ketones) are present in the spectra of freshwater samples only but not in treated effluents. Ketones are common as sugars (e.g. fructose), generated in the body and sometimes pass out with urine (Grabacka et al., 2016; Ho et al., 2019). Ketones have many applications as solvents in chemical industries, laboratories, rubber, paint and perfume manufacturing, printing and pesticides from where they find their way to the environment (William, 2019).

Aliphatic and olefinic esters show strong stretching vibrations of the C-O bond between 1300 cm^{-1} and 1160 cm^{-1} and usually with one or more weaker peaks in the region from 1300 cm^{-1} to 1000 cm^{-1}. Formates have peaks near 1185 cm^{-1}, acetates near 1256 cm^{-1}, and propionates near 1194 cm^{-1} (Bartels, 1978). The strong peak around 1705 cm^{-1} (Figure 1) alludes to the C=O bond stretching in various compounds and not only esters. It may also apply to ketones and others with such bonds. It is common to all the samples, although at different intensities.

Carboxylic acids are characterised by O-H stretch vibrations, a weak and broad spectrum between 3200 cm^{-1} and 2500 cm^{-1}. Since this is the same region of the strong C-H stretching vibrations of alkyl and aromatic compounds, they always

obscure the O-H stretch (Bartels, 1978). O-H peaks were not observed in the spectra bands of the samples, but other complementary peaks of carbo acids were listed. C-O stretching and OH in-plane deformation, coupled, near 1430 cm^{-1} and around 1300 cm^{-1} (two bands). Carbo-acid absorption peaks observed in this study were present in effluents, Bloukrans and Swartikops River samples and lower reaches of the other rivers. Carboxylic acids may enter the environment from various sources. They are essential constituents of foods such as fruits and vegetables and are used as food preservatives, flavours and antioxidants (Badea and Radu, 2018). They are present in various metabolic pathways in animals, various pharmaceuticals and personal care products (Kalgutkar and Daniels, 2010; Lukic et al., 2016). They are constituents of atmospheric aerosol, which is one of their sources in the surface waters (Mkoma et al., 2014). They are the dominant organic acids in the ambient air of many cities and contribute to the acidity of rainwater (Guo et al., 2015). Other sources include the burning of fossil fuels, photochemical oxidation of organic compounds, industrial and household wastes (Guo et al., 2015). Their presence in the upstream samples, in this work, might be due to plant products in the rivers, the upstream reach of Swartkops River, for example, had a lot of algae growth and the upstream of Bloukrans River had diverse aquatic macrophytes.

The diagnostic peaks for ethers are C-O vibrations. Ethers have double C-O bonds in their structures. From the spectra bands, medium symmetric stretching vibrations of heterocyclic ethers (940-860 cm^{-1}) were associated with river samples at all courses (Figure 1). Ethers are ingredients in flame-retardants and are present in many consumer products like furniture upholstery, cars, television sets and other household products. Ethers had been isolated in foods, household dust, human serum and milk (Czerska et al., 2012). Ethers are raw materials in the production of insecticides, fumigants, and in medicine as anaesthetics and pain relievers (Wade, 2019). Their applications in many products have made them available in the environment, especially in air and water as pollutants (Pan et al., 2018).

Medium-weak peaks at 1240-1170 cm^{-1} and 1038-1022 cm^{-1} observed in the spectra of effluent samples, characterise C-N stretching vibrations in aliphatic primary amines. The medium-weak peak at 1140-1080 cm^{-1} represents the C-N stretching vibrations in aliphatic primary amines. $(R)_2CH-NH_2$, which appeared in all samples except the middle and downstream samples of the Buffalo and Tyhume Rivers. Primary amines usually have two strong peaks at 3400 cm^{-1} and 3300 cm^{-1} while secondary has only one, and tertiary none (Bartels, 1978). The samples had no amine peaks above 3000 cm^{-1}. N-H bond absorption peaks appear around 1600-1500 cm^{-1} for primary and secondary amines, and around 1400 cm^{-1} and 1300 cm^{-1} for all amines (Coates, 2000). Most of the amine peaks were present in the midstream and downstream river samples. Aromatic amines are raw materials in the manufacturing of chemicals such as pesticides, dyes such as aniline, pharmaceuticals, cosmetics, rubber and textiles (Ferraz et al., 2012). Bacterial decarboxylation of amino acids in proteins, decaying plants, and animals will produce biogenic amines in contaminated water (Poste et al., 2014). Amines are natural components of many organic molecules such as histamine, dopamine, and some hormones such as epinephrine and norepinephrine; they may be available in urines (Poste et al., 2014). Amines enter the environment through natural processes, wastewater, and industrial sources. Tertiary amines are resistant to degradation and hence persist in the environment (Eide-Haugmo et al., 2009). Some amines such as aniline, aminophenols, naphthylamines and chloroanilines are carcinogenic pollutants (Ferraz et al., 2012). The presence of amine absorption peaks in the water samples is an indication of pollution. The amide group combines the features of amines and ketones because of the presence of both C=O and N-H bonds. They show strong to medium peaks around 3200 cm^{-1} for N-H and another peak around 1710 cm^{-1} for C=O bonds (Bryan, 1999). Hydantoin amide peaks are not present in the upstream river samples, their presence in midstream and downstream river samples is an indication of pollution. Their presence in wastewater effluents suggests the inability of WWTPs to remove them effectively from the influents. Vibrations of C-N bond in primary amides (1420-1400 cm^{-1}) were limited to wastewater effluents. Amides are present as structural components in materials such as nylons; they serve as heat-resistant and fire retardants in synthetic fibres. Amides are present in many drugs, such as paracetamol, penicillin, and lysergic acid diethylamide (LSD), among others (Zumstein and Helbling, 2019; Scott and Njardarson, 2019). Amides are also present as organic molecules in living things. The presence of hydantoin amide peaks in treated effluent samples indicates pollution with biocidal agents such as pesticides (Rai and Jayakrishnan, 2018). The presence of these peaks in treated effluent samples E2, E3, and E4 indicates that the amides were not effectively removed from the wastewater. Vibrations of C-N in primary amides were limited to wastewater effluents. Amides and amines are micropollutants that enter into surface and wastewater from various sources (Gulde et al., 2016; Zumstein and Helbling, 2019).

Peaks from 1290-1250 cm^{-1} (ortho- and meta-disubstituted benzene) and 1105-1065 cm^{-1} were observed in the river samples. Sources of these compounds in wastewater include but are not limited to, dyes (natural and synthetic), organic molecules such as DNA, drugs and cellulose (Talari et al., 2017; Kowalski et al., 2018). Their presence in the treated effluent samples indicates incomplete removal during treatment.

Medium absorption peaks of carbamate esters, N-C-O, (1265-1200 cm^{-1}) and C=O bonds peaks (1740-1680 cm^{-1}) relating to stretching vibrations in urethanes were observed in samples R1M, R1D and E2. In addition to the peak at 1225 cm^{-1}, they are characterised by C=O stretching vibrations at 1744-1739 cm^{-1} (strong intensity), vinyl and phenyl esters near 1770 cm^{-1}, esters of α and β unsaturated acids near 1720 cm^{-1} (Bartels, 1978). Urethanes are products of isocyanates, non-isocyanates and polyether, polyester or caprolactone glycol (Kim et al., 2016). They serve as monomers in the production of polyurethanes used in the manufacturing of various products, including foam. Urethanes might have entered the river through non-point sources.

Absorption peaks relating to halogen were present mainly among the wastewater effluents and downstream river samples. Halogen peaks in treated effluents might be due to the halogenation of wastewater during treatment. They were likely to have entered the watercourse through effluent discharge and may serve as a stream pollution index (da Costa et al., 2014; Mattingley, 2017). Absorption peaks (1280-1120 cm^{-1}) related to the C-F$_2$ bond (organoflourides) were observed in the Grahamstown wastewater effluent (E1), downstream Bloukrans River (R1D) and the downstream Swartkops River (R3D) samples. Organoflourides have many uses such as herbicides, pesticides, foams, refrigerants and propellants from where they find their way to water bodies and serve as pollutants with health implications (Khanna and Nag, 2019). The halogen peaks reported here may not be exhaustive of the actual peaks that should be available because of the problems explained by Bartels (1978).

Samples E4 and R4D showed strong peak intensities at 1415-1390 cm^{-1} representing SO$_2$ asymmetric stretching in sulphonyl halides. Samples R1M, E3 and E4 exhibited strong peak intensity of S=O asymmetric stretching in Sulphur compounds CO-SO$_2$-OC at 1200-1187 cm^{-1}. Strong peaks at 1090-990 cm^{-1} and 870-810 cm^{-1}, strong S=O stretching vibrations in sulphinic acids (-SO-OH) and S-O stretching vibrations in sulphonic acids (–SO-OH) were exhibited by R1M, R1D and E1 respectively. Sulphur peaks were associated mainly with treated effluents and midstream and downstream river samples. Sulphur compounds might have entered into the river bodies through run-offs and wastewater effluents. Sulphur is present in amino acids, some pharmaceuticals, personal care products and agrochemicals from where they find their way to water bodies through wastewater and runoffs (Kebede et al., 2019) They are indicators of pollution in water bodies.

The spectra of phosphorus-containing functional groups vibrate at the same wavenumbers for different compounds. For example, peaks 910-900 cm^{-1} are present in ethers P-O-C, (O=P)-O-C in esters, P-OH in alcohols, (O=P)-OH in carbo-acids and P=S in Sulphur Compounds. P-CH3 bond peaks appeared in all the river samples, and P=S peaks appeared limited to wastewater effluent samples. Compounds with P=S bonds are components of insecticides such as acephate, malathion, and dementon-S (Lim and Bolstad, 2019). Such compounds are additives in the production of plastics and as plasticisers (Li et al., 2019). They enter the environment, especially water, during production and usage (Khan et al., 2016; Lee et al., 2016). Other organophosphates in water may come from different sources such as microbial activities during decomposition of organic matter (Richardson and Simpson, 2011).

FT-IR peaks were subjected to multivariate statistics for proper analysis, classification and authentication of the water samples based on the composition of organic (metabolites profile) or chemical fingerprints. Sample normalisation was according to the method of Dieterle et al. (2006). The procedure for normalisation was row-wise, by sample median. Generalised log transformation (glog 2) was adopted for a better comparison of the features. Pareto (mean-centred and divided by the square root of the standard deviation of each variable) was used for data scaling (Dieterle et al., 2006). Figure 2 shows the result of data normalisation with the boxplots showing 50 features due to space limitations. The density plots were based on all the samples. The chart on the left shows the features before normalisation and that on the right after normalization.
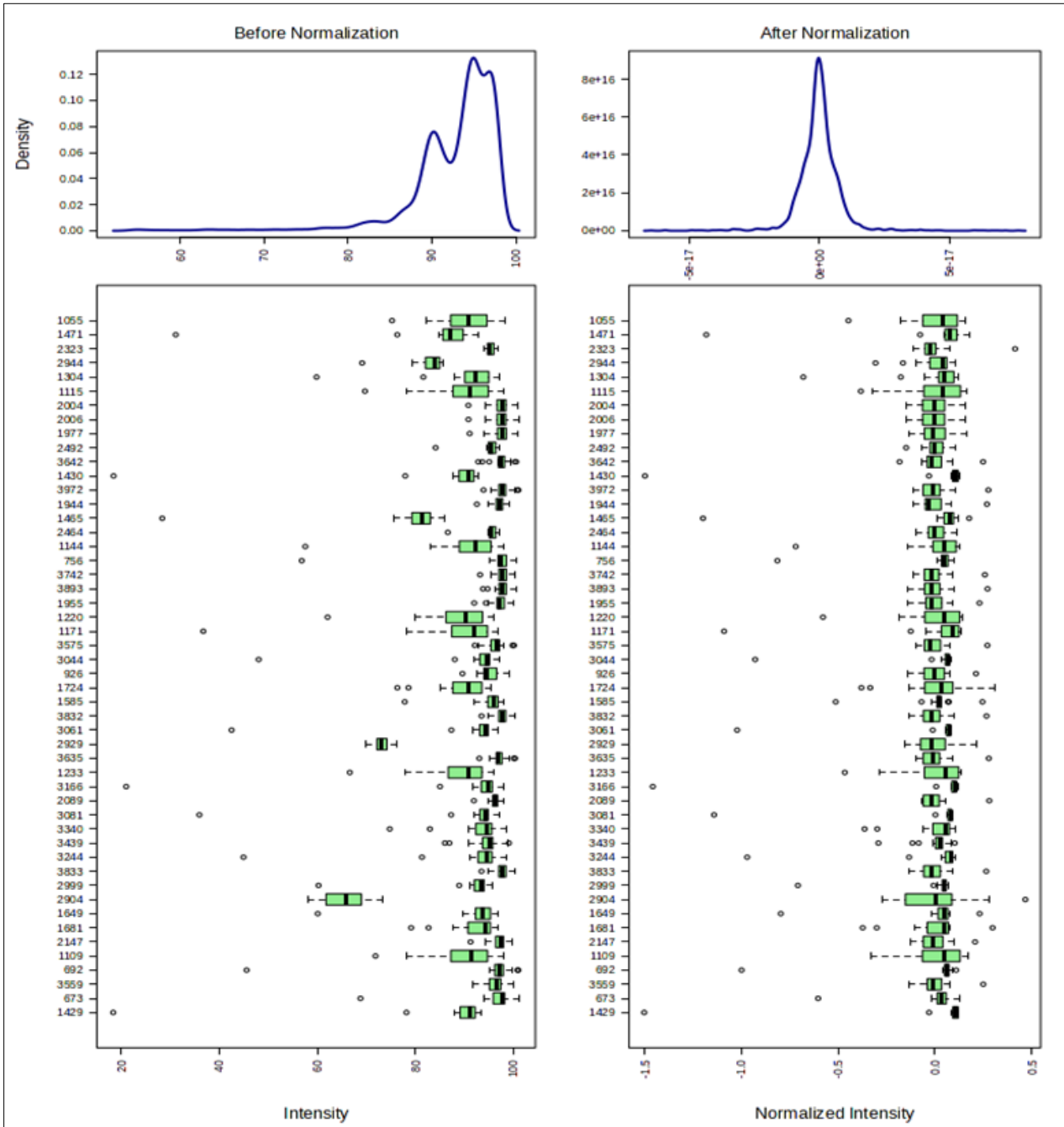
Figure 2 Box plots and kernel density plots before and after normalisation

The results of the ordination of the spectral features with the principal component analysis (PCA) are shown in Figure 3. PCA summarises the data in fewer variables called scores, which are the weighted average (loadings) of the original variables (Chong and Xia, 2018). It is a multivariate tool to observe trends graphically, guided by the correlation of components (Messai et al., 2016). The uniqueness of each water sample is due to the organic compound constituents, which vary in ranges and trends according to geographical origin. Ordination methods, including PCA, can show these differences. The PCA shows two main clusters with 79% of the samples in component 1. R2M and R3U did not cluster with the other samples, showing variation in composition.
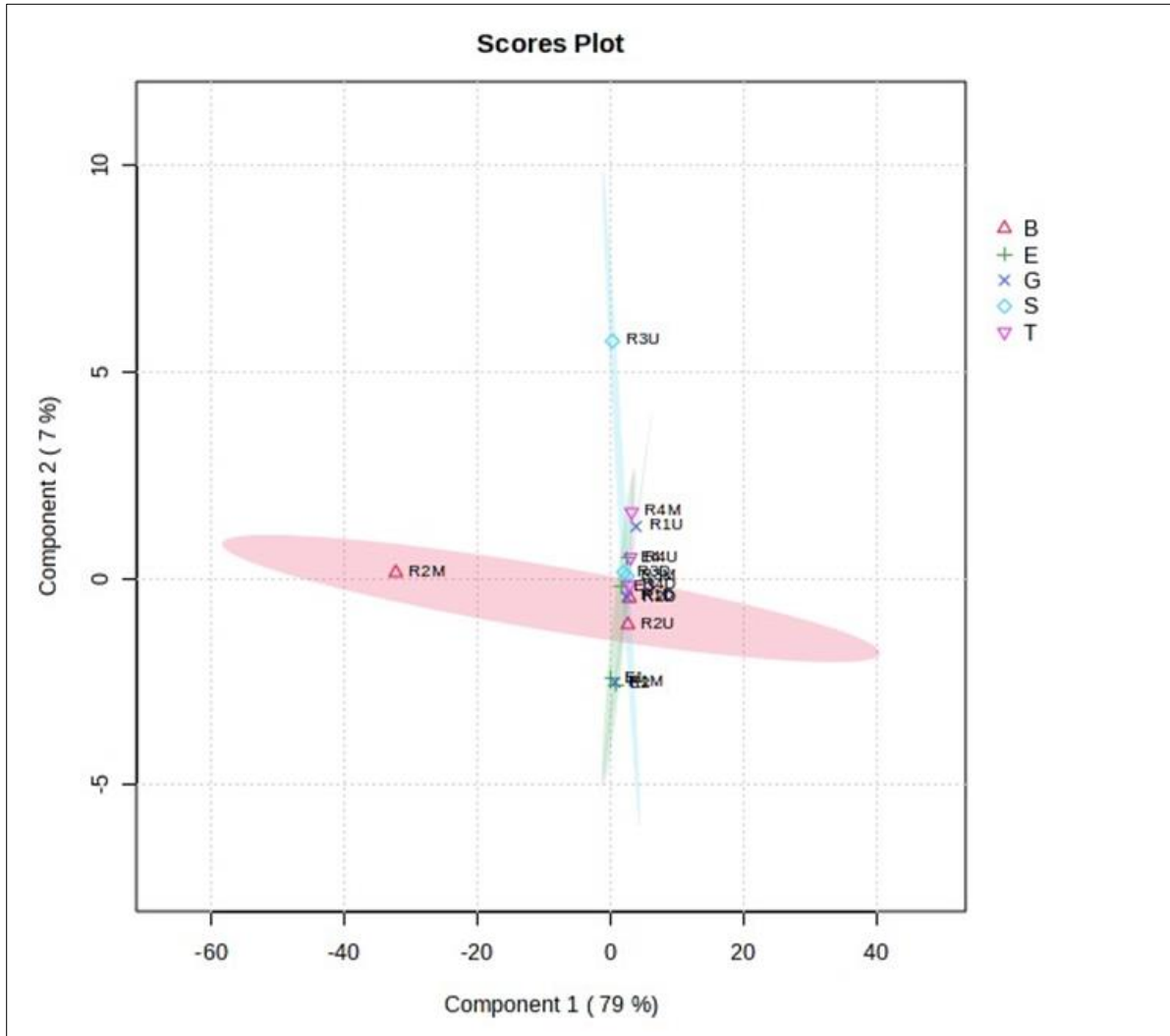
Figure 3 Scores plot between the selected PCs. The explained variances are in brackets

Figure 4 shows the result of Hierarchical clustering analysis of the samples as a dendrogram. Closely related samples (in terms of chemical composition) clustered together. The similarity is measured with Euclidean distance and clustering algorithm with Ward's linkage (clustering to minimise the sum of squares of any two clusters) (Murtagh and Legendre, 2014). At a distance of 30, there are two clusters, but at 20, there are 4 clusters.
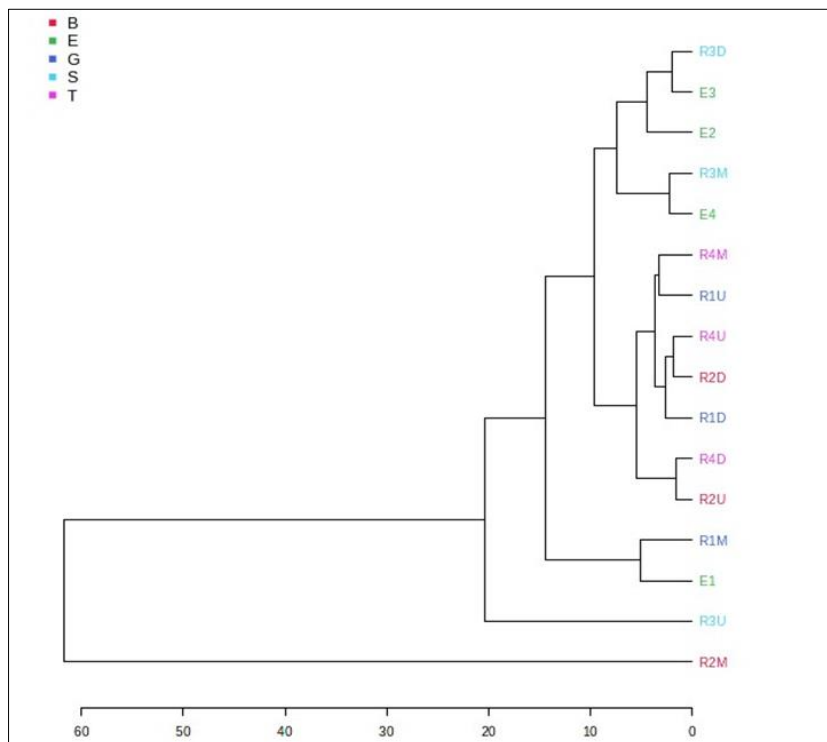
Figure 4 The clustering result is shown as a dendrogram (distance measure using Euclidean and clustering algorithm using ward.D)
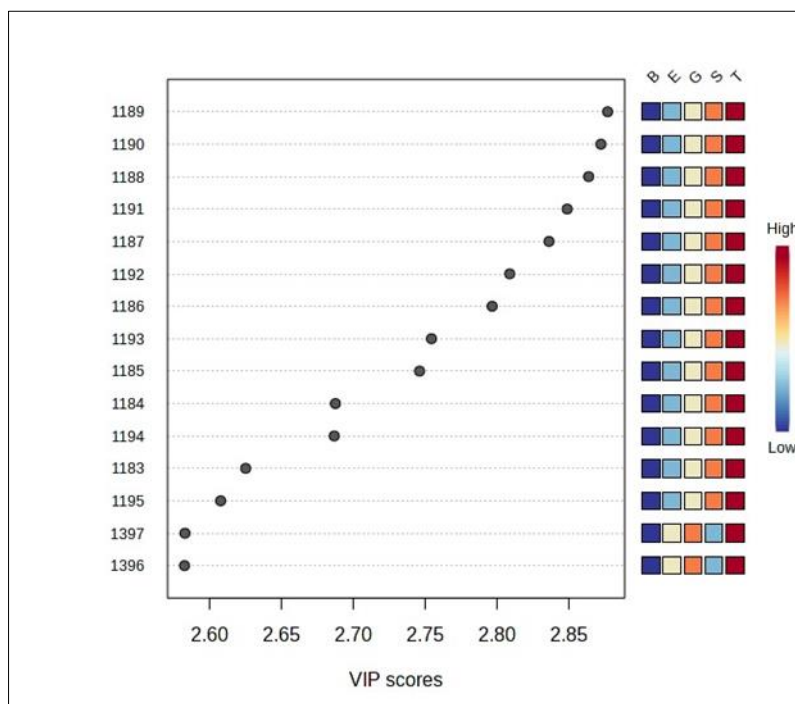

Figure 5 Important features identified by PLS-DA. The coloured box on the right indicates the relative concentrations of the corresponding metabolite in each group under study

Figure 5 shows the Variable Importance in Projection (VIP) scores for fifteen priority features identified by Partial Least Square-Discriminant Analysis (PLS-DA). These features correspond to the spectra of various substituted aromatic

compounds and phenols. Tyhume River samples had the highest concentration of these fifteen features. Grahamstown samples had features 794-804, which are peaks of aliphatic aldehyde and chlorinated aliphatic hydrocarbon compounds. Features 1007 to 1009, associated with substituted aromatic compounds were more abundant in Tyhume River samples. The VIP features may serve to distinguish these samples if taken further through elemental analysis.

4. Conclusion

The results of the water quality index and FT-IR analyses show that there are many chemical compounds in the water samples. Based on the result of the physicochemical parameters studied in this work, the quality of the four rivers was generally poor, and the concentrations of the attributes were above the recommended values for agriculture and aquatic ecosystems. This study shows that some of the parameters investigated in the effluents have concentrations above the recommended levels, thereby influencing the physicochemical qualities downstream. Lower reaches of the rivers show characteristics similar to the wastewater effluents received. The WWTPs were unable to remove chloride and nitrate ions from the wastewater. FT-IR report highlighted the differences in the water samples from different geographical backgrounds. These differences exist within samples from the same geographic location and among samples from different geographical areas. Some functional groups like aliphatic aldehydes, disubstituted alkenes, substituted aromatic compounds, alkynes, halogen and Sulphur compounds reported in the midstream and downstream samples of the rivers were not present in the upstream samples. Analyses revealed the presence of pollutants, especially functional groups of contaminants in the environmental water samples. The presence of functional groups of compounds such as alkyl halides in the treated effluent samples confirmed the inability of the treatment plants to remove them effectively from the wastewater. Certain primary amides, aryl aldehydes, organophosphates, fluorinated hydrocarbons, urethanes, and substituted aromatic compounds were not effectively removed from the wastewater at the WWTPs. They were present in the treated effluents, thereby contributing to river pollution. This study has been able to establish that some compounds filtered into freshwater through improperly treated wastewater effluents. Halogenated compounds, used in the treatment of some of the wastewater were reaching the receiving rivers. Multivariate analyses show a strong correlation between treated wastewater effluents and the receiving surface water samples, an indication that the rivers were polluted and the wastewater was poorly treated. If the current trend in water quality deterioration continues, it may prevent the achievement of social and economic growth and the elimination of poverty.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest is to be disclosed.

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