water

MDPI

Article

The Effect of Rainfall on *Escherichia coli* and Chemical Oxygen Demand in the Effluent Discharge from the Crocodile River Wastewater Treatment; South Africa

Thabang Maphanga [1], Benett S. Madonsela [1,*], Boredi S. Chidi [2], Karabo Shale [1], Lawrence Munjonji [3] and Stanley Lekata [4]

[1] Department of Environmental and Occupational Studies, Faculty of Applied Sciences, Cape Peninsula University of Technology, Corner of Hanover and Tennant Street, Zonnebloem, Cape Town 8000, South Africa
[2] Department of Biotechnology and Consumer Science, Faculty of Applied Sciences, Cape Peninsula University of Technology, Corner of Hanover and Tennant Street, Zonnebloem, Cape Town 8000, South Africa
[3] Risk and Vulnerability Science Center, University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa
[4] Centre for Postgraduate Studies (CPGS), Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville South Industrial, Cape Town 7530, South Africa
* Correspondence: madonselab@cput.ac.za

Abstract: The declining state of municipal wastewater treatment is one of the major contributors to the many pollution challenges faced in most parts of South Africa. *Escherichia coli* and Chemical Oxygen Demand are used as indicators for the performance of wastewater treatment plants. Wastewater treatment plant (WWTP) efficiency challenges are associated with susceptibility to seasonal variations that alter microbial density in wastewater. This study sought to investigate the effect of rainfall on *E. coli* and COD in the effluent wastewater discharged from the Crocodile River, Mpumalanga Province, South Africa. To cover the spatial distribution of the pollutant in the Crocodile River, water samples were collected from 2016 to 2021 at three strategic sites. The rainfall data was acquired from the South African Weather Services from 2016 to 2021, which contains daily rainfall measurements for each sampling site. Data analysis was carried out using Microsoft Excel 2019, Seaborn package, and Python Spyder (version 3.8). The White River, which is located on the upper stream, recorded the highest COD levels of 97.941 mg/L and 120.588 mg/L in autumn and spring, respectively. Matsulu WWTP was found to have the highest E. coli concentration per milliliter (72.47 cfu/100 mL) in the spring compared to any other location or time of year. The results also indicated that each of the sampling sites recorded above 60 (cfu)/100 mL of *E. coli* in Kanyamazane (spring), Matsulu (summer), and White River (winter). It was noted that the rainfall is a significant predictor ($p < 0.004$) of *E. coli*. Additionally, it was discovered during the data analysis that the rainfall parameter did not significantly affect COD prediction ($p > 0.634$), implying that rain was not a reliable predictor of COD.

Keywords: spatio-temporal variation; effluent; microbial quality; *Escherichia coli*; chemical oxygen demand

check for updates

Citation: Maphanga, T.; Madonsela, B.S.; Chidi, B.S.; Shale, K.; Munjonji, L.; Lekata, S. The Effect of Rainfall on *Escherichia coli* and Chemical Oxygen Demand in the Effluent Discharge from the Crocodile River Wastewater Treatment; South Africa. *Water* **2022**, *14*, 2802. https://doi.org/10.3390/w14182802

Academic Editor: Christos S. Akratos

Received: 9 August 2022
Accepted: 5 September 2022
Published: 9 September 2022

1. Introduction

Water quality is vital not only for the sustenance of human life but also for the health of the ecosystem [1,2]. Water quality is of particular importance in arid or semi-arid regions such as South Africa where water is scarce and access to safe piped water is uneven, as is the case in many Southern African countries [3–5]. In the rural areas of South Africa, especially in resource-poor settings, an inability to meet the increasing demand for drinking water has resulted in many people resorting to using polluted surface water such as rivers [3,6,7]. The country is currently classified as water-stressed, with only about 1200 m^3/person/year of fresh water available for a population of about 50 million [8]. One of the most significant contributors to the pollution of water resources, particularly surface water resources, is the state of municipal wastewater treatment facilities and infrastructure [9,10]. Other pollutant sources are the agricultural sector and industrial plants that channel their waste directly into rivers, thereby significantly compromising the microbial quality of nearby rivers [8,11,12]. In many instances, surface water resources are more vulnerable to pollution from a variety of sources because they are the most easily accessible for general use.

Water treatment plants and wastewater contain a wide variety of microbial communities that make effluent unfit for discharge into the environment [13]. *Escherichia coli* (*E. coli*) and chemical oxygen demand (COD) are the preferred indicators to monitor wastewater quality [4,14]. According to Edokpayi et al. [4], effluents generated from both industrial and domestic activities are the second most common source of chemical and microbial pollution in South Africa's water sources. The quality of recovered water sources for water reuse can also be evaluated using information from effluent wastewater and treated effluent. Wastewater treatment plants (WWTPs) were identified by Münze et al. [15] as important point sources of chemicals discharged in the aquatic environments, resulting in a much greater concentration of chemicals downstream of the effluent. Since wastewater is treated by microbes through a metabolic process [16], the water quality of a WWTP is highly dependent on parameters such as pH, COD, *E. coli,* and other contaminants [12,14,17]. Water treatment plants were established as central units to reduce pollution loads to acceptable levels before the resultant effluent was discharged into receiving water bodies to prevent potential health threats that might result from the occurrence of microbial pollutants in water resources. Thus, the effluent quality of municipal wastewater treatment plants is a crucial factor in determining the best treatment technologies [18,19].

Wastewater treatment plants are widely used around the world and play a vital role in improving wastewater quality before it is discharged into surface or groundwater and re-enters water systems [11,12]. However, given the ageing infrastructure of WWTP, mostly in developing countries which have been overburdened by rapid population growth, these treatment plants are unable to produce effluent that meets international and/or national discharge standards. South Africa is also facing significant pressure due to rapid population growth and urbanization. Many studies have been conducted regarding the poor infrastructure of WWTP and its impact on water quality [20–23]. Some studies have investigated the impact of poorly treated wastewater effluent from industries [8,24,25] and others from WWTPs [6,26].

During the last 50 years, many countries have worked to reduce the volume of untreated wastewater discharged into rivers and streams by closely monitoring and constantly improving municipal and industrial wastewater treatment plants [11,26]. Therefore, for countries to ensure the long-term sustainability of critical water supplies, particularly in urban areas, effective wastewater management is highly required. South Africa has developed a significant wastewater management industry, which includes approximately 850 municipal wastewater treatment plants, extensive pipe networks, and pump stations that transport and treat wastewater daily [27]. Ntombela et al. [28] reported that more than 19 WWTP were overflowing into water bodies because of ageing infrastructure and increasing pressure. Furthermore, the study revealed that approximately 49.6% of South Africa's WWTP scored below the required standards in 2012 and 2013. Given the poor state of WWTP infrastructure in South Africa, many rivers have poor water quality. The water quality of these rivers is critical for the health of the people who live in these water basins, and it is influenced by a variety of factors.

Concomitant to the above, water quality assessment is critical for identifying the major role of players and contributors to spatial and temporal variations in quality, which can be useful for integrated water resource management. Monitoring is necessary to ensure that water resources and their quality remain within acceptable limits for long-term use [29,30]. In light of the above, some researchers have conducted studies that seek to include other different factors that may contribute to the poor water quality in addition to the existing effluent discharged. Moreover, a small but growing number of researchers have additionally started assessing the influence of climatic and environmental variables on water quality in surface water [3,13,14,31]. Some of the studies discovered that the variation of physicochemical parameters demonstrates a general trend of higher concentrations during the wet season and lower concentrations during the dry season [8,14,17,32].

The above-mentioned studies focused on physicochemical factors such as temperature, turbidity, pH, and nutrient concentrations. However, the impact of rainfall on the spatio-temporal distribution of parameters such as COD and *E. coli* in discharged effluent has not been fully researched, particularly in South African rivers. In addition, previous studies have not compared these parameters at different locations or regions (upstream, midstream, and downstream). Therefore, the main aim of this study was to investigate the effect of rainfall on *E. coli* and COD in the effluent wastewater discharged on the Crocodile River. In order to address the overarching aim of this study, the following objectives were used: firstly, to assess the spatiotemporal distribution of physicochemical parameters (*E. coli* and COD) along the Crocodile River; secondly, to determine the influence of rainfall on the spatial variation of *E. coli* and COD along the river; and lastly, to assess the concentration of *E. coli* and COD across the Crocodile River and the influence of rainfall.

2. Materials and Methods

2.1. Study Area

The Crocodile River catchment area is approximately 10,500 km^2 and is located approximately 300 km east of Johannesburg in the Mpumalanga Province. This is the largest tributary of the Komati River, which it joins just before the Mozambique border. The Elands River, Upper Crocodile, Kaap, Middle Crocodile, and Lower Crocodile Rivers are tertiary sub-catchments of the Crocodile River catchment. The Kruger National Park's southern sector contains approximately 20% of the watershed (a north-eastern component). Crocodile River is a slow-moving river with a low gradient and primary bedrock and sandy pools. It has an average width of 45 m. The Lowveld region has grown significantly, with agricultural activity skyrocketing. Large volumes of water are pumped out of the river as a result of these activities, resulting in a decrease in flow, especially during dry seasons. The majority of the river's riparian zone is dominated by reeds. The Crocodile River's lower reaches are considered to have poor water quality due to agricultural runoff, mining activities, and poorly treated effluent from wastewater treatment plants. Figure 1 shows the study area map with sampling sites.

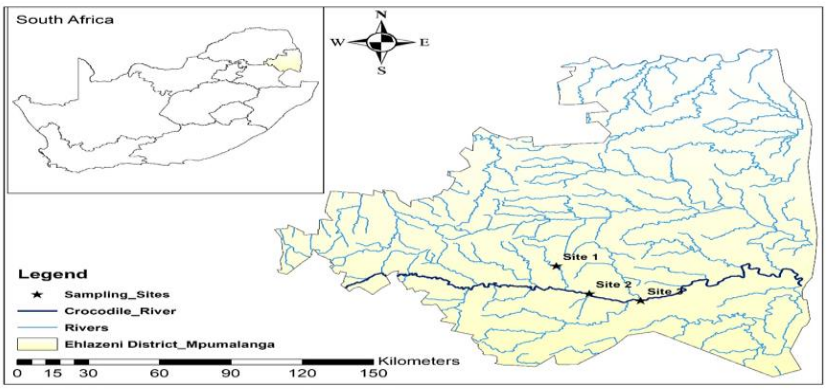

Figure 1. Map of the study area showing the location of sampling points.

2.2. Sampling Site Descriptions

2.2.1. White River WWTP (Site 1)

The White River wastewater treatment plant treats domestic wastewater from the town of White River and the nearby location Plaston and discharges the treated effluent into the White River, which is a tributary of the Crocodile River. The current land use around the area is mixed, especially towards the White River central business district, with residential settlements and retail centres. The surrounding areas are mostly agricultural lands dominated by tropical fruits, flowers, and timber.

2.2.2. Kanyamazane WWTP (Site 2)

Kanyamazane WWTP treats domestic wastewater from Kanyamazane township, and the treated effluent is discharged into the Crocodile River. The area is densely populated, and the water quality is mostly influenced by anthropogenic activities undertaken within the surrounding residential settlements situated next to the Crocodile River. Kanyamazane is the southernmost town in the Nsikazi activity corridor, located approximately 30 km east of Nelspruit, 17 km south of Kabokweni, and forms almost a continuously built-up area that links to Msogwaba in the north. The oblong north–south configuration of Kanyamazane can be ascribed to the surrounding mountainous areas and a prominent tributary.

2.2.3. Matsulu WWTP (Site 3)

Matsulu town is fairly secluded, situated in the easternmost part of the municipality, approximately 45 km east of Nelspruit. Matsulu is wedged between the Kruger National Park, Mthethomusha Nature Reserve, and the N4 highway and is bisected by the railway line to Phalaborwa. Matsulu consists of the formal townships of Matsulu A, B, C, and Matsulu West. Proximity to the N4 makes it a rapidly growing area with a high influx of people leading to the informal settlement. The Matsulu wastewater treatment plant treats domestic wastewater from Matsulu township and discharges treated effluent into the Crocodile River. The plant is situated in a residential area, and the area is also dominated by agricultural land use activities.

2.3. Sampling Methodology

The water quality sample bottles (polyethylene plastic bottles) were marked with the site code, date, and time of sample collection using a permanent marker. No additives were introduced in the microbial sample bottles as they were pre-sterilized. For sampling of chemicals and microorganisms to assess quality, the grab sample approach was employed [33]. Until the sample was ready to be collected, the bottle lids were left on. All the necessary samples were collected where the effluent is discharged into the river, downstream of the discharge points and at the confluence of tributaries. On the other hand, one (1) liter bottles (meant for chemical analysis) were rinsed three times before they were filled with sample. The 300 mL sample bottles (meant for microbial analysis) were not rinsed since they were sterilized, and ample air space was left in the bottle to facilitate mixing by shaking [33]. Both chemical and microbial water quality samples were stored in two separate cooler boxes and preserved with ice packs or cubes.

2.4. Rainfall Data

Rainfall data was attained from the South African Weather Service for the period of 2016 to 2021 which contained daily rainfall measurements for each study site. Rainfall data is crucial as it is highly variable, which can result in seasonal and inter-annual fluctuations, and therefore has the potential to influence the effluent discharge.

2.5. Physicochemical Parameters

The South African Department of Water and Sanitation (DWS) General Authorization guidelines (general and special limits) were used as benchmarks to evaluate the acceptability of the final effluent quality [34]. General limits apply to WWTPs that discharge less than 2000 m^3 of effluent and discharge into water resources that are not listed in the regulations, while special limits apply to WWTPs that discharge less than 2000 m^3 of effluent but discharge into a water resource listed in the regulations [34]. The information below shows the different effluent discharge quality limits per site as stipulated by the WWTP water use license. The standards were established by South Africa's Department of Water Affairs (DWA) in accordance with the government gazette no 39,614 issued on 22 January 2016 and issued water use licenses. The Crocodile River has a class C ecological status and is intended to support farming and commercial and subsistence fishing. Discharge limits vary from plant to plant depending on the characteristics of the receiving water, effects on aquatic life, recreational uses, and other factors. Table 1 shows the effluent discharge Quality Limits as per WWTP Water Use License for each site that is located in the Crocodile River.

Table 1. Effluent discharge Quality Limits as per WWTP in the three sites' Water Use License.

	pH	EC mS/cm	NO_2 + NO_3(mg/L)	*E. coli* (cfu)/100 mL	SS (mg/L)	PO_4 mg/dL	NH_3mg/L	COD mg/L
White River WWTP	5.5–9.5	70	15	0	25	1	1	75
Kanyamazane WWTP	5.5–9.5	75	15	0	25	1	6	75
Matsulu WWTP	5.5–9.5	70	15	0	25	1	3	75

3. Data Analysis

3.1. Analysis of Physicochemical Parameters

Water samples were collected from 2016 to 2021 at three different sites to cover the spatial distribution of the pollutant in the Crocodile River. Monthly samples were collected on each site following the same sampling procedure, as discussed above. The samples were transported to a laboratory accredited by the South African National Accreditation System (SANAS) for analysis, and microbiological samples were processed within 12 h from the point of collection.

Potassium Dichromate was used to analyse Chemical Oxygen Demand as an oxidizing agent. The sample was digested with dichromate, which oxidizes the COD in the sample. Potassium dichromate, sulfuric acid, and potassium hydrogen phthalate were used as reagents. At 610 nm, a spectrophotometer (Thermo Scientific Orion AquaMate 8100 UV-Vis, Labotec, Cape Town, South Africa) was used to analyse the sample, whilst on the other hand, the Hach USEPA membrane filtration method 8367 m-TEC Agar was used for *Escherichia coli*. The m-TEC method detects *E. coli* in samples of recreational freshwater in two steps. To revive injured organisms, membrane filters were incubated for two hours at 35 °C on m-TEC Agar. The thermos-tolerant organisms were subsequently selected by fermenting lactose at 44.5 °C. The second step distinguishes urease-negative *E. coli* from other thermotolerant coliforms that hydrolyze urea by using a substrate medium containing urea. Yellow or yellow-brown colonies without urease are positive for *E. coli*.

3.2. Statistical Analysis

Data analysis was carried out using Microsoft Excel 2019 and Python (version 3.8) Spyder. The generation of heat maps that were used for better visualization of the data was performed using the Seaborn package for Python. The daily rainfall from January 2016 to September 2021 was divided into four categories as follows: (1) rainfall below the lower quartile, (2) rainfall between the lower and median quartile, (3) rainfall between the median and upper quartile, and (4) rainfall above the upper quartile. As a result of rainfall data acquired from the South African Weather Service, it was observed that some months in a given year experienced zero rainfall, while others experienced above-average rainfall. The data were then clustered into the preceding quartile in order to eliminate severe outliers in the datasets, particularly the daily rainfall data. It is worth noting that rainfall below the lower quartile indicates periods when average rainfall was generally very low, and rainfall above the upper quartile indicates periods when average rainfall was extremely high. The daily average rainfall for each month was calculated from the measurement of the rainfall in each site. For the purpose of this study, the lower quartile is rainfall below 0.145 mm; the median quartile is 0.906 mm, and the upper quartile is 2.637 mm. The pivot tables or cross-tabulations for average *E. coli* and average COD for different categories were generated between the sites upstream, midstream, and downstream. In addition to evaluating single-variate patterns of water quality parameters, the study also examined multivariate patterns as proposed by Alberto et al. [35] and Singh et al. [36]. All statistical analyses were performed at the 95% confidence limit.

4. Results

4.1. Spatial-Temporal Distribution of Chemical Oxygen Demand (COD) in Three Strategic Sites of the Crocodile River

The heat maps were created to visualize data collected from 2016 to 2021 in the Crocodile River. The figures below illustrate the concentration of COD levels in the sampled sites (White River WWTP, Kanyamazane WWTP, and Matsulu WWTP) in years using heatmaps for visualization purposes. In order to ensure compliance, COD concentrations should be measured in effluent water at the time it enters the plant, before the mechanical screening process, and at the point of discharge after the treatment. The figures below show the seasonal concentration of COD for the data collected from 2016 to 2021 without the influence of rainfall.

The results above from in Figure 2B show the spatio-temporal distribution of COD from 2016 to 2021 and by seasons in all three sites that were sampled. Figure 2A shows the spatio-temporal distribution of COD across the Crocodile River over the years. It was observed that White River (site 1) recorded the highest concentration of COD in 2016 which was 137.66mg/L, almost double the required limits (see Table 1). Overall, the results show that White River WWTP recorded high levels of COD compared to the other sites. Moreover, it was noted that there is statistical significance in the levels of COD between Kanyamazane and White River at $p < 0.001$ (see Supplementary Table S1). To be precise, there are significant differences in COD concentrations at Kanyamazane, White River, and Matsulu. However, there is no significant difference between COD concentrations at Kanyamazane and Matsulu. In contrast to the results observed from site 1 (White River WWTP) from 2016 and 2017, the Matsulu WWTP observed very low COD concentration levels with 17.754 mg/L recorded in 2016. The White River WWTP was demonstrated to be non-compliant, not meeting the standards that were set by the Department of Water and Sanitation (Table 1) as gazetted in 2016 per the water use license of that site. The results from Figure 2A show that there is a variation in the spatio-temporal distribution of COD concentration in location and year. The concentration of COD in Kanyamazane was significantly lower than that in White River ($p = 0.001$), indicating lower concentration in COD.

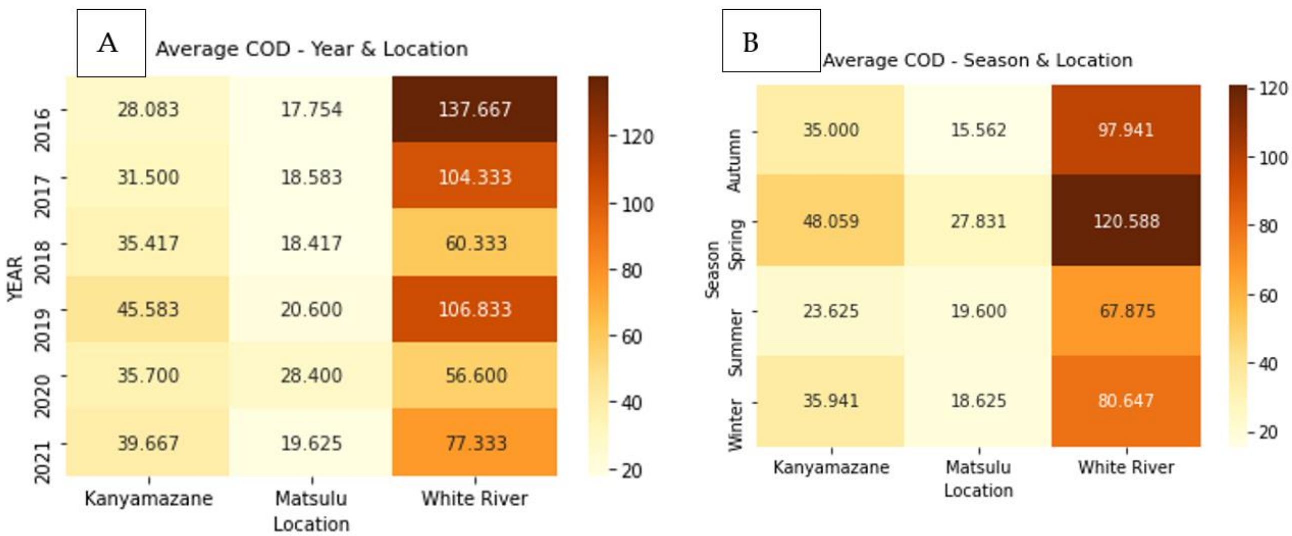

Figure 2. The distribution of COD concentration levels in the sampled sites (White River WWTP, Kanyamazane WWTP, and Matsulu WWTP) in years (**A**). Spatio-temporal distribution of Chemical Oxygen Demand (COD) seasons in the three sites (**B**).

Figure 2B shows the spatiotemporal distribution of COD across the Crocodile River for all the seasons to determine the seasonal variation of concentration levels of COD. The results above show that White River recorded almost double the levels of COD during the spring season, as per the approved limits. Figure 2B reflects the distribution of COD in seasons in all the three sites where it was noted that White River located on the upper stream recorded the highest COD levels of 97.941 mg/L and 120.588 mg/L in autumn and spring, respectively. The lowest COD concentration levels were recorded in Matsulu WWTP in all the seasons, whereby the lowest was 15.563 mg/L in autumn. According to the results above, the highest COD level at Kanyamazane WWTP was 48.059 mg/L recorded in spring, which still below the stipulated limits (Table 1). Figure 2B further illustrates that White River WWTP was compliant with the legal limits during 2018 and 2020. When compared with other sites, White River recorded high levels in winter, autumn, and spring. Therefore, it can be concluded that the White River site was non-compliant based on the findings above. This is further elaborated by the results in Figure 2A, which also showed a similar finding in terms of the spatial variation of the COD in the Crocodile River by years as opposed to seasons. Additionally, the results showed that the was no statistical significance in the seasons and location of the sites along the Crocodile River (see Supplementary Table S2).

4.2. Spatio-Temporal Distribution of Escherichia coli Levels from the Three WWTP Sampled in the Crocodile River

Wastewater treatment plants efficiencies are measured using many parameters, but the most common are chemical oxygen demand (COD) and *Escherichia coli* to determine the performance of the plant. Figure 3A below gives a conception of the spatio-temporal distribution of the *E. coli* levels depicted in the form of a heat map of the data collected from 2016 to 2021 in the three wastewater treatment plants. The occurrence levels of physicochemical properties in drinking water sources generally determine fate in the environment after discharge, and the relative contribution of treated wastewater to the overall flow. In light of this, Figure 3B illustrates that the concentration of *E. coli* in the Crocodile River varied according to the season and location for the time period sampled.

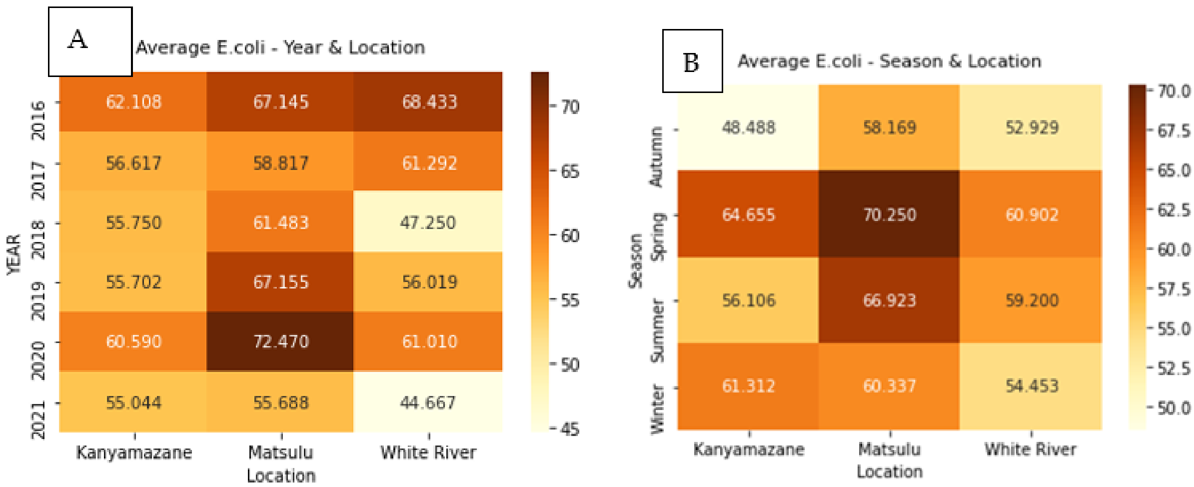

Figure 3. Spatial-temporal distribution of *Escherichia coli* levels from the three WWTP sampled in the Crocodile River (**A**). *E. coli* concentrations at each sampling site for all four seasons (**B**).

The above results show the spatio-temporal distributions of *E. coli* levels along the Crocodile River from 2016 to 2021, and these findings are evaluated against the effluent discharge quality limits as per WWTP in the three sites' water use license. The results show that Matsulu WWTP recorded its highest *E. coli* levels in 2020, while the other two sites recorded relatively low levels during the same period. It was also noted that in 2016 all three sites recorded almost the same levels of *E. coli,* which were above 60 (cfu)/100 mL.

Generally, the Matsulu WWTP was noncompliant with approved guidelines and in line with the water use license for the Matsulu effluent discharge limits. However, it was observed that White River WWTP was found to be not compliant with the stipulated water use license limits during the period of 2018 and 2021 even though it recorded the lowest level of concentration. The analysis from ANOVA (*E. coli* concentrations at different locations) shows that *E. coli* concentrations vary at different locations in the Crocodile River (see Supplementary Table S3). There was a significant difference (p = 0.004) between *E. coli* concentrations at Kanyamazane and Matsulu; White River and Matsulu. However, there is no significant difference between *E. coli* concentrations at Kanyamazane and White River.

It is observed that Matsulu WWTP had the highest level of *E. coli* count per ml in spring than any other site or season. The results also indicated that each of the sampling sites recorded above 60 (cfu)/100 mL. of *E. coli* in different seasons; Kanyamazane (spring), rainMatsulu (summer), and White River (winter). Moreover, there was a statistical significance in the level of concentration of *E. coli* between summer and autumn (p = 0.015), in addition to spring and winter (p = 0.042) (see Supplementary Table S4). All the sampling sites were noncompliant in terms of the South African water use license limits for effluent quality discharge of *E. coli* (see Table 1).

4.3. Spatio-Temporal Distribution of Chemical Oxygen Demand (COD) by Rainfall in the Three Sites

To better understand the latent spatial structure of the dataset, the study examined the seasonal dynamics of rainfall and chemical oxygen demand (COD) in water quality. Figure 4 therefore shows the effect of rainfall on the concentration of COD in all four seasons from 2016 to 2021 in all three sites, while Table 2 shows a regression model of the results depicted in the heatmaps in Figure 4. The variation of COD in all three sites as a results of rainfall is shown from 2016 to 2021 (see Appendix B).

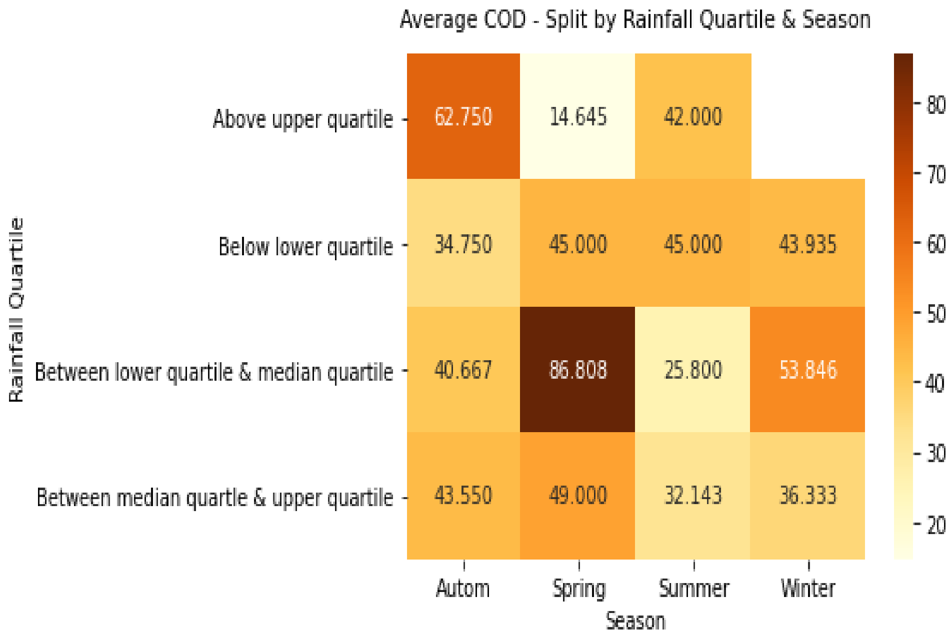

Figure 4. Spatial distribution of COD by seasons with the influence of rainfall. Lower quartile 0.145 mm, Median quartile 0.906 mm, and Upper quartile 2.637 mm.

The above results depict the spatio-temporal distribution of COD levels across seasons using a heat map to give better visualization. It was noted that rainfall had an effect on the concentration of COD in the three sites when compared to the previous analysis where rainfall was not factored in (see Figure 2). Spring recorded the highest levels of COD, which is slightly above the legal requirement. When rainfall was low during spring (Figure 4), high COD was recorded, which was in contrast to when there were high rainfall levels. In general, the level of COD was within the limits as per the license use. It was also observed during the data analysis that when predicting COD using rainfall, the rainfall parameter was not significant ($p > 0.634$), hence it can be noted that rainfall is not a significant predictor of COD. The results above illustrate that rainfall has no significant effect on the chemical oxygen demand concentration level in the Crocodile River. Furthermore, it was observed that only 0.1% ($R^2 = 0.001$) of the variation can be explained by the model and the independent variables of the model were not statistically significant (Table 2).

Table 2. Spatio-temporal distribution of Chemical Oxygen Demand (COD) by rainfall in the three sites.

OLS Regression Results						
Dep. Variable:		y		R-squared:		0.001
Model:		OLS		Adj. R-squared:		−0.004
Method:		Least Squares		F-statistic:		0.2278
Date:		Thu, 8 September 2022		Prob (F-statistic):		0.634
Time:		10:34:29		Log-Likelihood:		−1125
No. Observations:		195		AIC:		2254
Df Residuals:		193		BIC:		2261
Df Model:		1				
Covariance Type:		nonrobust				
	coef	std err	t	P > |t|	[0.025	0.975]
const	48.0056	6.809	7.051	0	34.577	61.434
Rainfall	1.0628	2.227	0.477	0.634	−3.329	5.455
Omnibus:		280.229		Durbin-Watson:		1.648
Prob (Omnibus):		0		Jarque-Bera (JB):		22,262.47
Skew:		6.433		Prob (JB):		0
Kurtosis:		53.739		Cond. No.		3.87

4.4. The Effect of Rainfall on E. coli in the Crocodile River across Four Seasons

Generally, water quality can provide useful information about the land use within a catchment area, such as deteriorating water quality. Figure 5 below illustrates the levels of *Escherichia coli* concentration in the Crocodile River in four different seasons under the influence of rainfall. Correlation tables (like correlation heat maps) show an inverse relationship between rainfall and *E. coli* (Table 3). The variation of *E.coli* in all three sites as a results of rainfall is shown from 2016 to 2021 (see Appendix A) .

The heat map shows the different concentrations of *E. coli* in the Crocodile River from 2016 to 2021, covering every season during this time, and there was a variation in *E. coli* levels at different rainfall levels and seasons. Spring recorded the highest level of *E. coli* when the rainfall was at median and lower quartiles. The data analysis shows that downstream (Matsulu) recorded a high level of *E. coli* compared to upstream (White River) with the influence of rainfall (Table 3). According to these findings, precipitation affects concentrations in a variety of ways. Increased precipitation reduces concentration, while decreased precipitation increases the proportion of wastewater in surface water and thus increases concentration. When predicting *E. coli* using rainfall, the study showed that the rainfall parameter is significant at 5% significance level, hence it can be noted that rainfall is a significant predictor of *E. coli* with $p < 0.004$. The results also showed that the regression model accounts for $R^2 = 0.043$ of the variance, and the independent variables of the model have statistical significance. This represents the mean change in the dependent variable when the independent variable shifts by one unit. A unit increase in rainfall decreases *E. coli* by 1.1017 units ($p < 0.004$). An inverse relationship between rainfall and *E. coli* is also indicated in the correlation table and correlation heat map.

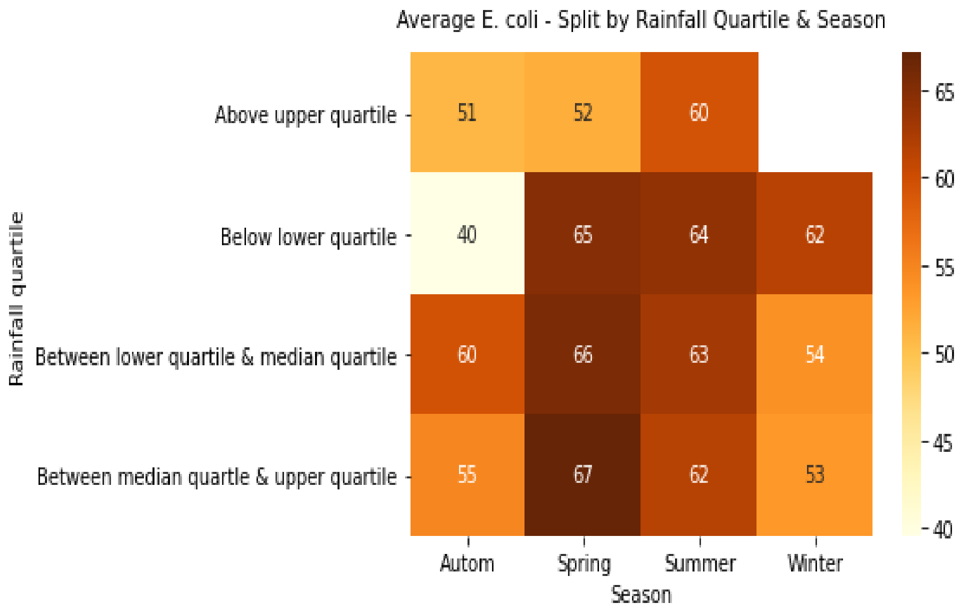

Figure 5. The distribution of *Escherichia coli* along the Crocodile River from 2016 to 2021 during different seasons.

Table 3. The regression model for Escherichia coli along the Crocodile River from 2016 to 2021 in the three strategic sampling sites.

OLS Regression Results					
Dep. Variable:		y		R-squared:	0.043
Model:		OLS		Adj. R-squared:	0.038
Method:		Least Squares		F-statistic:	8.738
Date:		Thu, 8 September 2022		Prob (F-statistic):	0.00351
Time:		11:45:03		Log-Likelihood:	−776.46
No. Observations:		195		AIC:	1557
Df Residuals:		193		BIC:	1563
Df Model:		1			
Covariance Type:		nonrobust			
	coef	std err	t	P > |t|	[0.025	0.975]
const	61.2524	1.14	53.75	0	59.005	63.5
Rainfall	−1.1017	0.037	−2.956	0.004	−1.837	−0.367
Omnibus:		33.863		Durbin-Watson:	1.174
Prob (Omnibus):		0		Jarque-Bera (JB):	218.581
Skew:		−0.347		Prob (JB):	3.43×10^{-48}
Kurtosis:		8.14		Cond. No.	3.87

5. Discussion

5.1. Spatio-Temporal Distribution of Chemical Oxygen Demand (COD) in Three Strategic Sites of the Crocodile River

Water and wastewater quality is measured by chemical oxygen demand (COD), which is commonly used to monitor water treatment plant efficiency. Therefore, the White River WWTP was inefficient and noncompliant with the WWTP's Effluent Discharge Quality Limits, as defined in the Water Use License for the three sites (Table 1). The White River WWTP site recorded 106 mg/L of COD levels (Figure 2A) and given that the site is located upstream, it was expected that during rainfall the physicochemical would be found in the lower sites. However, the model shows that when predicting COD using rainfall, it was noticed that the rainfall parameter was not significant ($p > 0.634$). The study found that rainfall was not a good predictor of COD with $R^2 = 0.001$ and $p = 0.634$ (Figure 4). This is supported by a finding by Wang et al. [1] whereby it was found that the concentration of

COD was not significantly different between the sampling sites during the four seasons. It was noted that the level of COD reduced only in one season (Figure 2B) which is in contrast to the findings of Joel et al. [37], which showed that COD was reduced from one point to the next during the two seasons of study.

Furthermore, higher levels of COD concentrations were observed in the upstream site (White River WWPT) recorded at 120.588 mg/L and 97.941 mg/L in spring and autumn, respectively, and these are considered dry seasons. These findings were similar to those of Abagale [38] where in the wet season, however, the concentration of COD ranged from 102.5 mg/L to 203.00 mg/L with an average concentration of 143.75 mg/L. Although the similarity was observed during the same months of March–May and September–November, the difference is that the wet and dry seasons as in the same months in the study by Abagale [38] were classified as wet seasons due to geographical locations. In addition, Abagale [38] found that COD concentration in wastewater by the different treatment units were statistically highly significant at *p* value of <0.001, which contradicts the findings of this study, as there was no tangible statistical significance (*p* value). Therefore, this study found that there was no spatial distribution of chemical oxygen demand in the Crocodile River and there was no seasonal variation in all three sites.

Although the rainfall did not have any significant effect on the distribution of the COD across the three sites, it was noted that the White River WWTP was discharging poorly treated effluent into the environment, which means that there was an introduction of organic pollution into receiving waters (Figure 2A). A high concentration of COD shows that there is poor efficiency in water treatment plants. This is because chemical oxygen demand is a measure of the quality of water and wastewater and is used to monitor the performance of WWTPs [4].

Figure 4 showed that rainfall had no significance in the spatio-temporal distribution of chemical oxygen demand in all the three sites along the Crocodile River. However, looking at the concentration levels of COD in Figure 2B, in which the highest was 120.588 mg/L in spring, compared to Figure 4 which factored influence of rainfall data in each season, it can be noted that there was a reduction in the concentration with the recorded highest at 86.808 mg/L in spring. Makuwa et al. [14] observed that, while rainfall does not predict COD, there may be a difference in COD during the wet season versus the dry season. However, lower COD concentrations, according to Osuolale and Okoh [39], are primarily associated with dilution by higher water flow during the rainy season. Given the results of Figure 4, it showed that rainfall was a poor predictor of the variation of COD as only 0.1% (R^2 = 0.001) of the variation could be explained by the model. This variation might be due to the fact that the study was performed in a more complex system not a homogeneous one. For example, splitting the seasons into dry and wet instead of considering rainfall for all the seasons as dry does not mean there was no rainfall but rather following the seasonal nomenclature of South Africa in terms of rain levels.

The high concentration of COD in wastewater indicates organic concentrations that can deplete dissolved oxygen in the water, resulting in negative environmental and regulatory consequences. There are concerns over the operation and maintenance of wastewater and sewage treatment infrastructure in South Africa. Mema [9] stated that water pollution is mainly caused by inadequate wastewater and sewage treatment infrastructure, which has a direct impact on both human health and the environment. Furthermore, the majority of wastewater treatment plants in South Africa are small (500–2000 m^3/day) [40]; thus, given the rapid population growth, the WWTPs are under severe pressure. The results in Figure 2A show that South Africa's WWTPs are in poor condition, and this is in line with a report issued by the Department of Water Affairs in 2012. The cumulative risk rating of South African wastewater treatment systems revealed that 72 percent of the various systems posed a high and critical threat to the environment [27]. According to Edokpayi et al. [40], water infrastructure in South Africa is in poor condition and does not meet regulatory standards 50 percent of the time.

5.2. Spatio-Temporal Distribution of Escherichia coli Levels from the Three WWTP Sampled in the Crocodile River

Figure 5 shows the seasonal variation of the *Escherichia coli* levels in the Crocodile River for the sampled period from 2016 to 2021. This variation was then benchmarked with the water use license as per the WWTP effluent discharge quality within the Crocodile River (Table 1). The results shown in Figure 3A,B further depict that there is variation in terms of the spatial distribution of *E. coli* from upstream to downstream of the Crocodile River. In the downstream area of the river (Matsulu), *E. coli* concentration levels averaged 63.92 (cfu)/100 mL and were higher during the spring and summer seasons. This is supported by a study by Abia et al. [3] conducted in the Apies River, where water contaminated with *E. coli* flows downstream, until it reaches an area where the flow conditions change (broader and flatter river channels and low water velocity).

The results observed in Figure 4 are in line with a finding by Makuwa et al. [14] where high levels of *E coli* were recorded during the wet season (summer). The analysis shows that there is an inverse relationship between rainfall and *E. coli*, and this is also indicated in the correlation table (see Table 3) and correlation heat map (Figure 5). The results in Figure 3A show a statistically significant difference in *E. coli* concentrations across all sites ($p = 0.004$) without the influence of rainfall. Although some studies have observed a similar seasonal variation in high concentration levels of *E. coli,* which is associated with water temperature [41] and heavy rainfall during the wet season [13,14]. This study observed a slight difference from previous studies as it factored in the effect of rainfall effect during the different seasons no matter the intensity. Furthermore, this study noted that the downstream site recorded high *E. coli* concentration levels without factoring in rainfall, and there was significant variation in the same site when rainfall was factored in.

According to Abagale [38], because of changes in temperature and, to a lesser extent, flow rate, which is influenced by rainfall in the wet season, there was a higher detection of *E. coli* in the wet season compared to the dry season. This concurs with this research as it noted the spatio-temporal variation of *E. coli* concentration levels during the spring and summer (September to March). The low microbiological quality of the effluents and receiving water bodies could put the local communities at risk. The presence of *E. coli* remains the most reliable indicator of recent fecal contamination in aquatic environments and is often used as a marker of effluents from WWTPs [42]. In order to identify potential water quality problems, the trend detection technique can be used to analyze long-term variations in water quality [43]. The formation and transformation of pollutants can be greatly influenced by hydro-meteorological factors such as precipitation and slope gradient [32]. Despite ongoing research, wastewater treatment plants continue to be a major source of environmental toxins in the environment [13]. Seasonal variations affected *E. coli* concentrations in both water and sediment, with concentrations increasing during the wet season [3]. These results are in line with the study as similar variations of *E. coli* levels in the different seasons were observed, but also worth noting is that in this research *E. coli* concentrations differ at different locations of the Crocodile River.

In line with the climate change and variable rainfall patterns, there have been changes where some places have received late winter rainfall, even though they were previously known to receive summer rainfall. With this climate challenge in mind, the study factored in all the rainfall in each site regardless of the season, which previous studies never took into account. When predicting *E. coli* using rainfall, this study noticed that the rainfall parameter was significant at 5% significance level, hence it can be concluded that rainfall is a significant predictor of *E. coli* with $p < 0.004$. Previous studies [3,9] suggested that rainfall correlates with decreasing aquatic ecosystem microbial quality. However, there are several factors contributing to this decrease, including an increase in microbial load from non-point sources entering the water bodies as runoff from nearby urban areas and farms [8,36].

Moreover, runoff from surrounding farms could also carry large amounts of organic nutrients. Other studies, however, have found lower levels of effluent pollutants in wet weather due to the dilution of wastewater by stormwater ingress [38,44]. As a result, the treatment facility must be evaluated stringently during heavy rainfall events in order to develop stormwater management policies that are appropriate [40]. Concentrations in surface water may rise following extreme precipitation events due to increased surface runoff, combined sewer overflows, and sediment re-suspension.

6. Conclusions

In terms of Section 39 of the National Water Act, 1998 [34], it is essential to assess whether a wastewater treatment plant's final effluent is in compliance with South African discharge guidelines (general and special limits). As a result of poorly treated effluent discharged into water resources, water resources and their ecosystems suffer from significant problems. As a result of several studies on the impact of wastewater effluents on the receiving environment, it was found that there is still much to be done in order to improve effluent quality in order to protect our water resources. During this study, rainfall fluctuations in *E. coli* in the White River were observed as a result of discharged pollutants from the WWTP. This study observed that rainfall is not significant when predicting COD using rainfall. As a result, rainfall is not a significant predictor of COD. It can be concluded that the COD concentrations vary across the Crocodile River. Additionally, this study observed that rainfall is significant at the 5% significance level when predicting *E. coli*, so it can be concluded that rainfall is a significant predictor of *E. coli.* The number of *E. coli* decreased by 1.1017 units for every unit of increased rainfall. Both the correlation data and correlation heat map indicated an inverse relationship between rainfall and *E. coli.* It can thus be concluded that *E. coli* concentrations differ at different locations of the Crocodile River. Seasonal variations did not affect the performance of the spatio-temporal distribution of chemical oxygen demand (COD) in the Crocodile River. The higher concentration levels of COD show the poor performance of the WWTP, especially in the upstream site (White River WWTP). The measurement of pollutant levels in wastewater effluents assists in identifying improvements to the treatment process that are needed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14182802/s1, Table S1: Spatial-temporal distribution of Chemical Oxygen Demand (COD) by years in the three sites; Table S2: Seasonal variation of the concentration of COD in the three sampled sites in the Crocodile River; Table S3: Spatio-temporal distribution of Escherichia coli levels from the three WWTP sampled in the Crocodile River; Table S4: Seasonal variation of E.coli levels in the three sampled sites in the Crocodile River.

Author Contributions: All authors contributed to the conception and design of the study. Material preparation conceived and contributed data or analysis were performed by T.M., B.S.M., B.S.C. and K.S. The performance of the analysis was done by S.L. and L.M. The first draft of the manuscript was written by T.M. and all authors commented on previous versions of the manuscript. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This paper does not contain any studies involving human participants or animals performed by any of the authors.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study is available on request from the corresponding author.

Acknowledgments: The authors would like to acknowledge Cape Peninsula University of Technology for encouraging and on the writing of the article. Gratitude is also extended to their families for support and encouragement.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Average of *E. coli*.

Average of *E. coli*	**Column Labels**			
Row Labels	**Kanyamazane**	**Matsulu**	**White River**	**Grand Total**
2016	**62.108**	**67.145**	**68.433**	**65.860**
Above upper quartile	59.90	67.37	64.37	64.38
Below lower quartile	67.10	63.55	74.43	69.57
Between lower quartile & median quartile	62.93	69.78	74.40	68.65
Between median quartle & upper quartile	57.50	60.50	53.60	56.70
2017	**56.62**	**58.82**	**61.29**	**58.91**
Above upper quartile	53.34	58.48	53.75	55.05
Below lower quartile	60.40	58.66	60.06	59.53
Between lower quartile & median quartile	59.23	63.05	79.25	66.04
Between median quartle & upper quartile	57.10	52.50	61.70	57.10
2018	**55.75**	**61.48**	**47.25**	**54.83**
Above upper quartile	55.25		44.10	48.56
Below lower quartile	56.90	60.70	49.17	55.43
Between lower quartile & median quartile	59.70	62.33	52.15	59.19
Between median quartle & upper quartile	53.12	61.28	45.73	53.92
2019	**55.70**	**67.16**	**56.02**	**59.18**
Above upper quartile	52.90	60.23	52.77	55.99
Below lower quartile	61.57	70.80	53.73	62.91
Between lower quartile & median quartile	53.84		68.12	59.55
Between median quartle & upper quartile	53.86	69.22	54.13	57.79
2020	**60.59**	**72.47**	**61.01**	**64.69**
Above upper quartile	48.60	77.70	56.62	59.52
Below lower quartile	63.70	76.30		71.26
Between lower quartile & median quartile	70.00	66.70	51.63	59.99
Between median quartle & upper quartile	60.33	69.00	120.50	71.10
2021	**55.04**	**55.69**	**44.67**	**51.65**
Above upper quartile	30.23	73.75	44.45	46.73
Below lower quartile	71.40	40.15		46.40
Between lower quartile & median quartile	67.85	75.00	45.73	53.90
Between median quartle & upper quartile	65.87	62.40	38.70	59.74
Grand Total	**57.66**	**63.87**	**56.84**	**59.37**

Appendix B

Table A2. Average of COD.

Average of COD	Column Labels			
Row Labels	**Kanyamazane**	**Matsulu**	**White River**	**Grand Total**
2016	**28.083**	**17.754**	**137.667**	**62.408**
Above upper quartile	23.00	15.43	88.67	44.79
Below lower quartile	26.67	27.50	87.00	53.67
Between lower quartile & median quartile	30.25	16.80	319.33	96.92
Between median quartle & upper quartile	30.00	10.00	40.00	30.00
2017	**31.50**	**18.58**	**104.33**	**51.47**
Above upper quartile	20.00	14.50	200.75	73.92
Below lower quartile	47.00	22.00	55.80	40.25
Between lower quartile & median quartile	37.67	18.50	47.00	34.86
Between median quartle & upper quartile	35.50	18.00	76.00	41.25
2018	**35.42**	**18.42**	**60.33**	**38.06**
Above upper quartile	32.50		51.67	44.00
Below lower quartile	33.00	15.33	40.00	29.00
Between lower quartile & median quartile	41.00	16.75	93.00	41.78
Between median quartle & upper quartile	34.20	21.60	65.75	38.71
2019	**45.58**	**20.60**	**106.83**	**59.85**
Above upper quartile	10.00	12.00	89.00	44.71
Below lower quartile	46.67	18.75	110.67	54.70
Between lower quartile & median quartile	47.67		149.00	88.20
Between median quartle & upper quartile	50.80	31.67	96.25	61.17
2020	**35.70**	**28.40**	**56.60**	**40.23**
Above upper quartile	29.00	12.00	58.00	41.33
Below lower quartile	39.50	56.33		49.60
Between lower quartile & median quartile	62.50	22.50	55.50	49.00
Between median quartle & upper quartile	23.75	15.33	54.00	24.38
2021	**39.67**	**19.63**	**77.33**	**46.54**
Above upper quartile	27.67	15.50	40.00	27.71
Below lower quartile	34.00	26.00		27.60
Between lower quartile & median quartile	47.00	16.00	100.67	79.33
Between median quartle & upper quartile	48.67	6.00	12.00	32.80
Grand Total	**35.84**	**20.42**	**92.12**	**50.05**

References

1. Wang, Y.; Wang, P.; Bai, Y.; Tian, Z.; Li, J.; Shao, X.; Mustavich, L.F.; Li, B.L. Assessment of surface water quality via multivariate statistical techniques: A case study of the Songhua River Harbin region, China. *J. Hydro. Environ. Res.* **2013**, *7*, 30–40. [CrossRef]
2. Gasim, M.B.; Khalid, N.A.; Muhamad, H. The influence of tidal activities on water quality of Paka River Terengganu, Malaysia. *Malays. J. Anal. Sci.* **2015**, *19*, 979–990.
3. Abia, A.L.; Ubomba-Jaswa, E.; Momba, M.N. Impact of seasonal variation on Escherichia coli concentrations in the riverbed sediments in the Apies River, South Africa. *Sci. Total Environ.* **2015**, *537*, 462–469. [CrossRef]
4. Edokpayi, J.N.; Odiyo, J.O.; Durowoju, O.S. Impact of wastewater on surface water quality in developing countries: A case study of South Africa. *Water Qual.* **2017**, *10*, 66561.

5. Phungela, T.T.; Maphanga, T.; Chidi, B.S.; Madonsela, B.S.; Shale, K. The impact of wastewater treatment effluent on Crocodile River quality in Ehlanzeni District, Mpumalanga Province, South Africa. *S. Afr. J. Sci.* **2022**, *118*, 8. [CrossRef]
6. Amoah, I.D.; Kumari, S.; Reddy, P.; Stenström, T.A.; Bux, F. Impact of informal settlements and wastewater treatment plants on helminth egg contamination of urban rivers and risks associated with exposure. *Environ. Monit. Assess.* **2020**, *192*, 1–13. [CrossRef] [PubMed]
7. Gqomfa, B.; Maphanga, T.; Shale, K. The impact of informal settlement on water quality of Diep River in Dunoon. *Sustain. Water Resour. Manag.* **2022**, *8*, 27. [CrossRef]
8. Iloms, E.; Ololade, O.O.; Ogola, H.J.; Selvarajan, R. Investigating industrial effluent impact on municipal wastewater treatment plant in Vaal, South Africa. *Int. J. Environ. Res. Public Health* **2020**, *17*, 1096. [CrossRef]
9. Mema, V. Impact of poorly maintained wastewater sewage treatment plants-lessons from South Africa: Wastewater management. *ReSource* **2010**, *12*, 60–65.
10. Guo, H.; Jiao, T.; Zhang, Q.; Guo, W.; Peng, Q.; Yan, X. Preparation of graphene oxide-based hydrogels as efficient dye adsorbents for wastewater treatment. *Nanoscale Res. Lett.* **2015**, *10*, 272. [CrossRef]
11. Medeiros, R.C.; Sammarro Silva, K.J.; Daniel, L.A. Wastewater treatment performance in microbiological removal and (oo) cyst viability assessed comparatively to fluorescence decay. *Environ. Technol.* **2022**, *43*, 962–970. [CrossRef] [PubMed]
12. Mbanga, J.; Abia, A.L.; Amoako, D.G.; Essack, S. Quantitative microbial risk assessment for waterborne pathogens in a wastewater treatment plant and its receiving surface water body. *BMC Microbiol.* **2020**, *20*, 346. [CrossRef] [PubMed]
13. Shuliakevich, A.; Schroeder, K.; Nagengast, L.; Wolf, Y.; Brückner, I.; Muz, M.; Behnisch, P.A.; Hollert, H.; Schiwy, S. Extensive rain events have a more substantial impact than advanced effluent treatment on the endocrine-disrupting activity in an effluent-dominated small river. *Sci. Total Environ.* **2022**, *807*, 150887. [CrossRef]
14. Makuwa, S.; Tlou, M.; Fosso-Kankeu, E.; Green, E. The effects of dry versus wet season on the performance of a wastewater treatment plant in North West Province, South Africa. *Water S.A.* **2022**, *48*, 40–49. [CrossRef]
15. Münze, R.; Hannemann, C.; Orlinskiy, P.; Gunold, R.; Paschke, A.; Foit, K.; Becker, J.; Kaske, O.; Paulsson, E.; Peterson, M.; et al. Pesticides from wastewater treatment plant effluents affect invertebrate communities. *Sci. Total Environ.* **2017**, *599*, 387–399. [CrossRef] [PubMed]
16. Bezuidenhout, C.C.; Mthembu, N.; Puckree, T.; Lin, J. Microbiological evaluation of the Mhlathuze river, KwaZulu-natal (RSA). *Water SA* **2002**, *28*, 281–286. [CrossRef]
17. Chokwe, T.B.; Magubane, M.N.; Abafe, O.A.; Okonkwo, J.O.; Sibiya, I.V. Levels, distributions, and ecological risk assessments of polybrominated diphenyl ethers and alternative flame retardants in river sediments from Vaal River, South Africa. *Environ. Sci. Pollut. Res.* **2019**, *26*, 7156–7163. [CrossRef]
18. Jin, Z.; Zhang, X.; Li, J.; Yang, F.; Kong, D.; Wei, R.; Huang, K.; Zhou, B. Impact of wastewater treatment plant effluent on an urban river. *J. Freshw. Ecol.* **2017**, *32*, 697–710. [CrossRef]
19. Luyt, C.D.; Tandlich, R.; Muller, W.J.; Wilhelmi, B.S. Microbial monitoring of surface water in South Africa: An overview. *Int. J. Environ. Res. Public Health* **2012**, *9*, 2669–2693. [CrossRef]
20. Masindi, V.; Duncker, L. *State of Water and Sanitation in South Africa*; Council for Scientific and Industrial Research (CSIR): Pretoria, South Africa, 2016; Available online: https://www.researchgate.net/publication/311451788_State_of_Water_and_Sanitation_in_South_Africa (accessed on 20 April 2022).
21. Narciso-da-Rocha, C.; Rocha, J.; Vaz-Moreira, I.; Lira, F.; Tamames, J.; Henriques, I.; Martinez, J.L.; Manaia, C.M. Bacterial lineages putatively associated with the dissemination of antibiotic resistance genes in a full-scale urban wastewater treatment plant. *Environ. Int.* **2018**, *118*, 179–188. [CrossRef]
22. Edokpayi, J.N.; Odiyo, J.O.; Msagati, T.A.; Potgieter, N. Temporal variations in physico-chemical and microbiological characteristics of Mvudi River, South Africa. *Int. J. Environ. Res. Public Health* **2015**, *12*, 4128–4140. [CrossRef] [PubMed]
23. Delanka-Pedige, H.M.; Munasinghe-Arachchige, S.P.; Abeysiriwardana-Arachchige, I.S.; Nirmalakhandan, N. Wastewater infrastructure for sustainable cities: Assessment based on UN sustainable development goals (SDGs). *Int. J. Sustain. Dev.* **2021**, *28*, 203–209. [CrossRef]
24. Kanu, I.; Achi, O.K. Industrial effluents and their impact on water quality of receiving rivers in Nigeria. *JATES* **2011**, *1*, 75–86.
25. Ngwira, L.; Lakudzala, D. Assessment of the quality of SOBO industrial wastewater and its impact on water quality in Nankhaka River. *Phys. Chem. Earth* **2018**, *108*, 9–12. [CrossRef]
26. Ebrahimi, M.; Gerber, E.L.; Rockaway, T.D. Temporal performance assessment of wastewater treatment plants by using multivariate statistical analysis. *J. Environ. Manag.* **2017**, *193*, 234–246. [CrossRef] [PubMed]
27. Department of Water Affairs. *Report on the Status of Sanitation Services in South Africa*; Department of Environmental Affairs: Pretoria, South Africa, 2012. Available online: https://www.dpme.gov.za/publications/Reports%20and%20Other%20Information%20Products/Sanitation%20Report.pdf (accessed on 17 June 2022).
28. Ntombela, C.; Funke, N.; Meissner, R.; Steyn, M.; Masangane, W. A critical look at South Africa's green drop programme. *Water SA* **2016**, *42*, 703–710. [CrossRef]
29. Bunce, J.T.; Ndam, E.; Ofiteru, I.D.; Moore, A.; Graham, D.W. A review of phosphorus removal technologies and their applicability to small-scale domestic wastewater treatment systems. *Front. Environ. Sci.* **2018**, *6*, 8. [CrossRef]
30. Cai, H.; Liang, J.; Ning, X.A.; Lai, X.; Li, Y. Algal toxicity induced by effluents from textile-dyeing wastewater treatment plants. *J. Environ. Sci.* **2020**, *91*, 199–208. [CrossRef]

31. Islam, M.M.; Iqbal, M.S.; D'Souza, N.; Islam, M.A. A review on present and future microbial surface water quality worldwide. *Environ. Nanotechnol. Monit. Manag.* **2021**, *16*, 100523. [CrossRef]
32. Song, H.M.; Zhu, L.J.; Wang, Y.; Wang, G.; Zeng, Z.X. Fe-based Prussian blue cubes confined in graphene oxide nanosheets for the catalytic degradation of dyes in wastewater. *Sep. Purif. Technol.* **2022**, *288*, 120676. [CrossRef]
33. United State Environmental Protection Agency. *Wastewater Sampling—US Environmental Protection Agency*; Environmental Protection Agency: Athens, GA, USA, 2013. Available online: https://www.epa.gov/sites/default/files/2015-06/documents/Wastewater-Sampling.pdf (accessed on 25 April 2022).
34. Department of Water and Sanitation. *Planning Level Review of Water Quality in South Africa*; Department of Water and Sanitation: Pretoria, South Africa, 2013. Available online: https://www.dws.gov.za/iwrp/iwqms/Documents/Report%201.1_Inception%20Report.pdf (accessed on 5 May 2022).
35. Alberto, W.D.; del Pilar, D.M.; Valeria, A.M.; Fabiana, P.S.; Cecilia, H.A.; de Los Ángeles, B.M. Pattern recognition techniques for the evaluation of spatial and temporal variations in water quality. A case study: Suquía River Basin (Córdoba—Argentina). *Water Res.* **2001**, *35*, 2881–2894. [CrossRef]
36. Singh, K.P.; Malik, A.; Mohan, D.; Sinha, S. Multivariate statistical techniques for the evaluation of spatial and temporal variations in water quality of Gomti River (India)—A case study. *Water Res.* **2004**, *38*, 3980–3992. [CrossRef] [PubMed]
37. Joel, C.; Kiprop, E.K.; Mwamburi, L.A. Effect of seasonal variation on performance of conventional wastewater treatment system. *J. Appl. Environ. Microbiol.* **2017**, *5*, 1–7.
38. Abagale, F.K. Seasonal Variation and Removal of Organic Pollutants in Wastewater Using Low-Cost Treatment Technologies in Tamale Metropolis, Ghana. *J. Water Resour. Prot.* **2021**, *13*, 271–282. [CrossRef]
39. Osuolale, O.; Okoh, A. Assessment of the physicochemical qualities and prevalence of Escherichia coli and vibrios in the final effluents of two wastewater treatment plants in South Africa: Ecological and public health implications. *Int. J. Environ. Res. Public Health* **2015**, *12*, 13399–13412. [CrossRef]
40. Edokpayi, J.N.; Enitan-Folami, A.M.; Adeeyo, A.O.; Durowoju, O.S.; Jegede, A.O.; Odiyo, J.O. Recent trends and national policies for water provision and wastewater treatment in South Africa. In *Water Conservation and Wastewater Treatment in BRICS Nations Technologies, Challenges, Strategies and Policies*; Singh, Y., Milshina, Y., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 187–211.
41. De Oliveira, M.; Frihling, B.E.; Velasques, J.; Magalhães Filho, F.J.; Cavalheri, P.S.; Migliolo, L. Pharmaceuticals residues and xenobiotics contaminants: Occurrence, analytical techniques and sustainable alternatives for wastewater treatment. *Sci. Total Environ.* **2020**, *705*, 135568. [CrossRef]
42. Dehghani, M.; Sorooshian, A.; Ghorbani, M.; Fazlzadeh, M.; Miri, M.; Badiee, P.; Parvizi, A.; Ansari, M.; Baghani, A.N.; Delikhoon, M. Seasonal variation in culturable bioaerosols in a wastewater treatment plant. *Aerosol. Air Qual. Res.* **2018**, *18*, 2826. [CrossRef]
43. Liu, G.; Chen, L.; Wang, W.; Sun, C.; Shen, Z. A water quality management methodology for optimizing best management practices considering changes in long-term efficiency. *Sci. Total Environ.* **2020**, *725*, 138091. [CrossRef]
44. Li, X.; Jin, X.; Zhao, N.; Angelidaki, I.; Zhang, Y. Efficient treatment of aniline containing wastewater in bipolar membrane microbial electrolysis cell-Fenton system. *Water Res.* **2017**, *119*, 67–72. [CrossRef]