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Research article

Occurrence of efavirenz, levonorgestrel, ibuprofen, and diclofenac in wastewaters of limpopo province, South Africa

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ABSTRACT

This study aims to investigate the occurrence of efavirenz, levonorgestrel, ibuprofen, and diclofenac in wastewaters of Vhembe and Mopane District Municipalities, Limpopo Province, South Africa. A total of 48 samples were collected at the inlet and outlet of the selected wastewater treatment plants during April, July, and October 2023. The physicochemical parameters such as pH, electrical conductivity, total dissolved solids, and dissolved oxygen of the influent and effluent were determined in the field, while total organic carbon (TOC) and chemical oxygen demand (COD) were determined in the laboratory. The LC-20 prominence High-pressure liquid chromatography with a Photo-diode Array (PDA) detector was employed to quantify and profile the targeted pharmaceutical compounds. Levonorgestrel and efavirenz were analyzed using the gradient elution method, while the isocratic method was applied to analyze diclofenac and ibuprofen. The obtained results showed that the concentration ranges for ibuprofen were $<$LOD-114.00 μg/L (influent) and $<$LOD-59.9 μg/L (effluent), levonorgestrel: 6.2–8.09 μg/L (influent) and 4.25–20.9 μg/L (effluent), diclofenac: 2.21–25.00 μg/L (influent) and 0.36–5 μg/L (effluent), and efavirenz: 3.81–11.9 μg/L (influent) and 0.69–6.3 μg/L (effluent). The presence of these pharmaceutical contaminants in wastewater, particularly in effluent, requires attention as they may pose significant ecological threats in the receiving water bodies. Therefore, this study recommends the development of efficient wastewater treatment technologies targeting pharmaceutical compounds and further routine monitoring of these compounds in surface water resources, as well as understanding their fate and effects in aquatic organisms.

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1. Introduction

Pharmaceutical contaminants are a group of different organic drugs such as antibiotics, non-steroidal anti-inflammatory, hormones, contraceptives, and lipid regulators that are often used to improve human health [1,2]. Regardless of their broad and continuous use to enhance human health, the presence of pharmaceutical residues in wastewater and surface water has gained global attention over the past decades, with hundreds of pharmaceutical compounds being detected in aquatic environments at concentrations ranging from ng/L to µg/L [3]. The sources of these compounds include raw and treated wastewater discharged into the environment, inadequate disposal of expired drugs from drug manufacturing industries, and leaching from landfill sites [4–6]. Since they are persistent in the environmental compartments, they have the potential to pose significant risks to aquatic life and human health. The identified risk includes feminization, masculinization of marine organisms, development of microbial resistance towards antibiotics, cancer, liver damage, and plant growth defects [7–10].

Wastewater treatment plants (WWTP) have limited removal efficiency towards pharmaceutical contaminants [11,12]. As a result, WWTPs are the primary contributors of pharmaceutical residues in receiving water bodies such as rivers, dams, and lakes, often used as domestic water sources [13,14]. Several pharmaceutical contaminants such as diclofenac, ibuprofen, efavirenz, naproxen, gemfibrozil, tenofovir, and emtricitabine have been reported in wastewater and water sources of Gauteng, Kwa-Zulu Natal, and North-West Provinces [15–17]. Diclofenac, ibuprofen, and efavirenz have been frequently detected among these different pharmaceutical contaminants. Diclofenac and ibuprofen are commonly used, cheap, and easily accessed pain relief medications, resulting in their increased consumption and load into the environment. While efavirenz's frequent detection could be attributed to its frequent use as the primary daily medication to boost the immune system of human immunodeficiency virus (HIV) [18,19]. Levonorgestrel is an emergency contraceptive pill that is commonly used and readily available in the market. However, its presence in wastewater has not been looked at. Thus, monitoring these compounds in remote areas of South Africa is essential.

There is limited literature on pharmaceutical compounds in rural areas, particularly in Limpopo Province. The lack of reports in other places does not imply the absence of these compounds; instead, it is due to insufficient research, which is influenced by a lack of resources such as consumables, analytical tools, and expertise [20,21]. This emphasizes the necessity to monitor and evaluate the presence of pharmaceutical pollutants in wastewater and receiving bodies of water in South Africa to influence decision-making and policy formation. Thus, this study aims to determine the occurrence of efavirenz, levonorgestrel, ibuprofen, and diclofenac in wastewater (influent and effluent) treatment plants of Vhembe and Mopani District, Limpopo Province, South Africa. The selected wastewater treatment plants were Thohoyandou, Malamulele, Giyani, Makhado, Siloam Nkowankowa, Tzaneen, and Kgapane. This work will provide insight into the extent to which different pharmaceutical contaminants are present in WWTP in receiving water bodies such as rivers and dams. The availability of up-to-date data on pharmaceutical contaminants in water bodies is essential for policymakers, researchers, practitioners, and public institutions to develop national and local action plans to ensure environmental water safety.

2. Materials and methods

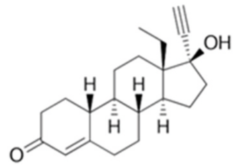
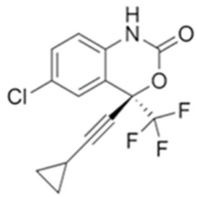
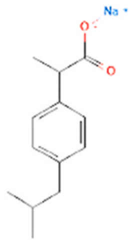
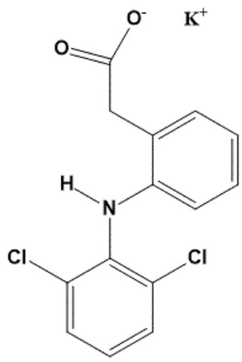
2.1. Materials and standard preparations

The analytical standards of ibuprofen, diclofenac, efavirenz, levonorgestrel, HPLC water (H_20), acetonitrile (ACN), trifluoracetic acid, and methanol (MeOH) were purchased from Sigma-Aldrich through Merck Life Sciences South Africa. All the reagents used in this study were of HPLC grade. The Oasis HLB cartridge (3 cc/60 mg), 2 ml amber vials, 5.8*315.5 mm transparent glass inserts, nylon syringe filters (0.22 µm), and 0.01–1 mL sterilized syringes were purchased from Stargate Scientific South Africa. Ultrapure water (18.2 MΩ/cm) generated from the Millipore Direct Q5 system (Millipore, Germany) and HPLC grade water were used for standard preparation. Individual stock solution of 1000 ppm was prepared by dissolving analyte (efavirenz, diclofenac, ibuprofen, and levonorgestrel) into 100 mL of 80 % of MeOH. The dilution method was used to prepare the respective analytes' working standards (0.5, 1, 5, 10, 15, 20, and 30 ppm). All the stock solutions were prepared in amber bottles to avoid sample degradation. Table 1 summarizes the respective pharmaceutical compounds' chemical formulas and molecular structures.

2.2. Study area

The study area is within the Vhembe District and Mopani District Municipalities in Limpopo Province, South Africa. Fig. 1 represents all the sampling sites. Thohoyandou (23°00′41.24" S, 30° 47′51.01" E), Malamulele (23°0.00′55.26" S, 30°71′45.01" E) Makhado (23°05′78.43" S, 30°89"71.11"E), and Siloam (23°90′22.09" S, 30°19"27.77"E) WWTP are in Vhembe District Municipality. Giyani (23°0.32′49.61"S,30°70′90.66" E), Nkowankowa (23°0.89′28.35"S, 30°31′41.68"E), Kgapane (23°63′38.61"S, 30°21′54.95" E) and Tzaneen (23°82′42.64"S, 30°17′21.62" E) WWTP are in Mopani District Municipality. These wastewater treatment plants (WWTPs) receive effluents from residential areas, health care facilities, light industry, educational institutions, and central business districts. Treatment plants of Thohoyandou, Malamulele, Giyani, Nkowankowa, Tzaneen and Kgapane employs processes such as sedimentation, biofiltration, oxidation processes and chlorination. In contrast, the Siloam wastewater treatment plant employs mainly grit sedimentation followed by oxidation with ponds now inversed by vegetation to form a wetland.

Table 1
Chemical formulae and molecular structures of selected pharmaceutical compounds.

Compound name	Chemical formula	Molecular structure.
Levonorgestrel	$C_{21}H_{28}O_2$	
Efavirenz	$C_{14}H_9ClF_3NO_2$	
Ibuprofen	$C_{13}H_{21}NaO_4$	
Diclofenac	$C_{14}H_{10}Cl_2KNO_2$	

2.3. Sample collection and preparation

A total of 48 wastewater samples ((24 of the influent and 24 of effluent) were collected in 200 mL amber bottles from eight different wastewater treatment plants in Thohoyandou, Malamulele, Giyani, Tzaneen, Kgapane, Nkowankowa, Makhado, and Siloam for a period of three months (April, July, and October 2023). Prior to sample collection, 200 mL amber bottles were washed, soaked in 10 % MeOH, rinsed with de-ionized water, and oven-dried to avoid sample contamination. Physicochemical parameters such as pH, temperature, salinity, dissolved oxygen, total dissolved solids, and electrical conductivity were measured using a Thermo-scientific Orion multi-meter, while turbidity was measured using a TB200 portable turbidimeter. All these physicochemical parameters were measured in the field. The samples were kept in ice and transported to the laboratory for further analysis. The total organic carbon (TOC) and chemical oxygen demand (COD) were measured using Spectro-quarts Pharo 100, Merck (made in Germany) in the laboratory. Upon arrival, samples were filtered and preconcentrated through the solid phase extraction (SPE) method using Oasis HLB cartridges prior to analysis of selected pharmaceutical compounds to enhance the detection potency of the high-pressure liquid chromatography (HPLC). As a pre-treatment step, the Oasis HLB cartridge was conditioned with 3 mL of 10 % MeOH and rinsed with 3 mL deionized water, then the filtered samples (100 mL) were added into the SPE column. The cartridge was rinsed with 3 mL of 10 % MeOH and 3 mL of deionized water to remove interfering organic compounds. 5 mL of 80 % MeOH was used to elute the samples within the SPE column into amber test tubes. To avoid photodegradation, the extract was transferred into 10 mL amber bottles, dried under nitrogen flow, and later reconstituted using 1 mL of 80 % MeOH for analysis [22].

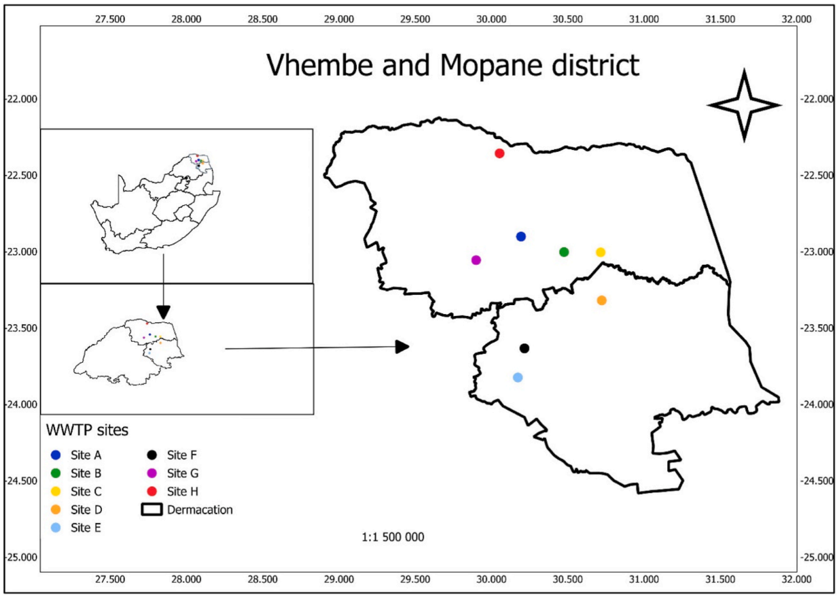


Fig. 1. Map showing the location of sampling points (Malamulele (A), Giyani (B), Nkowankowa (C), Tzaneen (D), Kgapane (E), Makhado (F), Siloam (G), and Thohoyandou (H)).

2.4. Analysis of the selected pharmaceutical contaminants

The LC-20 Prominence HPLC system (Shimadzu, Japan) was used for profiling and quantification of ibuprofen, diclofenac, efavirenz, and levonorgestrel. Chromatographic separation of the analytes was done using a Shim-pack Velox C18 column (100 × 2.1 mm, 2.7 μm) (Shimadzu Corporation, Kyoto, Japan) maintained at 37 °C. The gradient elution method used by Al Dalaty et al. [23] was adopted to profile and quantify efavirenz and levonorgestrel. The method used a gradient eluent of ACN and H_2O at 60:40 (v/v) for the first 4.4 min at a flow rate of 1 mL/min, increasing to 100 % ACN by 5 min, then returning to 60:40 until 7.5 min. A sample volume of 20 μL was utilized. Their absorbance was observed at wavelengths of 246 and 244 nm. The column temperature was set at 37 °C, and a single run lasted 7.5 min. The isocratic method used by Ajibola et al. [24] was adopted to profile and quantify ibuprofen and diclofenac. The isocratic elution of 70 % methanol and H_2O (MeOH: H_2O) 70:30 (v/v) with a flow rate of 0.8 mL/min to reach a time flow of 5 min was used as mobile phase, and the recorded absorbance was at 200 nm. The column temperature was set at 37 °C, and a single run lasted 13 min. A calibration curve was plotted to validate the method's accuracy for the selected pharmaceutical contaminants (efavirenz 5 points: 500-20 000 μg/L, levonorgestrel 7 points (500-20 000 μg/L, ibuprofen 5 points: 500-30 000 μg/L, and diclofenac 6 points: 500–30000 μg/L).

2.5. Quality assurance

Efavirenz, Levonorgestrel, diclofenac, and ibuprofen external standards were used for profiling and quantification. During sample analysis, a standard with a known solution was used as a spiked sample for method validation and quality control. Blank samples made of solvent methanol were analyzed, and no detection was observed. All the standards and environmental samples were analyzed in triplicates for quality control and data accuracy, and mean values were calculated. Deionized water was employed as a control for environmental samples for quality control, and no peak was observed. A calibration curve was plotted to validate the method's accuracy for the selected pharmaceutical contaminants. The LOD and LOQ were calculated using equations (1) and (2) [25]:

$$LOD = 3.3 * \sigma / S \tag{1}$$

Where σ is the standard deviation of the calibration curve, and *S* is the slope of the calibration curve.

$$LOQ = 10 * \sigma / S \tag{2}$$

3. Results and discussion

3.1. Physicochemical parameters

The results of physicochemical parameters of wastewater from the selected wastewater treatment plants are presented in Table 2. The influent pH ranged from 7.28 to 7.65, while the effluent pH ranged from 6.68 to 7.49. A slight decrease was observed between the

Table 2
The average and standard deviation (n = 3 except turbidity, TOC, and COD) of the recorded physicochemical parameters of the collected wastewater samples for three months (April, July, and October 2023).

Site	pH	EC (µs/cm)	TDS (ppm)	DO (mg/L)	Salinity psu	Temperature (°C)	Turbidity	TOC (mg/L)	COD (mg/L)
Influent									
Malamulele	7.31 ± 0.39	676.77 ± 171.43	320.37 ± 54.99	6.37 ± 1.17	0.32 ± 0.03	20.70 ± 7.45	100.00 ± 0.0	68.20 ± 0.0	81.10 ± 0.0
Giyani	7.34 ± 0.12	1156.80 ± 439.28	688.30 ± 313.02	3.43 ± 1.88	0.48 ± 0.09	20.60 ± 7.34	74.39 ± 0.0	81.60 ± 0.0	12.60 ± 0.0
Nkowankowa	7.28 ± 0.35	586.13 ± 198.94	390.73 ± 187.33	3.30 ± 0.22	0.25 ± 0.02	19.66 ± 5.91	6.64 ± 0.0	45.60 ± 0.0	LOD
Tzaneen	7.36 ± 0.47	722.90 ± 200.48	476.97 ± 203.80	3.37 ± 2.10	0.31 ± 0.01	15.80 ± 1.5	158.0 ± 0.0	48.20 ± 0.0	6.10 ± 0.0
Kgapane	7.28 ± 0.30	837.70 ± 391.42	576.57 ± 330.57	1.80 ± 1.30	0.39 ± 0.09	19.07 ± 4.53	76.55 ± 0.0	50.60 ± 0.0	61.80 ± 0.0
Makhado	7.63 ± 0.28	807.17 ± 182.59	452.13 ± 23.14	2.60 ± 2.35	0.65 ± 0.12	17.13 ± 2.00	352.70 ± 0.0	78.00 ± 0.0	22.70 ± 0.0
Siloam	7.65 ± 0.28	515.43 ± 76.23	296.67 ± 142.02	4.09 ± 4.78	0.23 ± 0.08	17.14 ± 3.05	89.78 ± 0.0	29.90 ± 0.0	36.10 ± 0.0
Thohoyandou	7.37 ± 0.32	797.40 ± 69.50	510.77 ± 131.38	4.25 ± 3.16	0.35 ± 0.05	18.73 ± 1.46	75.13 ± 0.0	59.50 ± 0.0	29.80 ± 0.0
Effluent									
Malamulele	6.98 ± 0.39	575.43 ± 144.02	377.57 ± 156.75	7.44 ± 1.46	0.24 ± 0.001	18.23 ± 2.76	24.96 ± 0.0	32.20 ± 0.0	LOD
Giyani	6.68 ± 0.41	1012.70 ± 345.52	668.73 ± 316.77	5.84 ± 1.91	0.55 ± 0.12	16.10 ± 1.91	18.00 ± 0.0	87.80 ± 0.0	LOD
Nkowankowa	6.91 ± 0.31	620.77 ± 201.87	407.57 ± 186.69	4.16 ± 3.09	0.28 ± 0.03	16.43 ± 1.45	41.91 ± 0.0	74.40 ± 0.0	LOD
Tzaneen	7.00 ± 0.06	290.13 ± 114.89	346.80 ± 131.91	4.52 ± 1.06	0.23 ± 0.0.01	17.63 ± 1.93	158.00 ± 0.0	19.60 ± 0.0	82.30 ± 0.0
Kgapane	7.40 ± 0.20	640.43 ± 141.99	409.03 ± 138.81	3.96 ± 1.94	0.31 ± 0.02	17.97 ± 2.05	5.55 ± 0.0	23.20 ± 0.0	77.70 ± 0.0
Makhado	6.93 ± 0.06	788.33 ± 117.20	498.67 ± 177.30	4.74 ± 0.89	0.50 ± 0.49	18.00 ± 1.73	33.99 ± 0.0	41.50 ± 0.0	50.60 ± 0.0
Siloam	7.30 ± 0.08	611.90 ± 164.25	406.05 ± 248.79	6.34 ± 0.23	0.26 ± 0.04	19.00 ± 0.35	20.68 ± 0.0	39.20 ± 0.0	86.80 ± 0.0
Thohoyandou	7.49 ± 0.63	608.60 ± 166.17	353.83 ± 286.05	4.31 ± 0.24	0.29 ± 0.08	19.60 ± 0.69	37.47 ± 0.0	48.00 ± 0.0	45.50 ± 0.0

influent and effluent samples in most samples except in Kgapane and Thohoyandou. The pH of the Thohoyandou wastewater treatment plant was slightly higher than that of other wastewater treatment plants. High pH values may be due to elevated levels of organic and inorganic contaminants that could have been retained during various purification stages [26,27]. Electrical conductivity is the measure of the ability of a substance to conduct electricity. In all the investigated sites, a decrease in EC (μs/cm) was observed from influent to effluent in most sampling sites except in Nkowankowa Kgapane and Siloam WWTP. The recorded EC data ranged from 515.43 to 1156.0 μs/cm (influent) and 529.13–1012.70 μs/cm (effluent), with the highest recorded at Giyani. Electrical conductivity is mainly influenced by the amount of dissolved ions due to the organic and inorganic inputs in wastewater [1]. Thus, increased EC concentration might be attributed to increased dissolved ions. Total dissolved solid measures all the dissolved content of inorganic and organic substances in water. The obtained values of TDS ranged from 296.67 to 688.30 ppm (influent) and 346.80–668.75 ppm (effluent), with the highest recorded at Giyani. TDS concentration increased from influent to effluent in most sampling sites except Tzaneen, Kgapane, and Thohoyandou WWTP. The concentration of dissolved salts was measured as salinity. The recorded salinity values ranged from 0.23 to 0.48 psμ (influent) and 0.23–0.55 psμ (effluent), with the highest recorded at Makhado WWTP. A decrease in salinity concentration was observed from influent to effluent samples within most of the sampling sites except Giyani, Nkowankowa, and Siloam. An increase in salinity concentration in effluent samples could be attributed to the introduction of chlorine during the chlorination process, which could have initiated salt precipitation [28,29]. Furthermore, since increased salinity was observed in WWTP plants composed of oxidative ponds, it could be associated with the evaporation of the wastewater, resulting in increased salt concentration. Thus, this can explain increased EC and TDS in effluent samples. Thohoyandou WWTP also receives sewage from the university community, which comprises different types of laboratory waste. Thus, this could increase the chemical loads in the WWTPs, which can leach further into effluent samples. Thus, this could have influenced the slight increase in pH values in effluent samples. An increase in dissolved oxygen (DO) was observed between the influent and effluent from all the investigated sites. This could be attributed to the reduced load of organic matter within the wastewater and the reaeration process. The obtained influent and effluent data for DO ranged from 1.80 to 6.37 mg/L and 3.96–7.44 mg/L, respectively. The TOC concentration data ranged from 29.90 to 81.60 mg/L (influent) and <LOD – 87.80 mg/L (effluent). A decrease in total organic carbon was observed in all WWTP except Giyani, Nkowankowa, and Siloam. An increase in TOC concentration in effluent samples could be related to the inability of the WWTP to remove organic contaminants since TOC is generally used to measure the available organic carbon present in water. The COD values ranged from <LOD – 81.10 mg/L in influent samples, while in effluent samples, it ranged from <LOD – 86.80 mg/L. COD concentration increased from influent to effluent samples except in Malamulele, Giyani, and Nkowankowa WWTP. COD measures the oxygen required to oxidize organic and inorganic material in water chemically. Increased COD concentration in effluent could relate to the inability of WWTP to reduce the presence of inorganic species such as nitrate and ammonia. All the recorded parameters correlated to those reported by Madikizela et al. [19], indicating that the WWTPs examined in this study operate similarly to most treatment works worldwide.

3.2. High-pressure liquid chromatography method validation

The instrument method was validated using standard solutions prepared in HPLC water. The linearity, limit of detection (LOD), and limit of quantification (LOQ) of the target pharmaceutical contaminants are summarized in Fig. 2 and Table 3. The typical chromatograms for the adopted method used for profiling and quantifying the selected pharmaceutical contaminants are presented in Fig. 2. A characteristic peak with a maximum wavelength of 246, 251, 254, and 276 nm was visible in the HPLC chromatographs of efavirenz, levonorgestrel, diclofenac, and ibuprofen at retention times 3.5, 4.25, 9.4, and 10.5 min. There was no change in retention times (R_t) for all the target compounds in the standard samples, validating the data accuracy and method quality control. A calibration curve was plotted to validate the method's accuracy for the selected pharmaceutical contaminants (efavirenz 5 points: 500-20 000 μg/L, levonorgestrel 7 points (500-20 000 μg/L, ibuprofen 5 points: 500-30 000 μg/L, and diclofenac 6points: 500–30000 μg/L).

The calibration curve's calculated correlation coefficient (R^2) values were >0.99, validating the satisfaction of the HPLC method results used for quantification. The limit of detection (LOD) and quantification were calculated using equations (2) and (3). A significant change in the retention times was observed compared to the adopted methods of profiling and quantification, which could be attributed to differences in reference standards, column type, and instruments. The obtained respective LOD and LOQ are summarized in Table 3. The respective LOD and LOQ ranged from 0.013 to 0.025 μg/L and 0.002–0.007 μg/L, respectively.

3.3. Occurrence of efavirenz, levonorgestrel, diclofenac, and ibuprofen in wastewater

The average concentrations of the four studied pharmaceutical contaminants in 8 sampling sites are presented in Fig. 3. The average concentrations of efavirenz, levonorgestrel, diclofenac, and ibuprofen ranged from the limit of detection (LOD) −17.7 μg/L, LOD-34.95 μg/L, LOD-71.95 μg/L, and LOD-342.98 μg/L in wastewater. Among the monitored pharmaceutical contaminants, efavirenz, levonorgestrel, and diclofenac were ubiquitous in all eight sampling points. The average concentration values of efavirenz ranged from <LOD – 3.81 μg/L (influent), <LOD – 0.69 μg/L (effluent) in Malamulele WWTP; <LOD – 11.87 μg/L (influent), <LOD – 6.26 μg/L (effluent) in Giyani WWTP; <LOD – 12.56 μg/L (influent), <LOD – 3.27 μg/L (effluent) in Nkowankowa WWTP; <LOD – 9.52 μg/L (influent), <LOD – 1.87 μg/L (effluent) in Tzaneen WWTP; <LOD – 8.34 μg/L (influent), <LOD – 2.26 μg/L (effluent) in Kgapane WWTP; <LOD – 8.31 μg/L (influent), <LOD – 2.38 μg/L (effluent) in Makhado WWTP; <LOD – 10.56 μg/L (influent), <LOD – 3.62 μg/L (effluent) in Siloam WWTP; and <LOD – 10.68 μg/L (influent), <LOD – 4.57 μg/L (effluent) in Thohoyandou WWTP. The highest concentration of 17.74 μg/L was obtained in the Kgapane WWTP influent for efavirenz, with a minimum concentration of 2.79 μg/L recorded at the Siloam WWTP influent. A maximum concentration of 11.73 μg/L of efavirenz was recorded in Giyani, with the

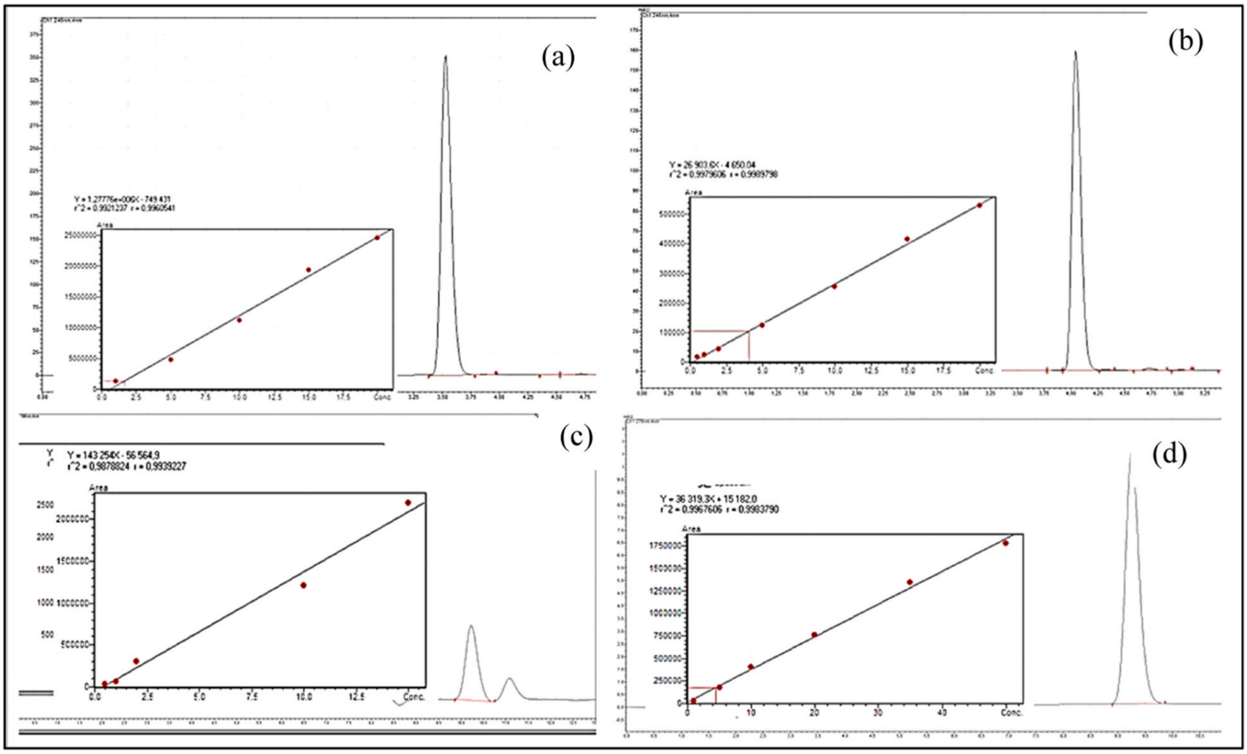


Fig. 2. HPLC Chromatograms and calibration curves of (a) efavirenz, (b) levonorgestrel (c) diclofenac and (d) ibuprofen.

lowest concentrations of 1.03 μg/L being recorded at Siloam and Thohoyandou WWTP effluent. Comparatively, a reduction in efavirenz concentration was observed in all sampling points from influent to effluent samples. The prevalence of efavirenz in Vhembe and Mopani District Municipalities validates that efavirenz is among the frequently detected pharmaceutical contaminants. Its prevalence in all sampling sites may be due to its daily intake by HIV patients, as South Africa has one of the highest HIV prevalence rates, and its persistence in the environment is due to low degradation [30,31].

The average concentration values of levonorgestrel ranged from <LOD – 7.42 μg/L (influent), <LOD – 6.38 μg/L (effluent) in Malamulele WWTP; <LOD – 7.14 μg/L (influent), <LOD – 8.29 μg/L (effluent) in Giyani WWTP; <LOD – 8.09 μg/L (influent), <LOD – 19.31 μg/L (effluent) in Nkowankowa WWTP; <LOD – 8.77 μg/L (influent), <LOD – 4.37 μg/L (effluent) in Tzaneen WWTP; <LOD – 6.20 μg/L (influent), <LOD – 4.61 μg/L (effluent) in Kgapane WWTP; <LOD – 6.81 μg/L (influent), <LOD – 4.24 μg/L (effluent) in Makhado WWTP; <LOD – 7.57 μg/L (influent), <LOD – 4.50 μg/L (effluent) in Siloam WWTP; and <LOD – 8.06 μg/L (influent), <LOD – 20.85 μg/L (effluent) in Thohoyandou WWTP. Comparatively, the concentration of levonorgestrel was generally low in Malamulele, Kgapane, Tzaneen, Kgapane, Siloam, and Makhado WWTP effluent samples except Giyani (8.30 μg/L), Nkowankowa WWTP effluent (19.32 μg/L), and Thohoyandou (20.86 μg/L) WWTP effluent. Levonorgestrel is one of the easily accessible emergency contraceptive pills on the market; its prevalence in all studied WWTP validates its daily consumption. High concentrations of levonorgestrel in effluent from wastewater treatment plants contribute to increased COD and TOC levels, highlighting the inadequacy of current wastewater treatment methods in eliminating specific pharmaceutical contaminants.

The average values of diclofenac ranged from <LOD – 2.21 μg/L (influent), <LOD – 0.36 μg/L (effluent) in Malamulele WWTP; <LOD – 5.45 μg/L (influent), <LOD – 3.09 μg/L (effluent) in Giyani WWTP; <LOD – 9.16 μg/L (influent), <LOD – 3.51 μg/L (effluent) in Nkowankowa WWTP; <LOD – 0.40 μg/L (influent), <LOD – 2.29 μg/L (effluent) in Tzaneen WWTP; <LOD – 18.81 μg/L (influent), <LOD – 3.35 μg/L (effluent) in Kgapane WWTP; <LOD – 24.98 μg/L (influent), <LOD – 5.00 μg/L (effluent) in Makhado WWTP; <LOD – 3.89 μg/L (influent), <LOD – 1.78 μg/L (effluent) in Siloam WWTP; and <LOD – 4.76 μg/L (influent), <LOD – 1.78 μg/L (effluent) in Thohoyandou WWTP. In contrast, diclofenac concentrations decreased from influent to effluent sample in every sampling location except the Tzaneen WWTP effluent, which might be attributed to malfunctioning of the WWTP or the adsorbed analyte being mobilized from sediments or debris in activated sludge since sampling was done during rainy days.

Ibuprofen was detected in all sampling points except Thohoyandou WWTP for all the sampling periods. The concentration values ranged from <LOD – 114.33 μg/L (influent), <LOD (effluent) in Malamulele WWTP; <LOD – 21.90 μg/L (influent), <LOD – 25.67 μg/L (effluent) in Giyani WWTP; <LOD – 78.73 μg/L (influent), <LOD – 31.41 μg/L (effluent) in Nkowankowa WWTP; <LOD – 89.23 μg/L (influent), <LOD – 59.91 μg/L (effluent) in Tzaneen WWTP; <LOD – 81.83 μg/L (influent), <LOD – 31.11 μg/L (effluent) in Kgapane WWTP; <LOD – 105.53 μg/L (influent), <LOD (effluent) in Makhado WWTP; <LOD (influent), <LOD – 34.55 μg/L (effluent) in Siloam WWTP; and <LOD (influent), <LOD (effluent) in Thohoyandou WWTP. Although ibuprofen was not ubiquitous in all sampling points, it had the highest concentration compared to diclofenac and efavirenz, with levonorgestrel recording the lowest concentration; this

Table 3
Method validation of the target pharmaceutical compounds.

Compound	R_t	R^2	LOD (μg/L)	LOQ ((μg/L)
EFA	4.019	0.99	0.007	0.025
LVG	3.526	0.99	0.006	0.022
DICLO	9.4	0.98	0.003	0.013
IBU	10.5	0.98	0.002	0.015

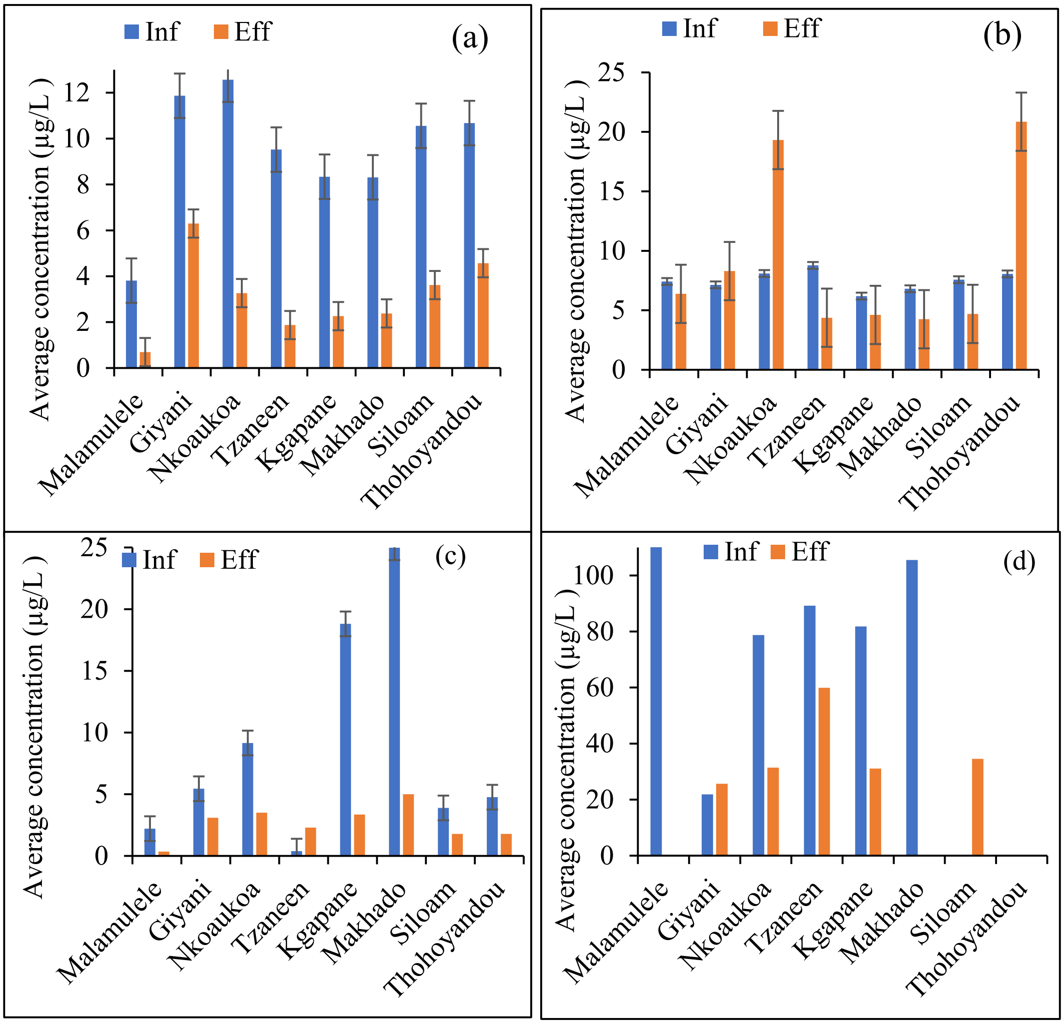


Fig. 3. Average concentrations of efavirenz (a), levonorgestrel (b), diclofenac (c), and Ibuprofen (d) for the three-sampling period.

could be attributed to its use for general pain relief.

Table 4 compares efavirenz, levonorgestrel, ibuprofen, and diclofenac recorded in the present study with those reported in the literature. Efavirenz, ibuprofen, and diclofenac are among the most studied pharmaceutical contaminants in South Africa compared to levonorgestrel. Comparatively, the recorded levels of these compounds in the present study are generally lower than the ones reported in the available literature, particularly for ibuprofen and diclofenac. Furthermore, diclofenac and ibuprofen showed the highest concentrations compared to efavirenz and levonorgestrel. This could be linked to their high intake since they are among the most consumed pharmaceutical contaminants in South Africa, and they can be easily accessed since one can buy from available pharmacies [32]. With regard to levonorgestrel, few studies have reported its occurrence in wastewater [32,33]. The highest concentration of levonorgestrel was reported in the present study compared to the available literature.

Table 4
Concentration of efavirenz, diclofenac, ibuprofen and efavirenz found in South African wastewaters.

Compound	Influent	Effluent	Location	Reference
Efavirenz	1400–34000 ng/L	2–34 μg//L	Wastewater, Kwa-Zulu Natal, South Africa	[34]
	17400 ng/L	7100 ng/L	Wastewater, Gauteng, South Africa	[35]
	>LOD – 1.17 μg/L	>LOD – 1.04 μg/L	Surface and wastewater, Gauteng, South Africa	[36]
	>LOD – 11.90 μg/L	>LOD – 4.57 μg/L	Wastewater, Limpopo, South Africa	Current study
Diclofenac	>LOD – 115.1 μg/L	>LOD – 23.5 μg/L	Surface and wastewater, Kwa-Zulu Natal, South Africa	[37]
	>LOD – 22.3 μg/L	>LOD – 19.0 μg/L	Surface and wastewater, Kwa-Zulu Natal, South Africa	[38]
	>LOD – 2.31 μg/L	>LOD – 0.90 μg/L	Surface water, Kwa-Zulu Natal, South Africa	[39]
	>LOD – 24.98 μg/L	>LOD – 5.00 μg/L	Wastewater, Limpopo, South Africa	Current study
Ibuprofen	>LOD – 220 μg/L	>LOD –67.9	Surface and wastewater, Kwa-Zulu Natal, South Africa	[37]
	>LOD – 117.5	>LOD – 58.7	Surface water, Kwa-Zulu Natal, South Africa	[40]
	>LOD – 15.83 μg/L	>LOD – 2.50 μg/L	Surface and wastewater, Gauteng, South Africa	[36]
	>LOD – 105.53 μg/L	>LOD – 59.91 μg/L	Wastewater, Limpopo, South Africa	Current study
Levonorgestrel	>LOD – 0.2 ng/L	>LOD – 0.6 ng/L	Wastewater, Australia	[41]
	>LOD – 8.06 μg/L	>LOD – 8.29 μg/L		Current study

3.4. Removal efficiency of pharmaceutical compounds selected WWTP

Several factors influence the presence of pharmaceutical pollutants in WWTP and their subsequent removal during wastewater treatment. The removal efficacies of both WWTPs for the targeted pharmaceutical contaminants were calculated by employing the following:

$$removal = \frac{Ci - Cf}{Ci} * 100 \quad (3)$$

Where C_i is the inlet detected concentration, and C_f is the effluent concentration.

Fig. 4 presents the removal efficiency of the selected pharmaceutical contaminants in sampled WWTP. The removal efficiency of efavirenz varied between 47 %–82 %, with Giyani WWTP registering the lowest percentage of removal and Malamulele exhibiting the highest removal efficiency. Levonorgestrel removal efficiency ranged from <0 % to 50 %, with Malamulele WWTP showing the highest removal efficiency and Tzaneen WWTP recording the lowest removal percentage. The removal efficiency of diclofenac varied between <0 % and 84 %, with Tzaneen WWTP having the lowest removal percentage and Malamulele exhibiting the highest removal efficiency. Ibuprofen removal efficiency ranged between <0 %–100 %, with Malamulele demonstrating the highest removal efficiency and Giyani WWTP having the lowest removal percentage. The observed differences in the removal efficiency of pharmaceutical contaminants at different sampling sites can be primarily attributed to the distinct wastewater treatment methods implemented at each site. Briefly, the treatment facilities in Thohoyandou, Malamulele, Giyani, Kgapane, and Nkowankowa employ biofilters in conjunction with oxidation ponds following chlorination, and notably, these sites do not process industrial sludge. In contrast, Tzaneen and Makhado utilize activated sludge systems without oxidation ponds and they receive a small proportion as industrial waste. Siloam's treatment infrastructure incorporates grid sedimentation and oxidation ponds that exclusively handle hospital sludge.

The obtained results indicated that ibuprofen and diclofenac demonstrate significantly higher removal efficiencies in wastewater treatment plants utilizing biofiltration coupled with oxidation ponds compared to those employing activated sludge followed by chlorination. The limited removal efficiency observed in WWTPs utilizing activated sludge may be attributable to the retention and immobilization of pollutants within the sludge matrix [42]. Furthermore, an inconsistency in efavirenz removal efficiency was documented among different WWTPs utilizing varying treatment methodologies. The highest removal efficiency for efavirenz was noted at the Tzaneen facility, which also relies on activated sludge treatment. In contrast, levonorgestrel exhibited its highest removal efficiency at WWTPs employing the activated sludge process rather than those utilizing biofiltration combined with oxidation ponds. Additionally, a facility incorporating grid biofiltration and oxidation ponds, augmented by vegetated wetland formations, exhibited a

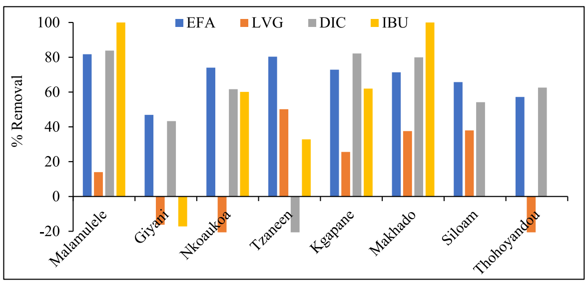


Fig. 4. Levonorgestrel, efavirenz, diclofenac, and ibuprofen removal efficiency by WWTP.

Table 5
Pearson correlation of the physicochemical parameters and pharmaceutical contaminants in wastewater samples.

Pearson Correlation													
Parameters	**pH**	**EC (μs/cm)**	**TDS (ppm)**	**DO (mg/L)**	**SAL (PSU)**	**TEMP (°C)**	**TUR**	**TOC (mg/L)**	**COD (mg/L)**	**EFA (μg/L)**	**LVG (μg/L)**	**IBU (μg/L)**	**DIC (μg/L)**
pH	1												
EC (μs/cm)	−0.095	1											
TDS (ppm)	−0.235	0.909[b]	1										
DO (mg/L)	−0.232	0.142	0.11	1									
SAL (PSU)	0.152	0.634[b]	0.478[b]	−0.092	1								
TEMP (°C)	0.105	−0.412[b]	−0.402[b]	−0.197	−0.157	1							
TUR (mg/L)	0.267	0.061	−0.029	−0.191	0.409[b]	−0.11	1						
TOC (mg/L)	0.067	0.501[b]	0.344[a]	−0.016	0.605[b]	0.005	0.224	1					
COD (mg/L)	0.053	−0.201	−0.198	0.116	−0.2	0.154	−0.008	−0.474[b]	1				
EFA (μg/L)	0.322[a]	−0.041	−0.111	−0.444[b]	0.052	0.178	0.147	0.251	−0.305[a]	1			
LVG (μg/L)	0.121	−0.113	−0.181	0.086	−0.11	−0.076	−0.077	0.183	−0.203	0.064	1		
IBU (μg/L)	0.022	−0.245	−0.274	−0.112	−0.058	0.372[a]	0.312[a]	0.119	0.129	0.342[a]	−0.048	1	
DIC (μg/L)	0.138	−0.066	−0.01	−0.14	0.088	0.18	0.315[a]	0.161	−0.026	0.351[a]	−0.048	0.547[b]	1

[a] Correlation is significant at the 0.05 level (2-tailed).
[b] Correlation is significant at the 0.01 level (2-tailed).

commendable removal efficiency. Given the differential removal efficiencies of various pharmaceutical contaminants across diverse WWTPs employing distinct treatment processes, it is imperative to critically assess the effectiveness of available treatment methodologies in addressing the removal of various pharmaceutical compounds across different classes.

3.5. Correlations of pharmaceutical compounds with physicochemical properties of wastewater

Table 5 shows the correlation between physicochemical parameters and pharmaceutical contaminants in wastewater samples. The values of the Pearson correlation coefficient indicated multiple positive correlations between efavirenz and pH, salinity, temperature, turbidity, and total organic carbon, with pH showing a strong positive correlation ($p > 0.05$). While a strong negative correlation ($p < 0$) between dissolved oxygen, electrical conductivity, chemical oxygen demand, and total dissolved solids implies an indirect correlation. A positive correlation between levonorgestrel efavirenz, total organic carbon, dissolved oxygen, and pH was observed, suggesting a direct correlation with a strong negative correlation on electrical conductivity, total dissolved solids, salinity, temperature, and chemical oxygen demand. The Pearson correlation coefficient values of $p > 0.05$ were observed between ibuprofen with temperature, turbidity, and efavirenz, indicating a direct correlation. A weak positive correlation was observed with total organic carbon, chemical oxygen demand, and pH. With regard to diclofenac, a strong positive correlation was observed with turbidity ($p > 0.05$), efavirenz ($p > 0.05$), and ibuprofen ($p > 0.01$) with weak interdependence between salinity, temperature, pH, and total organic carbon. A strong positive correlation ($p < 0$) was observed between electrical conductivity, total dissolved solids, dissolved oxygen, and total organic carbon. The monitored physicochemical parameters and pharmaceutical contaminants showed weak to strong positive and negative correlations. A negative correlation implies that as one parameter increases, the other decreases, whereas a positive correlation suggests that as one parameter increases, the other also increases. The obtained results correlate with a study by Ohoro et al. [43], which reported that the concentrations of these contaminants also depend on levels of physicochemical parameters such as pH, temperature, electrical conductivity, salinity, turbidity, and dissolved oxygen.

4. Conclusion

The study reported the occurrence of efavirenz, levonorgestrel, ibuprofen and diclofenac in both the influent and effluent of wastewater treatment plants of the Vhembe and Mopani Districts of Limpopo Province. The study revealed the prevalence of the selected pharmaceuticals in the wastewater, with ibuprofen and diclofenac being detected at the highest concentration. Wastewater treatment plants only partially remove these compounds, leading to their release into receiving water bodies. Thus, future studies should monitor these compounds in receiving water bodies to further assess the associated ecological risks. In addition, it is recommended that studies looking into ways to improve the removal of these compounds by WWTPs be conducted to avoid their ecological impacts on the aquatic environment.

CRediT authorship contribution statement

E.P. Munzhelele: Writing – review & editing, Writing – original draft, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **W.B. Ayinde:** Writing – review & editing, Visualization, Validation, Supervision, Data curation, Conceptualization. **W.M. Gitari:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Funding acquisition, Data curation, Conceptualization. **G.K. Pindihama:** Validation, Software, Formal analysis, Data curation. **R. Mudzielwana:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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