AQUATIC ECOSYSTEM AND HUMAN HEALTH RISK ASSESSMENT OF COVID-19 DRUGS WITHIN A TRANSBOUNDARY WATER BASIN

Report
to the Water Research Commission

by

Belle GN[1], Omotola EO[2], Moodley B[3], Olatunji OS[2]; Truter JC[4]; Moodley R[5]; Oberholster P[1]

[1]Centre for Mineral Biogeochemistry, University of the Free State
[2]School of Chemistry and Physics, University of KwaZulu-Natal
[3]Department of Chemical Sciences, Tai Solarin University of Education, Nigeria
[4]Stellenbosch University Water Institute
[5]Department of Chemistry, University of Manchester, England

WRC report no. 3261/1/26
ISBN 978-0-6392-0800-8

June 2026

This is the final report of the WRC project no. C2023/2024−01308

EXECUTIVE SUMMARY

Given the significant concerns about the potential risks posed by widely used pharmaceuticals during the COVID-19 pandemic to ecosystems and human health, the current study investigated how selected pharmaceuticals in the Orange-Senqu Transboundary River Basin, a critical shared water resource, could pose risks and affect environmental fate and distribution. The study targeted four widely used COVID-19-related drugs, namely the antibiotic azithromycin, the corticosteroids dexamethasone, prednisone, and prednisolone. Prednisolone, a metabolite of prednisone, was included because transformation products can exhibit toxicity that is equal to or greater than that of the parent compound. To quantify these pharmaceuticals in surface water and sediments during summer and winter, a validated solid-phase extraction-liquid chromatography-mass spectrometric method was developed and utilised. The depth-dependent distribution was evaluated using sediment core analyses and provided insight into historical deposition linked to pandemic-related use. The study underscores that pollution originating in one region may adversely affect downstream ecosystems and communities across national borders. Hence, understanding contamination patterns in this transboundary basin is essential. An integrated risk assessment framework was applied following chemical analysis to link environmental concentrations to ecological and human health impacts. Risk-based indices were combined with bioassays using yeast and zebrafish embryos to assess endocrine disruption, developmental toxicity, and broader toxicological effects. The findings revealed that prednisone exhibited the highest concentrations in water, whereas dexamethasone dominated sediment samples, highlighting compound-specific partitioning behaviour. In addition, Azithromycin showed the highest detection frequency in water (79%), but was less prevalent in sediments, indicating variable transport and retention mechanisms among pharmaceuticals. Spatial and vertical variations were observed across sampling locations and sediment layers. This reflects the dynamic exchange between water and sediment compartments. These distribution patterns were consistent with transport modelling results, which indicated that pharmaceutical mobility is influenced by both convection and diffusion, which in turn are shaped by channel morphology and thermal conditions. The findings also revealed that azithromycin exhibited limited mobility and a tendency to settle, whereas dexamethasone showed greater transport potential. In addition, toxicological assessments revealed significant estrogenic and anti-androgenic activities, particularly at discharge points, with dioxin-like activity detected at nearly all sites. The study suggests that these effects translate into severe biological outcomes, as evidenced by 100% embryotoxicity at selected sites during summer, followed by reduced mortality in winter. Risk-based indices identified localised hotspots driven mainly by dexamethasone, while overall ecological risk remained low to moderate relative to international benchmarks. The findings also revealed that human health risks via fish consumption were generally low, although persistent antibiotic residues raise concerns regarding antimicrobial resistance and long-term endocrine effects. Overall, the study concludes that, despite the moderate ecological risk posed by pharmaceutical contamination at the basin scale, there are significant threats to aquatic ecosystems from localised discharges and persistent residues. This, therefore, underscores the need for targeted monitoring and improved wastewater management.

ACKNOWLEDGEMENTS

The project team would like to thank the following people for their contributions:

Reference Group	**Affiliation**
Dr Eunice Ubomba-Jaswa	Water Research Commission
Ms Penny Jaca	Water Research Commission
Dr Minashree Kumari	Ministry of Science and Technology, New Delhi, India
Dr Ntombikayise Mahaye	Agricultural Research Council
Prof Oke Saheed	Central University of Technology
Mr Michael Ramaano	Orange−Senqu River Commission (ORASECOM)
Prof Beatrice Opeolu	BEE Solutions and Consultancy Services
Prof Leslie Petrik	University of the Western Cape
Prof Wilmien Powell	University of Limpopo
Ms Dephney Kabini	Department of Water and Sanitation
Project Team	
Dr Gladys Belle	University of the Free State
Prof Paul Oberholster	University of the Free State
Prof Brenda Moodley	University of KwaZulu-Natal
Dr Elizabeth Omotola	Tai Solarin University of Education, Ogun State, Nigeria
Prof Roshila Moodley	University of Manchester
Prof Stephen Olatunji	University of KwaZulu-Natal
Dr Johannes Truter	University of Stellenbosch

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

AChE	Acetylcholinesterase
ADI	Acceptable daily intake
AhR	Aryl hydrocarbon receptor
ANOVA	ANALYSIS of variance
AR	Androgen receptor
AZI	Azithromycin
BAFs	Bioaccumulation factors
BCFs	Bioconcentration factors
βNF	β-naphthoflavone
CDER	Centre for Drug Evaluation and Research
CECs	Contaminants of Emerging Concern
DEX	Dexamethasone
ECOSAR	Ecological Structure Activity Relationships Class Program
EC	Electrical conductivity
EC50	Half maximal effective concentration
FD	Fluorescence detectors
EDCs	Endocrine-disrupting chemicals
EDI	Estimated daily intake
EEQ	β-estradiol equivalents
ESI	Electrospray ionisation
FEQ	flutamide equivalents
GC	Gas chromatography
GDS	Green Drop Score
GRR	Green Drop Risk Rating
HI	Hazard index
HLB	Hydrophilic-lipophilic balance
hpf	Hours post-fertilisation
HPLC	High-performance liquid chromatography
HQ	Hazard quotient
ICH	International Conference on Harmonisation
LC	Liquid chromatography
LC50	Lethal concentration
LC−MS	Liquid chromatography mass spectrometry
LM	Local Municipality
LoD	Limit of detection
LoQ	Limit for quantification
MEC	Measured Environmental Concentration
MeOH	Methanol
m-PRD	Prednisolone

MS	Mass spectrometry
NDoH	National Department of Health
ORASECOM	Orange−Senqu River Basin Commission
PCs	Pharmaceutical compounds
PNEC	Predicted No-Effect Concentration
PoD	Point of Discharge
PPCPs	Pharmaceuticals and personal care products
PRD	Prednisone
RQ	Risk Quotient
RSD	Relative standard deviation
SPE	Solid-phase extraction
SPE−LC−MS	Solid-phase extraction−liquid chromatography−mass spectrometry
SRM	Selected reaction monitoring
UHPLC–(ESI)MS/MS	Ultra-high performance liquid chromatography−electrospray ionisation tandem mass spectrometry
US	United States
WHO	World Health Organisation
WRC	Water Research Commission
WWTP	Wastewater Treatment Plant
YAS	Yeast androgen screen
YAAS	Yeast anti-androgen screen
YES	Yeast Oestrogen Screen

CHAPTER 1
BACKGROUND

1.1 Introduction

The expansion of industrialisation and rapid population growth has driven increased chemical production, including the production of contaminants of emerging concern (CECs). Among other environmental contaminants, pharmaceutical residues remain significant pollutants due to increased use, driven by population growth. These residues affect water, soil, and sediments and pose fatal risks to living organisms upon environmental release, which has gained the attention of researchers (Omotola et al. 2023a, b). These pharmaceuticals are essential to human health for preventing or treating diseases. The 2019 outbreak of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in China, leading to COVID-19, resulted in significant global fatalities. As of July 26, 2023, 768 560 727 global cases and 6 952 522 deaths were recorded (World Health Organisation [WHO] 2023). South Africa reported 4 072 533 cases and 102 595 deaths, while Lesotho had 34 490 cases and 706 deaths (WHO 2023).

The devastating consequences of the COVID-19 pandemic prompted the scientific community to research suitable drugs with minimal side effects (Hendricks et al. 2021). During the early years of the pandemic, clinical trials identified existing drugs that were repurposed for the effective treatment of COVID-19-related symptoms, as well as preventing COVID-19-related hospitalisations and deaths. The South African National Department of Health (NDoH 2021) recommended several tested drugs for COVID-19 treatment. Corticosteroids, such as dexamethasone and prednisone, reduced inflammation in the latter infection stages in hospitalised patients requiring oxygen (NDoH 2021). Antibiotics such as azithromycin were suggested for use only for patients in whom there was a clear antimicrobial indication, to destroy opportunistic bacterial infections in early infection stages (Hendricks et al. 2021; NDoH 2021). Paracetamol was used for pain and fever (Hendricks et al., 2021). Antivirals such as remdesivir were recommended for hospitalised and unvaccinated patients to reduce the burden on the medical system, despite limited use in South Africa due to cost (NDoH 2022). Blood thinners, including low molecular weight heparin (Clexane), were used to reduce blood clots associated with damage to blood vessels in hospitalised patients needing oxygen (NDoH 2021).

Research indicates that these pharmaceuticals and their metabolites enter water resources during manufacturing, usage, or disposal via effluents from wastewater treatment plants (WWTPs) (Gwenzi et al. 2022). The increased use of COVID-19 drugs raises concerns about their environmental transport; consequently, WWTP effluent is a significant point source of these pharmaceuticals in water resources (Archer et al. 2017; Swartz et al. 2018).

Pharmaceuticals in surface water pose a severe threat to public health and the environment (Ebele et al. 2017). The presence of COVID-19 drugs in aquatic ecosystems, including antimicrobial pharmaceuticals,

may lead to the emergence of antimicrobial-resistant pathogens in water, posing a risk of disease spread through the food chain and affecting aquatic ecosystems (Almeida et al. 2023). Additionally, the toxic effects of these compounds on human health, as well as their persistence and their ability to bioaccumulate in different environmental media, make these contaminants a public health concern (Ebele et al. 2017; Jamil 2019; Oluwole et al. 2020; Reyes et al. 2020). Moreover, the possible health and environmental risks of exposure to these drugs in water resources during the COVID-19 pandemic are unknown. Investigating the risk of these pharmaceuticals on aquatic ecosystems is crucial for remediation, as threats to aquatic organisms may affect humans consuming contaminated fish (Kumari and Kumar 2022).

Researchers such as O'Flynn et al. (2021) reviewed pharmaceutical sources and pathways in aquatic ecosystems using life cycle assessment. Studies by Gwenzi et al. (2022) and Kumari and Kumar (2021, 2022) assessed the environmental and human health risks of the COVID-19 drugs, including ribavirin, ritonavir, and lopinavir, on three trophic levels of aquatic organisms, algae, Daphnia, and fish, in environmental waterbodies. This was done using the recommended half-maximal effective concentration (EC50) or lethal concentration (LC50) values obtained from the Ecological Structure Activity Relationships Class Program (ECOSAR database) of the U.S. Environmental Protection Agency (2008), which sometimes underestimates toxic effects (Raimondo et al., 2010). Tarazona et al. (2021) also reviewed the environmental impact of COVID-19 therapeutic solutions. Their study was a prospective analysis that used multiple models and available data to predict the environmental risk of various COVID-19 drugs (Tarazona et al., 2021). Almeida et al. (2023) predicted the ecotoxicological risk of 22 antimicrobial pharmaceuticals. They found that rifaximin and atovaquone exhibited predicted ecotoxicological risks to aquatic organisms. At the same time, flucloxacillin, piperacillin, tazobactam, meropenem, ceftriaxone, phosfomycin, and metronidazole indicated a major potential for antibiotic resistance (Almeida et al. 2023). Similarly, Desgens-Martin and Keller (2021) modelled the fate and aquatic ecosystem toxicity risk of remdesivir and dexamethasone, used for COVID-19 treatment, based on predicted environmental concentrations derived from the ChemFate model.

In this study, field data were used to assess the aquatic and human health risks associated with four COVID-19 drugs within the Orange-Senqu Transboundary River Basin. The different COVID-19 drugs assessed in this study were those used extensively during the COVID-19 pandemic, including Corticosteroids (Dexamethasone, Prednisone, and Prednisolone, a metabolite of prednisone) and antivirals (Azithromycin). Although some drugs currently used for COVID-19 have been used to treat other conditions, such as corticosteroids for anti-inflammatory and immune-modulating therapy, their use has escalated since the outbreak of COVID-19. The extent of contamination of these drugs in water resources and the risk they may pose to human health and aquatic organisms remains indeterminate in the scientific literature. This lack of comprehensive data undermines the ability to assess and effectively mitigate the risks. Addressing these gaps is crucial for informing post-pandemic water quality monitoring programmes in South Africa and globally, ensuring the safety of aquatic environments and public health. To bridge these gaps, it is imperative to advance research on the environmental impact of COVID-19 drugs. This includes developing

more robust detection methods and conducting thorough risk assessments. Additionally, integrating these findings into post-pandemic water quality monitoring programmes is crucial for safeguarding both aquatic ecosystems and public health. Failure to address these issues could lead to prolonged environmental and health implications, highlighting the urgent need for coordinated scientific and regulatory efforts.

This project utilised an integrated environmental risks assessment approach to investigate the selected pharmaceuticals within the Orange-Senqu River Basin. The approach integrated several assessment components, including the occurrence of pharmaceuticals in water and sediments, their vertical distribution in sediment layers, seasonal variability, ecological and human health risk assessments, bioassay-based biological activity screening, and predictive deposition modelling. By combining these elements, the study enhanced knowledge of the occurrence, environmental fate, and potential risks of these pharmaceuticals in water resources. The findings provide important evidence to guide better water management and governance in the basin. Beyond that, the recommendations could help raise awareness in local communities, encouraging safer disposal practices, like take-back programmes, that reduce the impact of pharmaceuticals on our shared water resources.

1.2 Project aims

1. To develop a compromise multi-analyte method for separation, detection, and quantitation of COVID-19 drugs, as well as a solid-phase extraction procedure for recovery of COVID-19 drugs from aqueous solution.
2. To determine the occurrence and fate of COVID-19 drugs and their metabolites in water and sediments.
3. To determine the toxicological risk of COVID-19 drugs using yeast bioassays and zebra fish embryos.
4. To assess the human health risk and aquatic ecosystem risks from the consumption of contaminated fish species using risk-based indices.
5. To predict the risk of COVID-19 drugs and their metabolites on aquatic organisms using modelling.

1.3 Scope and Limitations

This study examined the occurrence and fate of four pharmaceuticals, Azithromycin, Prednisone, Dexamethasone, and one of Prednisone's metabolites, Prednisolone, in water and sediment samples from a transboundary water basin. The study assessed the fate of these pharmaceuticals in terms of their forms in sediments, their potential toxicological risks to aquatic organisms and human health, and their risks using risk-based indices. Additionally, the fate of the PCs was assessed using the Normalised Stokes-Based Model to predict their long-term behaviour in water and sediments. The case study for this research is the Orange−Senqu River Basin. Water pH, conductivity, salinity, total dissolved solids, suspended solids, dissolved oxygen, and chemical oxygen demand were also assessed at each of the eight sampling sites

selected along the transboundary river basin. A toxicological assessment using yeast bioassays and zebrafish embryos was conducted to evaluate the risks of exposure to these drugs in water samples. In this toxicology study, the researchers chose to test entire environmental samples rather than isolating individual pollutants. This approach provides a more comprehensive understanding of how both dissolved and solid contaminants affect aquatic ecosystems. It also helps reveal how different toxic substances interact with one another and how their effects shift under natural conditions, such as changes in temperature or pH. To complement this, the study used modelling techniques to estimate the long-term risks posed by pharmaceuticals to aquatic life. However, the research did not examine how these pollutants degrade over time, whether through biological processes involving microorganisms or through chemical processes such as hydrolysis.

1.4 Study area and sampling sites

This research focuses on the Orange-Senqu Transboundary River Basin in Southern Africa, one of the largest in the region, spanning about 1,000,000 km² and crossing four countries: Lesotho, South Africa, Botswana, and Namibia. The river begins in the Lesotho highlands as the Senqu River, flowing through the lowlands and receiving input from smaller rivers such as the Malibamatso, Mashai, and Sequnyane. The Caledon River, a major tributary, forms part of Lesotho's western boundary before joining the Orange-Senqu system. The river's quality is vital for the nations along its course, particularly South Africa, which accounts for 63% of total water withdrawals, compared to Lesotho's 0.2%. For this study, sampling began at the Thetsane River in Maseru and extended downstream to the Gariep Dam.

Monitoring pharmaceutical pollutants within a transboundary river basin is especially important because contamination in one area can affect multiple countries and communities. Pollutants such as chemicals, sewage, pharmaceuticals, and metals threaten both human populations and fragile ecosystems. Regular assessments not only protect these resources but also strengthen cooperation among nations by providing credible evidence, fostering trust, and enabling fair, timely decisions.

The data collected on emerging contaminants in the Orange-Senqu River Basin will be invaluable. While most existing water quality studies focus on issues such as nutrient enrichment, sediment loads, flood regimes, and algal blooms. Incorporating pharmaceutical pollution into this research will significantly enhance its research depth, which may further lead to the development of more effective and comprehensive management strategies. Safeguarding the basin's water resources is not just important for environmental health; it is also critical for fostering sustainable socio-economic growth and ensuring fair resource distribution among the nations that depend on these vital waterways.

1.5 Sampling site description

The research was conducted by collecting water samples from eight different points along the Caledon (Mohokare) River and the Orange-Senqu River. The points were chosen based on their accessibility for sampling and proximity to WWTPs. The samples were collected from upstream, downstream, and at the

discharge point of the WWTPs. Emphasis is given to sampling at these points because it helps identify the composition of the water flowing out from these plants into the river, thereby highlighting its impact on the ecosystem of the river. Two sets of sampling were conducted: one in February for summer conditions and another in May for winter conditions.

These operations spanned various geographical points along the Orange-Senqu River, including Site 1: Gariep WWTP (30°35'7.56"S and 25°30'54.62"E); Site 2: Gariep Dam (30°35'11.41"S and 25°29'11.45"E); Site 3: Aliwal North (30°41'12.71"S and 26°41'39.43"E); Site 4: Zastron (30°17'57.91"S and 27° 6'43.95"E); Site 5: Ladybrand (29°11'12.81"S and 27°29'7.82"E); Site 6: Maseru (29°18'53.23"S and 27°28'2.11"E); Site 7: Ficksburg (28°53'26.53"S and 27°53'45.73"E); and Site 8: Clarens (28°31'17.23"S and 28°25'50.80"E). Three sites were added along the Mohokare (Caledon) River, including Lesotho (Maseru), Ladybrand, and Ficksburg. The Mohokare River is a major tributary of the Orange−Senqu River (Chatanga et al., 2019), which justifies its inclusion in this study. Figure 1.1 illustrates the locations of the sampling sites.

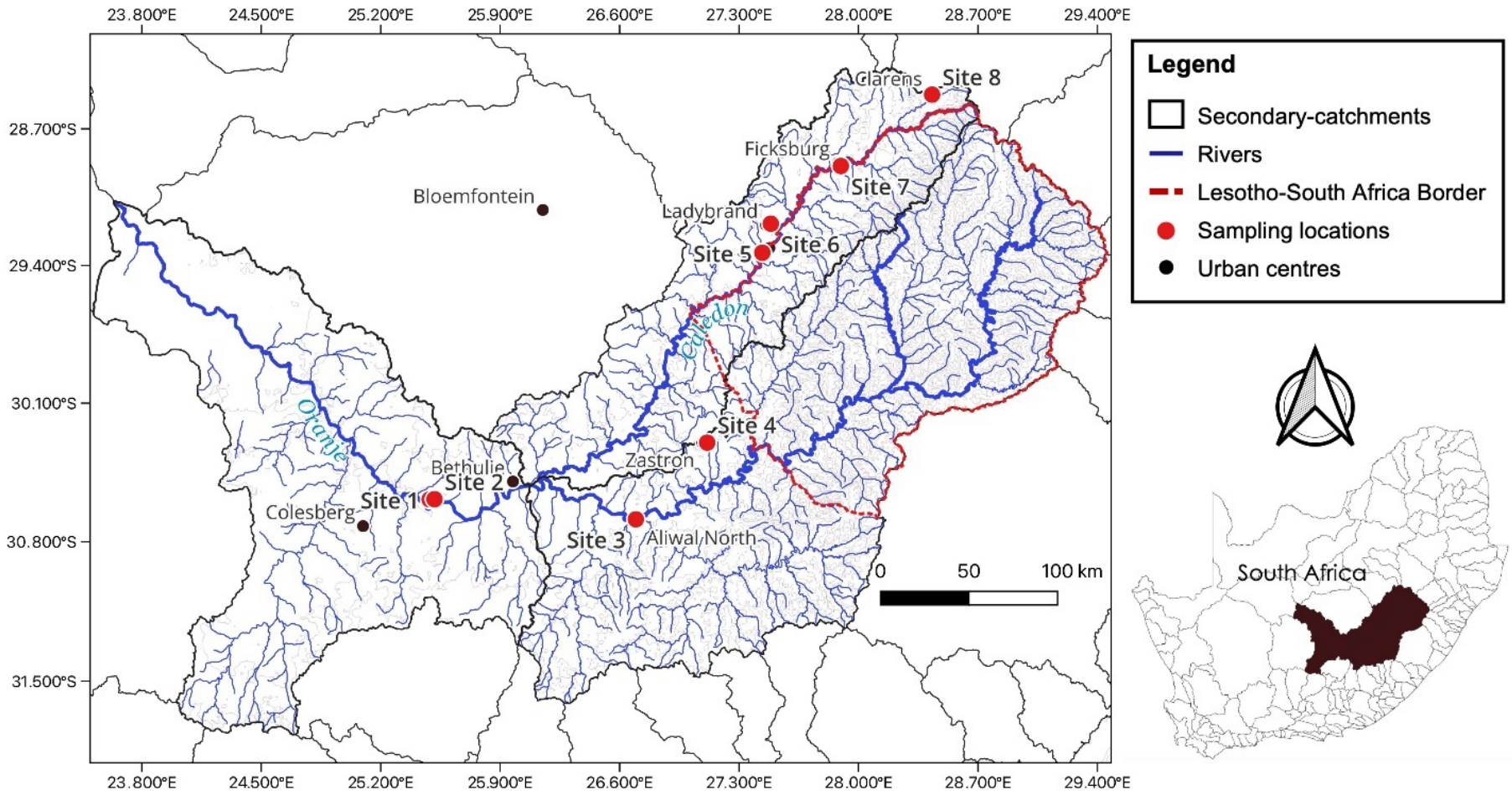

Figure 1.1 Location of sampling sites along the Orange−Senqu River

Table 1.1 Description of WWTP sampling sites within the Orange−Senqu River basin

Sampling sites	Name of WWTP	Coordinates	River System into which effluent is discharged	Local Municipality (LM)	-Treatment option of WWTP and Characteristics of WWTP
Site 1	Gariep Dam WWTP	30°35'7.56"S and 25°30'54.62"E	Effluent is channelled into a farm Dam, which then flows into a wetland and then into the Upper Orange System	Kopanong Local Municipality, Free State	-Small-sized plant (2,50Mℓ/D) -Bio-filter & Oxidation Ponds
Site 2	Gariep Dam	30°35'11.41"S and 25°29'11.45"E	Section of the Upper Orange System	Kopanong Local Municipality	-Large size plant (12.20Mℓ/D) -The entire treatment works is not operational due to vandalism
Site 3	Aliwal North	30°41'12.71"S and 26°41'39.43"E,	Upper Orange River System	Walter Sisulu LM, Eastern Cape / Joe Gqabi DM, EC	-Medium-sized plant (2.0Mℓ/D) -Oxidation pond
Site 4	Zastron WWTP	30°17'57.91"S and 27°6'43.95"E	Effluent falls into the Caledon River System	Mohokare LM, Free State	-Small-sized plant (1.0Mℓ/D) -Ponds are poorly maintained, with aged sludge accumulation. Inconsistent disinfection.
				Joe Gqabi District, Eastern Cape	Large size plant (18.8Mℓ/D)
Site 5	Ladybrand WWTP	29°11'12.81"S and 27°29'7.82"E	Effluent falls into a wetland, which later seeps into the Caledon River System	Mantsopa Local Municipality	-Medium-sized plant (2.80Mℓ/D) -Bio-filter and Oxidation ponds
Site 6	Maseru Thetsane River)	29°18'53.23"S and 27°28'2.11"E			The plant makes use of both the conventional and waste stabilisation pond methods to treat or purify sewage

Site 7	Ficksburg Sewage Work Final Effluent	28°53’26.53"S and 27°53’45.73"E	Effluent falls into the Caledon River System	Setsoto LM, FS	Large size plant (12.20 mℓ/D) The entire treatment works is not operational due to vandalism
Site 8	Clarens WWTP	28°31’17.23"S and 28°25’50.80"E	Effluent falls into the Little Caledon River System	Dihlabeng LM, FS	Small size plant (2,50Mℓ/D) -Bio-filter & Oxidation Ponds -Upper orange

1.6 Study design

This study employed an integrated environmental assessment approach to investigate selected pharmaceuticals within the Orange-Senqu River Basin. The approach integrated several assessment components, including the occurrence of pharmaceuticals in water and sediments, their vertical distribution in sediment layers, seasonal variability, ecological and human health risk assessments, bioassay-based biological activity screening, and predictive deposition modelling. The case study area for this study was the transboundary Orange−Senqu River Basin. Water and sediment samples were collected from eight sampling sites identified in sections of the Orange−Senqu River Basin, where wastewater treatment plants discharge effluent. In the current study, a solid-phase extraction-liquid chromatography mass spectrometry (SPE−LC−MS) method was developed to isolate, detect, and separate the analytes of interest in water and sediment samples. This method was then validated in accordance with the International Conference on Harmonisation (2005) standard procedures.

The field data obtained was then analysed, and, using an integrated risk assessment approach, the fate of the analysed pharmaceuticals was determined by computing their ecological and human health risks, as well as their toxicological risks to aquatic organisms and human health, using yeast bioassays and Zebrafish embryos. Moreover, modelling was performed to predict the long-term risks to human health posed by COVID-19 drugs arising from the consumption of fish grown in water contaminated with these drugs. The study was divided into seven broad investigations; each was conducted in different phases to facilitate effective assessment and to make the overall findings easier to report and understand. Whereas Investigation 1 involved method development, Investigation 2 involved the investigation of the occurrence of PCs in water, and Investigation 3 involved the investigation of the occurrence of PCs in sediments. In order to assess the risks associated with the PCs, Investigation 4 involved the use of ecological and human health risk indices to assess the impacts of the PCs on aquatic organisms and human health. In addition, Investigation 5 involved the investigation of toxicological risks, where the possible impacts of the waters containing the contaminants on aquatic life were assessed using yeast bioassays, as well as the health impacts of the contaminants on humans who consume fish grown in waters containing the contaminants, using zebrafish embryos as a model for the investigation. Finally, the long-term risks associated with the contaminants in relation to human health were modelled in order to assess the possible health effects of long-term exposure to the contaminants.

Each of the seven investigations was conducted in phases. These phases included: scouting the study area and identifying sampling sites; sampling; measuring variables; analysing data and interpreting results; discussing findings; and drawing conclusions. Details about each phase of each investigation were presented in separate chapters. These deliverable chapters had already been submitted to the Water Research Commission, approved, and published in accredited journals. The overall study design is illustrated graphically in Figure 1.2.

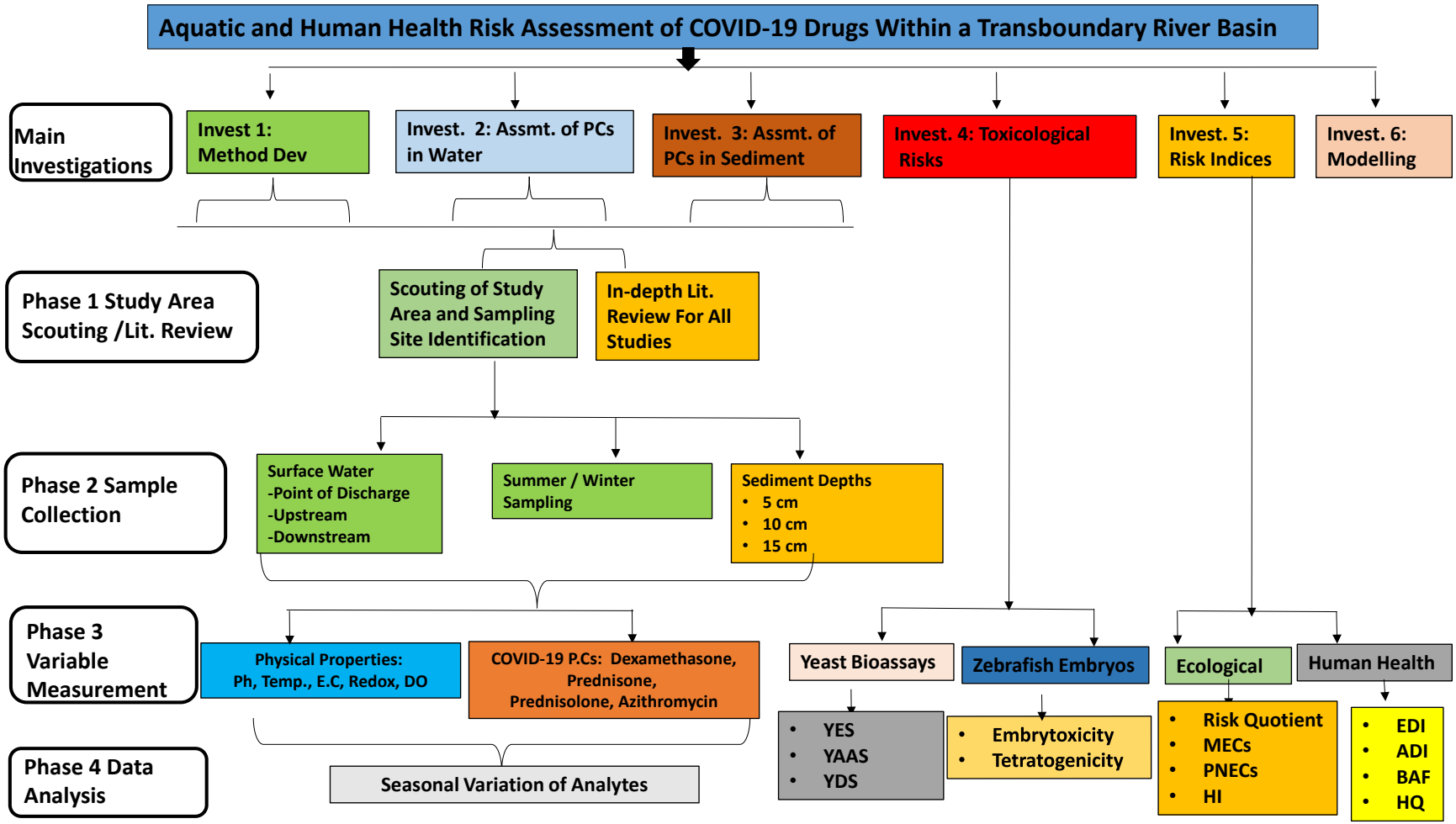

Figure 1.2 Study design for the entire study

CHAPTER 2 [1]
LITERATURE REVIEW

2.1 Introduction

The increase in industrialisation and rapid population growth has driven a surge in chemical production to sustain and enhance the quality of life. These chemicals include, but are not limited to, pharmaceuticals and personal care products. Consequently, the production and consumption of these substances have been linked to the presence of their residues in the immediate environment. Pharmaceutical compounds (PCs) remain significant environmental pollutants due to increased use. Their widespread use in the medical and agricultural fields has led to their rise as dangerous environmental pollutants. Pharmaceutical contamination of aquatic habitats is primarily caused by wastewater effluent released by pharmaceutical industries (Eapen et al., 2024). With quantities ranging from trace to ultra-trace amounts, these compounds are present in various environmental niches, including soils, groundwater, surface water, and wastewater treatment facilities (Nippes et al., 2021; Gwenzi et al., 2022). It is worrisome that the treatment plants are not effective enough to eliminate the residues of pharmaceuticals (Archer et al., 2017; Swartz et al., 2018) before effluent discharge, as observed in the literature (Munzhelele et al., 2024). The treatment plants would not be listed as sources of pharmaceutical contamination in the environment. Unfortunately, these compounds have been reported to pose fatal risks to living organisms upon environmental release from diverse sources. Pharmaceutical contaminants that come from sources such as wastewater, farming, and inappropriate disposal linger and negatively impact organisms through bioaccumulation and biomagnification (Ebele et al., 2017; Oluwole et al., 2020; Eapen et al., 2024). Reproductive problems and neurotoxic impacts are two of the many detrimental health effects these compounds have been connected to in humans and aquatic life (Eapen et al., 2024). Since these compounds have been established to pose certain health risks to living entities, they have gained the exigent attention of researchers (Omotola et al., 2023a; Omotola et al., 2023b).

Despite growing research on pharmaceutical contaminants in aquatic environments, existing studies have focused mainly on a limited group of well-known compounds such as acetaminophen, ibuprofen, carbamazepine, and ciprofloxacin (Patel et al., 2019; Ortúzar et al., 2022; Szarka et al., 2024; Wada and Olawade, 2025). However, pharmaceuticals like azithromycin, dexamethasone, and prednisone, although widely used, especially during the COVID-19 pandemic, have received insufficient scientific attention regarding their environmental fate, detection techniques, and ecotoxicological risks (Morales-Paredes et al., 2022; Bhardwaj et al., 2024; Maremane et al., 2024). A few recent studies have reported their presence in wastewater and surface waters (Wu et al., 2023; Lin et al., 2024; Manbohi et al., 2024; Scaccia et al., 2024; Sun et al., 2024; Xu et al., 2025). Comprehensive assessments of their occurrence, detection challenges, environmental persistence, and potential impacts on aquatic ecosystems and human health remain limited and fragmented.

[1] Chapter 2- https://doi.org/10.1007/s42452-025-07716-5

Moreover, existing reviews and monitoring efforts often lack specificity regarding corticosteroids and macrolide antibiotics, especially their transformation products, environmental persistence, and potential to induce resistance. This gap is critical given that these compounds of interest, when introduced into aquatic systems, can bioaccumulate and contribute to antimicrobial resistance (AMR) and endocrine disruption, thereby affecting aquatic organisms and potentially entering the human food chain (Guiloski et al., 2015; Kusi et al., 2022; Ranjan et al., 2022; Kock et al., 2023).

This study addresses these gaps by critically evaluating the occurrence patterns, detection methods, environmental behaviour, and risks of azithromycin, dexamethasone, and prednisone. These compounds, as shown in Figure 2.1, were selected for their extensive use during the COVID-19 pandemic for both prophylactic and therapeutic purposes (Hendricks et al., 2021; National Department of Health, South Africa, 2021) and for their continued relevance in public health. Corticosteroids, such as dexamethasone and prednisone, reduced inflammation in the later stages of infection in hospitalised patients requiring oxygen (National Department of Health, South Africa, 2021). Unlike more commonly studied pharmaceuticals, data on these three compounds remain scarce in key environmental matrices, such as water, which act as reservoirs for these pollutants (Sardar et al., 2025).

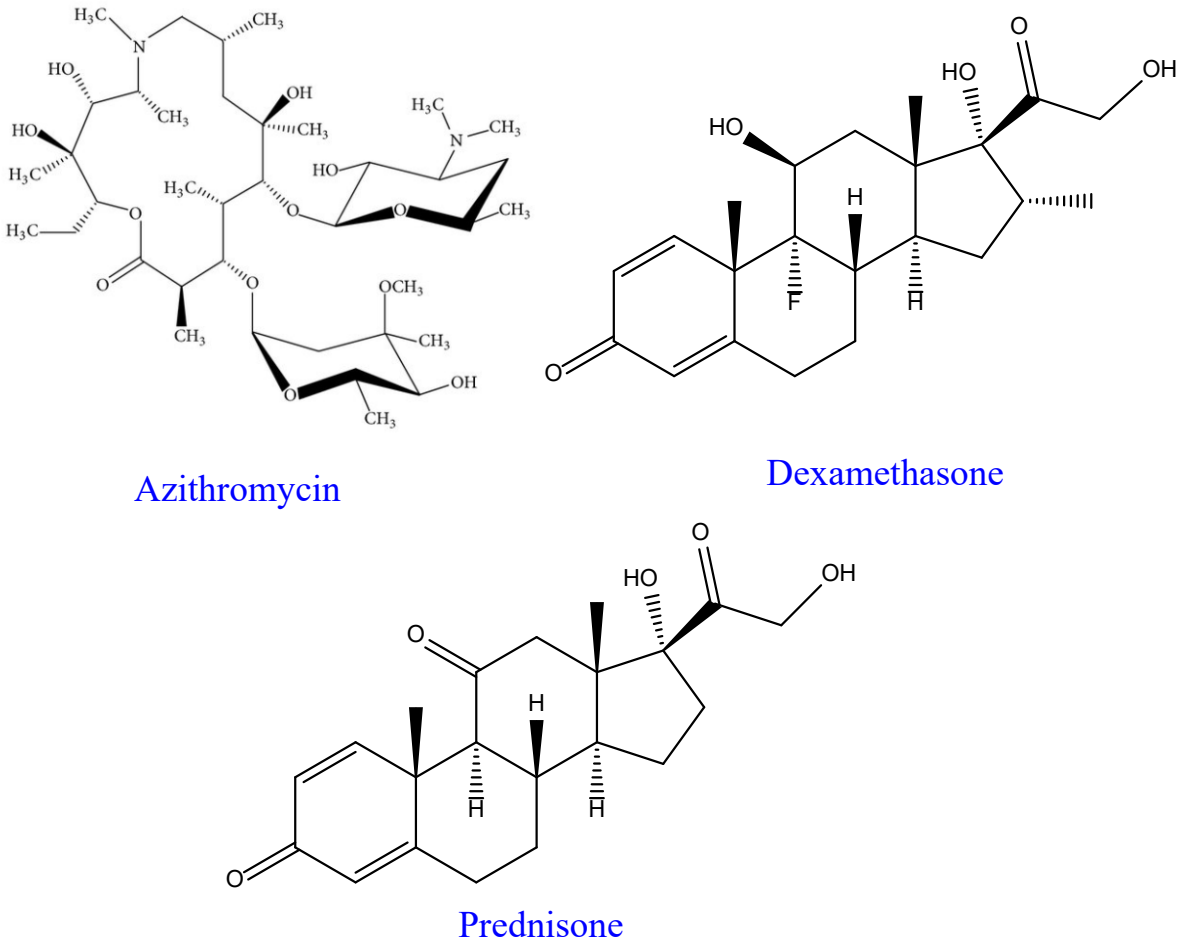

Figure 2.1 Chemical structures of selected pharmaceuticals of the study

The novelty of this study lies in its focused synthesis of available data on these understudied but widely used pharmaceuticals, identification of emerging detection trends, analysis of environmental and

toxicological risks, and highlighting of key knowledge gaps. In doing so, the article contributes to developing future research directions, especially concerning risk mitigation and environmental monitoring of emerging pharmaceutical contaminants. Specifically, this review includes a consolidated, systematic review of the literature on azithromycin, dexamethasone, and prednisone in aquatic systems. It also identifies their primary sources, environmental pathways, and transformation processes affecting these compounds. Additionally, it evaluates various detection techniques, occurrence levels, and associated human and ecological health risks for the target compounds.

2.2 Methodology

This review employed a narrative-based literature synthesis approach to analyse the environmental occurrence, detection techniques, and ecological and human health risks associated with the selected pharmaceuticals in aquatic ecosystems. A systematic search for relevant literature was conducted across multiple scientific databases, including Scopus, Web of Science, PubMed, ScienceDirect, and Google Scholar, with a focus on the last decade. The search strategy combined relevant keywords and Boolean operators, such as “pharmaceutical residues”, “azithromycin”, “dexamethasone”, “prednisone”, “aquatic environment”, “bioaccumulation”, “toxicological risk”, “detection methods”, and “environmental monitoring”. To ensure the inclusion of high-quality and relevant literature, specific inclusion and exclusion criteria were applied. Inclusion criteria comprised studies published in English; peer-reviewed original research articles, systematic reviews, or meta-analyses; studies addressing the environmental occurrence, detection, transformation, or ecotoxicological effects of the selected compounds; and research conducted in aqueous, marine, or estuarine environments. Exclusion criteria involved articles focused on clinical or therapeutic aspects without environmental relevance and studies lacking methodological rigour. Following abstract and full-text screening based on the above criteria, over a hundred articles were selected for this review article. This methodology ensured that the review presents a balanced, evidence-based discussion of the state of knowledge regarding the environmental presence, detection, and impacts of these emerging pharmaceuticals of concern.

2.3 Contaminants of emerging concern

Contaminants of emerging concern (CECs) are natural or synthetic chemicals increasingly detected in the environment, but not routinely monitored or regulated (Ali et al., 2022). Historically, limitations in analytical sensitivity hindered their detection. However, advancements in analytical techniques, such as liquid chromatography mass spectrometry (LC−MS), have significantly improved the ability to detect and quantify these contaminants even at ultra-trace levels in various environmental matrices (Tang et al., 2019; Kuskopf et al., 2020; Wang et al., 2024). These improvements have uncovered the widespread presence of compounds once considered negligible, redefining the boundaries of what constitutes a “pollutant”.

CECs include pharmaceuticals and personal care products (PPCPs), pesticides, flame retardants, and newer classes like nanomaterials (Kuskopf et al., 2020; Mhuka et al., 2020; Selwe et al., 2022; El-Kalliny et al., 2023). Their classification based on origin, spatial distribution, and persistence reflects the complexity of their environmental dynamics (Goldscheider, 2010; Mathew et al., 2017). Their detection in diverse environmental compartments, from aqueous matrix to biota, raises concern due to their

bioactive nature, persistence, and ability to disrupt endocrine and neurological functions (Barrios-Estrada et al., 2018; Khan et al., 2022; OECD, 2023). The pharmaceuticals azithromycin, dexamethasone, and prednisone are particularly interesting in this review, as they were widely used during the COVID-19 pandemic. Unlike legacy pollutants, these compounds reflect contemporary usage patterns, but their environmental implications remain poorly understood. This review aims to fill that gap by linking global occurrences, risk profiles, and detection trends for these three pharmaceuticals, expanding the scope of CEC discussions to include pandemic-era pharmaceutical pollution.

2.4 Pharmaceuticals as emerging contaminants

Pharmaceuticals are used worldwide to treat human and animal health. Even though these PCs are used to save lives, they have emerged as a unique group of CECs because of their chronic and acute effects on aquatic organisms and human health, as well as a significant contributor to water pollution in aquatic ecosystems (Patel et al., 2019; Khan et al., 2020b; Ngqwala and Muchesa, 2020; Wilkinson et al., 2022). Evidence from research studies has proven that the presence of PCs in water may disrupt biological processes in non-target lower organisms upon exposure, as well as posing a potential risk to biotic components, leading to disruption of ecosystem functions and trophic interactions in aquatic organisms (Khan et al., 2020a; Gwenzi et al., 2022). These PCs are the most persistent group of contaminants in aquatic ecosystems, and as such, they may result in ecotoxicological effects on aquatic organisms (Ngqwala and Muchesa, 2020; Okeke et al., 2022).

The occurrence of PCs in aqueous ecosystems has been widely documented. It is known to result in their uptake and accumulation in aquatic organisms, posing direct and indirect risks across trophic levels. Studies have reported the bioaccumulation of PCs in various fish tissues, including liver, gills, muscles, and blood plasma, demonstrating their persistence in biological systems (Popek, 2018; Nkoom et al., 2020; Carter et al., 2024). Such accumulation not only threatens the health of aquatic organisms but also poses risks to humans through dietary exposure, particularly in communities reliant on contaminated water bodies for fish consumption (Kayode-Afolayan et al., 2022; Pinto et al., 2022; Eapen et al., 2024). Antibiotics such as azithromycin are of particular concern due to their role in promoting antimicrobial resistance in aquatic environments. This resistance can transfer horizontally among bacterial species, transforming aquatic systems into reservoirs of resistance genes (Ngqwala and Muchesa, 2020). This phenomenon has been observed globally, including in river sediments in Asia and Africa, where resistant genes persist even in areas with minimal direct pharmaceutical input, suggesting long-term ecological memory and exposure (Gwenzi et al., 2022).

Additionally, endocrine-disrupting PCs such as dexamethasone have been shown to alter fish behaviour, reproductive physiology, and hormone regulation (Guiloski et al., 2015; Adeel et al., 2017). For example, exposure to corticosteroids can interfere with vitellogenin synthesis and gonadal development, leading to reproductive toxicity in fish populations (Solé et al., 2003; Islam et al., 2024). In humans, prolonged exposure, even at low concentrations, has been linked to hormonal imbalances and elevated risks of breast and prostate cancer (Lacouture et al., 2022; Al-Shami et al., 2023). While surface waters serve as transport pathways for these compounds, river sediments function as long-term sinks, storing PCs and their transformation products and releasing them gradually under certain environmental conditions (Khan et al., 2020a; Zhang et al., 2023). This sediment-water interaction

complicates risk assessments, as it creates secondary exposure routes for benthic organisms and bioaccumulation through sediment-dwelling species (Bowman et al., 2002; Zhou and Broodbank, 2014). Despite growing awareness of these issues, knowledge gaps persist, particularly regarding the combined effects of pharmaceutical mixtures, their metabolites, and long-term sublethal exposures. This highlights the need for expanded sediment monitoring, bioaccumulation modelling, and the application of adverse outcome pathways in ecotoxicological risk frameworks.

Pharmaceutical residues have been extended to their parent compounds, metabolites, and transformation products (Quesada et al., 2019). The major source of these compounds has been documented as wastewater treatment plants, which are significant point sources in aquatic ecosystems (Samal et al., 2022; Munzhelele et al., 2024). However, other point sources exist, such as terrestrial runoff from livestock farms, hospitals, and pharmaceutical industrial areas (Ngqwala and Muchesa, 2020). A study conducted by Mheidli et al. (2022) indicated that between 2008 and 2022, about 210 different PCs have been detected in rivers in the Middle East and North Africa. From these matrices, identified concentrations of PCs were up to 66 400 ng/ℓ. Ecotoxicity tests revealed that 39 of the pharmaceuticals tested in these rivers posed a risk to aquatic organisms, with six of the PCs revealing an extremely high-risk quotient (> 1 000), indicating high toxicity to aquatic organisms. However, eight of the PCs revealed a possible human health risk. Hormones showed the highest risk to human health, with an antibiotic revealing the highest risk of resistance, with a risk quotient of 133 (Mheidli et al., 2022).

In another study conducted in Malaysia, some antibiotics were detected in three rivers, namely the Lui, Gombak, and Selangor rivers (Praveena et al., 2018). An antibiotic was detected at all sampling sites, with the highest concentration of 299.88 ng/ℓ. The human health risk assessment indicated a low risk of contamination by these pollutants, whereas the ecological health risk revealed a moderate risk of contamination (Praveena et al., 2018). In the Eastern Cape province of South Africa, PCs have also been detected in surface waters reaching 11,800 ng/ℓ (Vumazonke et al., 2020). Moreover, different studies in KwaZulu-Natal province in South Africa have reported the occurrence of PCs along the Umgeni River (Agunbiade and Moodley, 2014; Matongo et al., 2015; Gumbi et al., 2017; Rimayi et al., 2018), as well as in the coastal areas of the city of Durban (Sigonya et al., 2023). Similar studies in KwaZulu-Natal measured different pharmaceuticals in surface water and dams along the Umgeni River, which are used for water supply (Ebele et al., 2017). These pharmaceuticals in surface water pose a severe threat to public health and the environment due to their potential to cause physiological effects in humans, even at very low concentrations. Generally, PCs have been detected consistently in the environment, as reported in the literature surveyed in this study. Their occurrence has been investigated and detected worldwide; their toxic nature, as established by the literature search, is of concern. Due to the adverse effects they pose to the ecosystem, urgent efforts must be made to continuously monitor their levels and implement remediation strategies to eliminate them from the environment.

2.5 Sources of pharmaceutical compounds and pathways into aquatic ecosystems

There are several main ways that PCs might get into aquatic systems, as shown in Figure 2.2: one such is via inefficient treatment plants and human excretion (Malmqvist et al., 2023). The residues of pharmaceuticals undergo partial metabolism after ingestion, with the unmetabolised parts being eliminated through urine and faeces. These leftovers make their way into sewage systems and then

WWTPs. Nevertheless, these substances are frequently released into surface waters since traditional WWTPs are ill-equipped to eliminate them (Malmqvist et al., 2023). Another entry route of PCs into the aqueous ecosystem is through the improper disposal of unused medications (Omotola et al., 2022). These compounds are introduced into wastewater systems when unused or expired medications are flushed down sinks or toilets because treatment facilities might not completely remove all PCs; this adds to environmental contamination (Omotola et al., 2022). Agricultural practices and runoff are another source of pharmaceutical contamination, as PCs such as antibiotics are administered to livestock in production and eventually end up in manure. Consequently, releasing this manure can affect aquatic ecosystems when used as fertiliser because it can seep into the soil and then infiltrate adjacent water bodies through runoff (Mosharaf et al., 2024). Aquatic ecosystems can get contaminated by the direct entry of PCs from these pollutants into nearby waters.

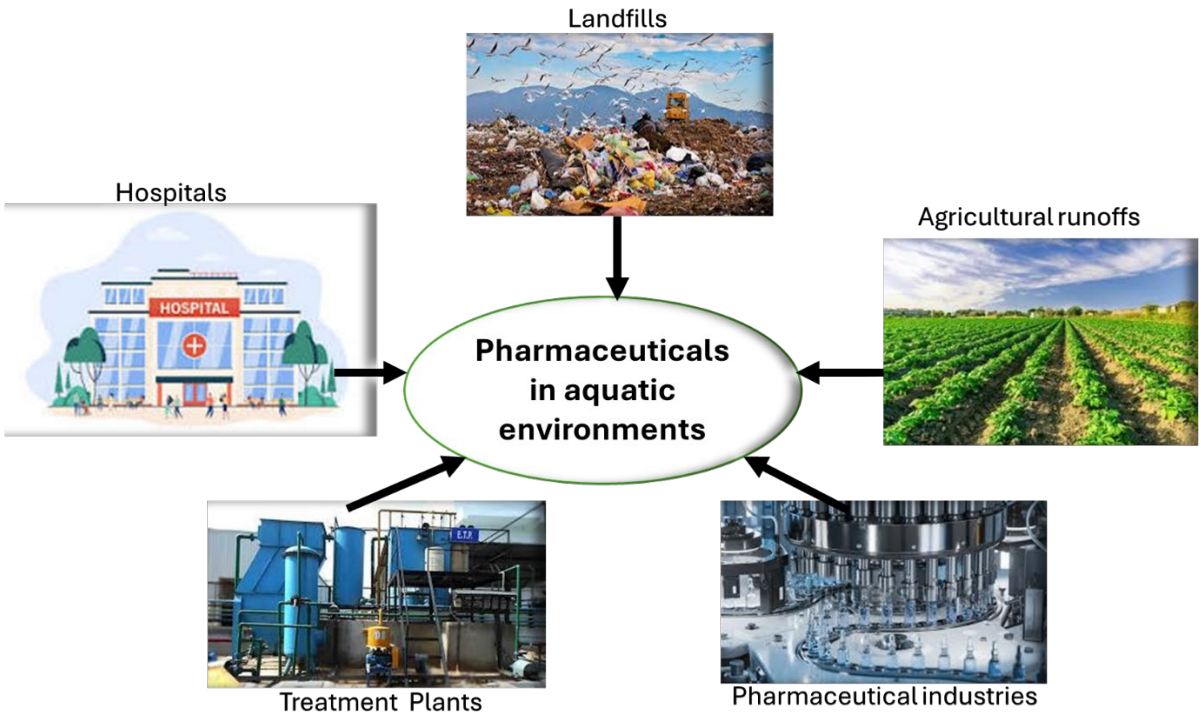

Figure 2.2 Pathways of pharmaceuticals in the aquatic environments

Furthermore, wastewater discharges from pharmaceutical manufacturing facilities may transfer PCs into aquatic bodies. Although this source is regarded as minimal globally in comparison to excretion, manufacturing sites may have local hotspots with high levels of contamination (Wang et al., 2022). A potential source of pharmaceutical contamination of the aquatic ecosystem is the dumping of PCs in landfills; they may also seep into nearby surface and groundwater. These compounds may break down in rainwater that percolates through landfills and eventually reaches aquatic ecosystems (Lu et al., 2016; Yu et al., 2024). These pathways highlight how PCs contaminate aquatic ecosystems, raising questions about their environmental and human health effects.

2.6 Detection of pharmaceutical residues in aquatic ecosystems

Various methods have been adopted to effectively detect PC residues in aquatic environments. Fundamentally, the instrumental technique precedes the detection of PCs. An appropriate analytical method development must first be carried out on neat standards of PCs of interest before sample collection commences. Besides, physicochemical characteristics such as salinity, dissolved organic carbon, total organic carbon, dissolved oxygen, pH, temperature, and electrical conductivity, to mention a few, must be determined on the sample matrices before assessing pharmaceuticals in the environmental matrix. This sometimes assists in understanding the presence of certain compounds in the sample matrix. For instance, Omotola and Olatunji (2020) reported that the low levels of PCs detected in their study were perhaps attributed to the dilution effects elicited by offshore mixing from pristine water. This, in turn, was attributed to the significant impact on the water's salinity.

Similarly, high salinity has been reportedly linked with low levels of polyfluorinated alkyl substances in water (Ohoro et al., 2024). Moreover, Rahman et al. (2021) studied the seasonal variations of water quality of the Turag River to investigate the extent of water pollution using the physicochemical properties of the water, among which are temperature, pH, electrical conductivity, dissolved oxygen concentration, chloride ion concentration, and total alkalinity. Furthermore, the occurrence pattern of polyfluorinated alkyl substances was recently linked to the physicochemical properties of specific environmental matrices (Ohoro et al., 2024). Therefore, evaluating the samples' physicochemical nature before instrumental analysis is expedient.

After sample collection and pretreatment, analyte extraction from the sample matrices follows. It is essential to state that extraction procedures depend solely on the sample matrix. For example, unlike sediments, which are usually mixed with extracting reagents that can efficiently desorb the pharmaceuticals (Gumbi et al., 2017). Water samples are typically extracted without going through this step. Generally, the water samples might be filtered, especially if they are wastewater or dirty surface water samples, using appropriate filter papers (Omotola and Olatunji, 2020; Omotola et al., 2022). Once filtration is complete, a solid-phase extraction (SPE) is performed directly on the filtered water sample under suitable extraction conditions. Finally, the eluate is detected by a chromatograph or a liquid chromatograph coupled to a mass spectrometer. Sometimes, the solution is acidified before the SPE procedure to ensure acceptable analyte recovery (Gumbi et al., 2017; Amos Sibeko et al., 2019). Moreover, for the preservation of organisms' lives, this is not appropriate, given the number of organisms (fish and others) that would be used for analytical method development. Hence, researchers mostly conduct ecotoxicity studies in the laboratory and use lower organisms to assess the risks posed by pharmaceuticals (Mezzelani et al., 2016; Mezzelani et al., 2018a; Mezzelani et al., 2018b; Omotola et al., 2021). Worth noting are the molar masses (g/mol) and mass-to-charge (m/z) ratios of analytes of interest for instrumental analysis, such as spectrometric techniques. The information on the three pharmaceuticals considered in this review is presented in Table 2.1.

Table 2.1 Specific characteristics of selected pharmaceutical compounds in this study

Pharmaceutical Compound	Molecular formula	Molecular weight (g/mol)	$[M+H]^+$	*m/z* of major fragments	Log P	λmax (nm)	Reference
Dexamethasone	$C_{22}H_{29}FO_5$	392.5	393	147, 237, 337, 355, 373	1.83	240	(Haneef et al., 2013; StressMarq Biosciences, 2023)
Prednisone	$C_{21}H_{26}O_5$	358.4	359	34, 147, 267, 323, 341	1.46	240	(Haneef et al., 2013; National Centre for Biotechnology Information, 2023a)
Azithromycin	$C_{38}H_{72}N_2O_{12}$	749.0	749.5	The addition of two transitions 749.5 → 591.0 *m/z* ad 749.5 → 158.0 *m/z* was applied to monitor azithromycin	–	–	(Filist et al., 2014; Wu et al., 2020; National Center for Biotechnology Information, 2023b)

Note: λmax means wavelength of maximum absorption; Log P means octanol-water partition coefficient

Generally, PCs are detected first using an appropriate analytical method designed to separate and detect the analytes of interest. Until this step is achieved, collecting samples for analysis is usually unsuitable, as prolonged storage may compromise their integrity. Subsequently, the collected samples are extracted to isolate targeted analytes, which are then analysed using the developed analytical method, an instrumental technique. Among the instrumental techniques employed for the detection of PCs are the chromatographic methods, including liquid and gas chromatography (LC and GC) coupled to mass spectrometry (MS) or fluorescence detectors (FD), such as GC−MS/MS, LC−MS/MS, HPLC−MS/MS, UHPLC−MS/MS, and HPLC−FD (Caban et al., 2016; Edwigetchadji et al., 2018; Guettai et al., 2024). Although using gas chromatography to analyse pharmaceutical residues may require some derivatisation, the method suffices for this subject (Suleman et al., 2015). Before instrumental detection, SPE is the most employed pre-treatment method for analysing PCs and other emerging micro-pollutants in aqueous solutions (Gorito et al., 2022; Guettai et al., 2024). SPE coupled with enzyme-linked immunosorbent assay (ELISA), followed by spectrophotometric analysis, has also effectively detected environmental PCs (Fang et al., 2016; Praveena et al., 2019). Moreover, the recent use of sensors for detecting PCs has gained researchers' attention (Letsoalo et al., 2023). However, as previously stated, this technique has not been sufficiently proven to replace the chromatographic-spectrometric techniques. Like these techniques, the use of sensors has not been reported to detect

50 or more pharmaceuticals simultaneously. This limitation may soon be a research focus for stakeholders in the environmental monitoring of pollutants.

2.7 Trends in the methods of detection and occurrence patterns of selected pharmaceuticals

2.7.1 Prednisone

Generally, prednisone belongs to the family of glucocorticoids and specifically to a class of medication known as corticosteroids (Flower, 2009; De Oliveira et al., 2021). It is one of the most used synthetic corticosteroids for patients with autoimmune diseases (De Oliveira et al., 2021). One of its metabolites is 20β-hydroxyprednisone, which is a hydroxylated metabolite (Garg and Jusko, 1991). It is worth mentioning that prednisone is a prodrug to prednisolone; it is converted to the active form, prednisolone, in the liver after ingestion (Flower, 2009; Papich, 2016). Prednisone detection can be traced back over three decades, when it was detected in urine samples by extraction with two cycles of ethyl acetate (6 mℓ) using an inert, high surface area diatomaceous earth extraction column (Garg and Jusko, 1991). After separating the organic layer, the eluate was washed with sodium hydroxide and dried with anhydrous sodium sulphate. The mobile phase, consisting of methylene chloride, glacial acetic acid, and methanol (91.3:7.5:1.2), was used to reconstitute the residue. The reconstituted residue was then analysed using a high-performance liquid chromatograph (Garg and Jusko, 1991).

Aside from the studies reported by Teng and Benet (1989) and Garg and Jusko (1991) over three decades ago, research on the occurrence patterns of prednisone in aqueous environmental matrices was relatively scarce. One of the few studies conducted on the occurrence pattern of prednisone was reported by Gong et al. (2019). Prednisone was detected in at least 50% of the investigated surface water in the Pearl River Delta, South China, at a 50% occurrence. They carried out sample pretreatment by filtering the surface water through pre-combusted glass fibre filters. The filtrates were stored in the dark at 4 °C until SPE was performed.

Furthermore, the SPE cartridges were conditioned with 6 mL each of ethyl acetate and acetonitrile, followed by 10 mℓ of water (high-performance liquid chromatography [HPLC] grade). The samples were loaded onto the cartridges and, after extraction, washed with water and acetonitrile (9:1). After the washing step, the PC was eluted with 6 mL each of a 1:1 mixture of ethyl acetate and acetonitrile and ethyl acetate. The extracts were concentrated using a rotary evaporator to less than 2 mℓ , dried under a gentle nitrogen stream, and then redissolved in 0.5 mL of methanol for analytical instrumentation. An ultra-high performance liquid chromatographic system, coupled with a triple tandem quadrupole mass spectrometer with an electrospray ionisation ultra-high performance liquid chromatography−electrospray ionisation tandem mass spectrometry (UHPLC–(ESI)MS/MS) source, was used for the detection of the analytes. This method, used by Gong et al. (2019), was reported to be based on the UHPLC–(ESI)MS/MS method employed by Herrero et al. (2012). Herrero and team members used the instrument with a degasser, an automatic injector, a column oven, and a binary pump. A chromatographic column (50 mm × 4.6 mm, 1.8 µm) was used to separate the target analytes via gradient elution. Water/acetonitrile (78:22) and methanol/acetonitrile (78:22) with formic acid (0.1%) were the solvents A and B, respectively. Starting at 0.8% B, the gradient was increased to 5% in five

minutes, 15% in 6.5 minutes, 50% in half a minute, maintained constant for 1.5 minutes, increased to 99.9% in half a minute, maintained constant for half a minute, and finally decreased to 0.8% B in half a minute. The oven temperature was maintained at 50 °C. An injection volume of 50 µℓ was employed, and the flow rate was 1 mℓ/min.

Moreover, this method had been previously employed by Wu et al. (2019). Recently, prednisone was among the glucocorticoid compounds detected in wastewater effluents in the Netherlands via effect-directed analysis (Jonkers et al., 2023). The effect-directed analysis combines high-resolution fractionated bioassay testing with nontarget and suspect screening of high-resolution mass spectrometry data (Jonkers et al., 2023). Furthermore, four different authors recently reported the presence of prednisone in different aquatic niches in China. The concentrations of prednisone reported were 5.13 ng/ℓ in tributary water (Xu et al., 2025), 29.8 ng/ℓ in surface water from a drinking water source area (Wu et al., 2023), 26.5 ng/ℓ in the surface water of an agricultural region of Guangzhou City (Lin et al., 2024) and 78.3 ng/ℓ in water from the Yangtze River (Zhang et al., 2024). Of concern is the relatively high concentration of 8105 ng/ℓ of prednisone detected in a Brazilian drinking water treatment plant (Reis et al., 2019). This medium should be continuously monitored, and efforts should be made to remediate the environment for the safe health of the Brazilian populace. From the literature reviewed, it can be concluded that studies on the environmental monitoring of prednisone in aquatic environments are still evolving, with few or no studies available for African and European aquatic ecosystems.

2.7.2 Dexamethasone

Dexamethasone is a long-acting glucocorticoid with the highest efficacy, having a biological half-life of 1.5 to 2.25 days (Yasir et al., 2023). During the COVID-19 pandemic, there was speculation that this PC could lower patients' mortality while being hospitalised (Alessi et al., 2020). Hence, there is interest in studying its occurrence and detection pattern in the environment, as they are becoming increasingly widespread. A while ago, Suzuki et al. (2015) investigated the presence of dexamethasone in effluents from Japanese sewage treatment plants. According to the report by Suzuki and colleagues, the treatment plants were unable to effectively remove dexamethasone residues. Therefore, researchers should aim to develop a technique (as part of treatment processes in treatment plants) that effectively eliminates diverse bioactive compounds before they are discharged into the environment. Gong et al. (2019) reported a 54% occurrence pattern of dexamethasone within 50% of the investigated surface water within the Pearl River Delta in South China. This method adopted for dexamethasone was also used to detect prednisone using liquid chromatography-tandem mass spectrometry (LC–tandem MS) simultaneously.

Surprisingly, dexamethasone was reported to be present in Malaysian drinking water in Putrajaya at 0.37 ng/ℓ (Praveena et al., 2019). Also, in Malaysian estuarine water, 1.51 ng/ℓ of dexamethasone was detected by Ismail et al. (2019). Aside from the Lima River estuary and Douro River estuary in Portugal, where dexamethasone was monitored but not quantified (Gorito et al., 2024), other studies found in the literature were conducted in China. For instance, Lin and colleagues reported 22.5 ng/ℓ of dexamethasone in the surface water of an agricultural area in Guangzhou City, China (Lin et al., 2024). Also, most recently, 1.72 ng/ℓ of dexamethasone was reported in the tributary water of Guangzhou City in China (Xu et al., 2025). Other reports on the presence of dexamethasone and the other PCs under

study in the aqueous environments are presented in Table 2.2. Based on the surveyed literature, it is safe to conclude that the occurrence and detection of dexamethasone were relatively rare. Hence, future work should investigate this aspect analytically.

2.7.3 Azithromycin

One of the most often-used antibiotics is azithromycin (Guo et al., 2021). Azithromycin is a broad-spectrum antibiotic, a 15-membered macrolide, and a semi-synthetic derivative of erythromycin with a methyl-substituted nitrogen atom of the lactone ring at the 9-α position (Guo et al., 2021). It is the third most widely consumed group of antibiotics (Klein et al., 2018), and it serves as a therapeutic agent to treat illnesses caused by specific bacteria. Continuous utilisation of antibiotics subsequently results in their presence in the water ecosystem, consequently leading to drug resistance (Ahmed et al., 2015). Thus, antibiotic prescriptions should not be taken lightly. Quite a few studies have examined the patterns of azithromycin use. Recently, Scaccia et al. (2024) reported a relatively high concentration of azithromycin, ranging from 1 140 ng/ℓ to 1 210 ng/ℓ, in effluents from Cameroon using UHPLC−MS/MS for detection. Numerous studies have revealed the effectiveness of this technique for the efficient detection of residues of PCs over the past decades till the present (Ebrahimzadeh et al., 2021; Li et al., 2021; Omotola et al., 2022). According to Table 2.2, all the published articles presented on the detection of azithromycin showed that the instrumental technique employed for this purpose was LC-tandem MS. Next to the relatively high levels of azithromycin reported by Scaccia and team members is the 672 ng/ℓ of azithromycin detected in another effluent in Spain (Solaun et al., 2022). Azithromycin levels in the Iranian coastal water of the south Caspian Sea reached up to 85.6 ng/ℓ (Manbohi et al., 2024), which is similar to those recently observed in Indian surface water (87 ng/ℓ), reported by Brauns et al. (2024). Conversely, in Portugal, residues of azithromycin were found in Douro estuary water at a lower level (14 ng/ℓ) than those mentioned earlier. From these findings, it could be inferred that the Portuguese treatment plants may be more effective, as trace amounts of azithromycin were detected in selected aqueous environments. Details of the available reports on azithromycin are seen in Table 2.2. From the few published works on azithromycin detection, it can be concluded that little work has been done on the occurrence pattern of this compound in the global environment, an area of focus for future studies.

As noted in the surveyed literature, the three pharmaceuticals have not been detected simultaneously. Meanwhile, previously developed analytical methods might not suffice, as they target different classes of pharmaceuticals. This makes detecting pharmaceuticals challenging, as there are still not enough suitable methods to detect most PCs simultaneously. Researchers must, therefore, investigate a compromise method that could serve this purpose in future work.

Table 2.2 Occurrence and detection of selected PCs, as reported in the literature

Pharmaceutical Compound	Country of Detection	Sample Matrix	Method of Detection	Amount Detected, as Reported	References
Dexamethasone	-	Pond water and sewage water	molecularly imprinted electrochemical aptasensor	10^{-13} M to 10^{-5} M with a detection limit as low as 1.79×10^{-14} M	(Sun et al., 2024)
Dexamethasone	Portugal	Lima River estuary and Douro River estuary	SPE−LC−MS/MS	Not quantified	(Gorito et al., 2024)
Dexamethasone	China	Surface water (agricultural area of Guangzhou City, China)	SPE−LC−MS/MS	22.5 ng/ℓ	(Lin et al., 2024)
Dexamethasone	Malaysia (Putrajaya)	Drinking water	SPE−LCELISA-Spectrophotometric detection	0.37 ng/ℓ	(Praveena et al., 2019)
Dexamethasone	Malysia (Pulau Kukup, Johor)	Estuarine water	SPE−LC−MS/MS	<1.00 - 1.51 ng/ℓ	(Ismail et al., 2019)
Dexamethasone	China (Pearl River Delta, South China)	Surface water	SPE−LCUHPLC−MS/MS	<0.3 -3.5 ng/ℓ	(Gong et al., 2019)
Dexamethasone	China (Guangzhou city)	Surface water	SPE−LC−MS/MS	22.5 ng/ℓ	(Lin et al., 2024)
Dexamethasone	China (Guangzhou city)	Tributary water	SPE−LC−MS/MS	1.72 ng/ℓ	(Xu et al., 2025)

Pharmaceutical Compound	Country of Detection	Sample Matrix	Method of Detection	Amount Detected, as Reported	References
Prednisone	China	Surface water (Agricultural area of Guangzhou City, China)	SPE−LC−MS/MS	26.5 ng/ℓ	(Lin et al., 2024)
Prednisone	China (Pearl River Delta, South China)	Surface water	SPE−UHPLC−MS/MS	<0.2 – 2.3 ng/ℓ	(Gong et al., 2019)
Prednisone	China (Yangtze River)	Surface water	SPE−LCHPLC−HRMS/MS	78.3 ng/ℓ	(Zhang et al., 2024)
Prednisone	Brazil	Surface water (used as a drinking water source)	SPE−LCHPLC−micrOTOF−QII MS	2800 ng/ℓ	(Santos et al., 2020)
Prednisone	China (Yangtze River)	Surface water (from the drinking water source area)	SPE−LCHPLC−quadrupole orbitrap MS	29.8 ng/ℓ	(Wu et al., 2023)
Prednisone	Brazil	Drinking Water Treatment Plants	SPE−LCmicrOTOF−QII MS	8105 ng/ℓ	(Reis et al., 2019)
Prednisone	China (Guangzhou city)	Tributary water	SPE−LC−MS/MS	5.13 ng/ℓ	(Xu et al., 2025)
Azithromycin	Spain	effluent	SPE−LC−MS/MS	672 ng/ℓ	(Solaun et al., 2022)
Azithromycin	Iran	Seawater	SPE−LCHPLC–MS/MS	7 ng/ℓ	(Mirzaie et al., 2022)
Azithromycin	Brazil	Effluent	UHPLC−MS/MS	0.32–7.37 µg/ℓ	(Scaccia et al., 2024)

Pharmaceutical Compound	Country of Detection	Sample Matrix	Method of Detection	Amount Detected, as Reported	References
Azithromycin	Cameroon	Effluent	UHPLC−MS/MS	1.14 to 1.21 µg/ℓ	(Scaccia et al., 2024)
Azithromycin	China (Estuary of Rongjiang River)	Estuary water	SPE–UPLC–MS/MS	4.30 ng/ℓ	(Ohore et al., 2023)
Azithromycin	India	Effluent	SPE–LC–MS/MS	2.7 ng/ℓ	(Arun et al., 2022)
Azithromycin	Portugal	Douro Estuary water	SPE–LC–MS/MS	14 ng/ℓ	(Gorito et al., 2024)
Azithromycin	Iran	Coastal water of the south Caspian Sea	SPE–LC–MS/MS	85.6 ng/ℓ	(Manbohi et al., 2024)
Azithromycin	India	Surface water	SPE−LC(Q-TOF) LC/MS	0.087 µg/ℓ	(Brauns et al., 2024)
Azithromycin	Italy	Lagoon water (Lagoon of Venice)	LC–MS/MS	Not quantified	(Pizzini et al., 2024)

2.8 Fate of the selected PCs in aquatic ecosystems

Pharmaceutical residues are present in the environment as parent compounds or metabolites. A parent pharmaceutical may have one or more metabolites until it can no longer be broken down into another product. The final product of a parent compound could help determine the fate of that compound. Metabolites are often formed through decomposition or degradation processes induced by light and heat. For instance, light exposure to aqueous dexamethasone and prednisolone suspensions results in partial phototransformation (DellaGreca et al., 2004). Specifically, 60% of prednisone was converted into 7 photoproducts after 8 hours of exposure to a solar simulator. Similarly, dexamethasone was also transformed after being irradiated by a solar simulator for eight hours (DellaGreca et al., 2004). Some of the photoproducts of prednisone and dexamethasone, identified by Dellagreca et al. (2003), are illustrated in Figure 2.3. These photoproducts, typically isolated using chromatographic techniques, are identified by spectroscopic methods. These transformation products are sometimes more toxic than their parent compounds, as reported by Dellagreca et al. (2003), who found no significant adverse impact on *Thamnocephalus platyurus* after 24-hour exposure to prednisone. Still, the transformation product exerted a lethal impact within the same period. Conversely, azithromycin has been reported not to be metabolised (Liu et al., 2023). Liu and colleagues observed that azithromycin entered the surface water without being metabolised. However, this does not entirely rule out its metabolism, suggesting the need for further research. This remains a critical focus for future studies.

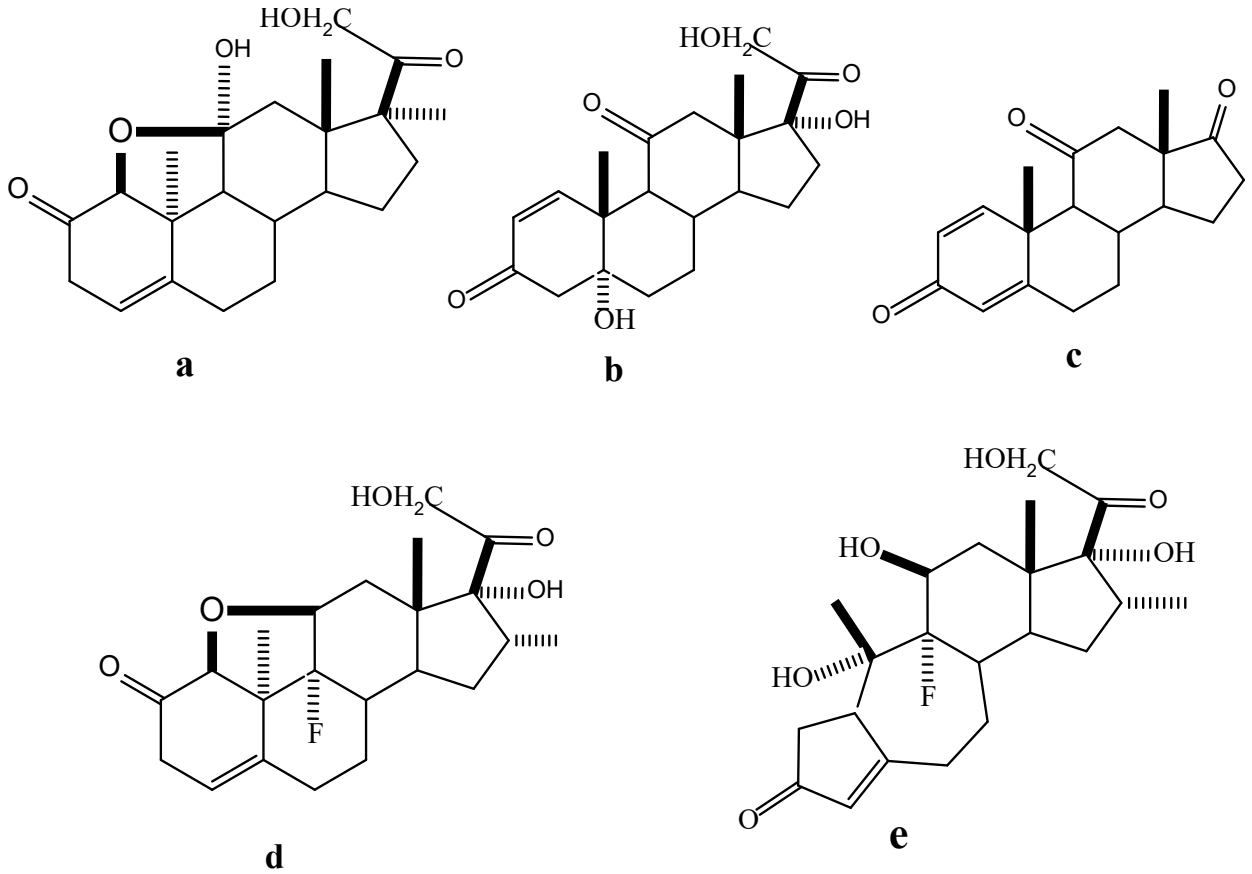

Figure 2.3 Some metabolites/photoproducts of prednisone (a to c) and dexamethasone (d and e)

2.9 Ecotoxicology of PCs of study on aquatic ecosystems and humans

The release of pharmaceuticals into the environment is of utmost concern, as they can pose ecological risks, even at low concentrations in the ng/ℓ range. Generally, the ecotoxicity of pharmaceutical residues is key in assessing the risk these compounds can pose to living organisms. Such an assessment will provide insight into the risk associated with the investigated PCs.

Ecotoxicological studies evaluate the potential risks posed by chemical contaminants to aquatic ecosystems and the effects of such exposure on human health (Li et al., 2019). Different approaches have been used to assess the long-term ecotoxicological risks posed by low concentrations of PCs to aquatic organisms (Oliveira et al., 2015; Kidd et al., 2024). One approach involves exposing sensitive organisms, such as algae, zooplankton, invertebrates, and fish, to different concentrations of PCs in water to determine the acute or chronic effects of these PCs on the test organisms (Oliveira et al., 2015; Kidd et al., 2024). Other approaches involve using biomarkers, which assess specific physiological responses in organisms exposed to PCs in water, including oxidative stress in cells of different test organisms. Moreover, prediction models inform the long-term risk of PCs to aquatic organisms (Kidd et al., 2024). Apart from the above-mentioned approaches, certain risk-based indices have also been applied to assess the ecotoxicity risks posed by PCs to aquatic organisms and human health. The ecological risks posed by mixtures of chemical contaminants to aquatic organisms have also been assessed. This is because, if several PCs with a similar mode of action exist in an aquatic ecosystem, their toxicity will be higher than for a single PC (Kumari and Kumar, 2022). The study by Kumari and Kumar (2022) found that the ecotoxicity of PC mixtures indicated that algae are the most vulnerable species among the three trophic levels. The maximum allowable concentration of a pharmaceutical mixture was found to be 0.53 mg/ℓ.

2.10 Assessment of potential risks of PCs on human health and aquatic ecosystems using risk-based indices

The risk of pharmaceuticals to aquatic life is undoubtedly recognised, particularly over prolonged exposure in water resources. For this reason, several researchers developed risk assessment methods to identify risks and classify them as acute or chronic and as high, medium, or low. Such risk assessment processes provide insight into the lethality of PCs to aquatic life. One of the methods used is the Risk Quotient (RQ) parameter that assesses risk arising from pharmaceuticals in the compartments of the environment, such as soils, water, animals, and plants (Sackey et al., 2024).

Capelli and team members earlier reported that the environmental risk of azithromycin was on the high side after being evaluated by calculating its RQ alongside other PCs by dividing the Predicted No-Effect Concentration (PNEC) by the Measured Environmental Concentration (MEC) (Cappelli et al., 2022). The RQ of PC can be calculated as

$$RQ = \frac{MEC\ 95^{th}\text{perc}}{PNEC}$$

where the $MEC\ 95^{th}\text{perc}$ is the 95th percentile of all the surface water measured concentrations, stated by Capelli and co-authors.

According to Villa et al. (2020), inferences to the risk posed by a specific contaminant are made from the RQ values following the criterion: RQ > 10, high risk; 1 < R < 10, medium risk; 0.1 < RQ < 1, low risk; RQ < 0.1,

negligible risk. Worthy of note is the importance of PNEC data when assessing the risk posed by a contaminant (Gwenzi et al., 2022). When PNEC values are used to calculate RQs, they are sometimes extracted from an ecotoxicology database. One such database is the NORMAN Ecotoxicology Database (Cappelli et al., 2022). Moreover, the mixture toxicity of some PCs has been assessed using the RQ value, which revealed that algae appeared to be the most sensitive species to targeted PCs, followed by daphnia and fish (Kumari and Kumar, 2022).

Another way of assessing risk is the multiple-level ecological risk assessment (MLERA), which was employed to evaluate the possible ecological risk for selected PCs in surface water from Wuhan City's lakes and WWTP-river-estuary system (Chen et al., 2021). This study solely assessed the potential risks associated with aquatic exposure to the target analyte PCs, given the absence of toxicity data for benthos and risk assessment techniques for sediment. The RQs for glucocorticoids were all less than 0.01 in the summer or fall, suggesting negligible hazards (Chen et al., 2021).

Similarly, the environmental risk assessment of some contaminants of concern, including azithromycin, was conducted in London’s waterways throughout the COVID-19 pandemic. A report showed that azithromycin was one of the PCs with the most severe danger to aquatic life among all those evaluated in freshwaters, using the RQ value obtained (Egli et al., 2023). In addition to assessing the historical effects of the COVID-19 pandemic in near real-time, the study by Egli et al. (2023) provided a baseline for predicting future changes in their occurrence and sources at high spatiotemporal resolution.

Other methods, including the scenario–evaluation-based approach and the exposure-related parameter approach, have also been proven to successfully demonstrate the risks of drugs in environmental compartments on human health (Kumar and Xagoraraki, 2010). Other studies have assessed the ecotoxicological and human health risks from exposure to different PCs based on their treatment efficiencies. However, for these studies, no experimental data were available; instead, the data were estimated using the recommended half maximal effective concentration (EC50) or lethal concentration (LC50) values obtained from the Ecological Structure Activity Relationships Class Program (ECOSAR) database (Kumari and Kumar, 2022). The data estimated the risk posed by toxic substances to aquatic organisms. They were then used to compute the PNEC, which employed an assessment factor based on the lowest effective concentration. Once the PNEC has been obtained from the ECOSAR database, it can be used to quantify the risk quotient, which is used to determine the risks of such chemical substances on aquatic organisms (Kumari and Kumar, 2022).

On the other hand, the human health risk of PCs might be estimated based on consuming contaminated fish species that thrive in PC-contaminated water (Kumari and Kumar, 2022). Thus, a six-step approach is used to estimate the risk of these drugs on human health, which includes hazard assessment, dose-response assessment, exposure assessment, risk estimate, risk characterisation, and management. The human health risk of five repurposed PCs used to treat patients was estimated in a study performed in India, using a proposed six-step approach (hazard identification, dose−response assessment, exposure assessment, risk estimation, risk characterisation, and management) to determine the human health risk from the exposure of these pharmaceuticals (Kumari and Kumar, 2022).

2.11 Assessment of potential risks of pharmaceutical compounds on the aquatic ecosystem

Several researchers have reviewed pharmaceutical sources and pathways in aquatic ecosystems using life cycle assessment. Studies by Gwenzi et al. (2022) and Kumari and Kumar (2022) assessed the environmental

and human health risks of certain PCs on three trophic levels of aquatic organisms – algae, *Daphnia*, and fish – in environmental waterbodies. This was done by using the recommended half maximal effective concentration (EC_{50}) or lethal concentration (LC_{50}) values obtained from the Ecological Structure Activity Relationships Class Program (ECOSAR database) of the U.S. Environmental Protection Agency (2008), which sometimes underestimates toxic effects (Raimondo et al., 2010). Tarazona et al. (2021) also reviewed the environmental impact of COVID-19 therapeutic solutions. Their study was a prospective analysis in which different models and available information were used to predict the risk of different PCs in the environment (Tarazona et al., 2021). Almeida et al. (2023) predicted the ecotoxicological risk of some pharmaceuticals. They found that they portrayed a predicted potential ecotoxicological risk for aquatic organisms and indicated a significant potential for antibiotic resistance (Almeida et al., 2023). Similarly, Desgens-Martin and Keller (2021) modelled the fate and aquatic ecosystem toxicity risk of dexamethasone based on predicted environmental concentrations using the ChemFate model.

Living organisms have been used to assess the potential risk of exposure to different PCs in water. Such an ecotoxicity risk approach uses bioassays, which allow for toxicity measurement upon exposure to contaminated water samples by different indicator organisms, such as fish, algae, invertebrates, and plants (Hassan et al., 2016). Such ecotoxicity risk approaches have proven advantageous over regular approaches that involve the quantification of PCs using sophisticated machines, since they provide rapid responses, and test methodologies are simple, sensitive, and cost-effective (Hassan et al., 2016). For example, Kumari and Kumar (2022) assessed the risk of some PCs on three trophic levels of aquatic organisms – algae, *Daphnia*, and fish – as well as the risk to human health from the consumption of PC-contaminated fish species and found that lopinavir metabolites showed maximum risk to aquatic organisms, while ritonavir indicated a potential risk to human health from the ingestion of contaminated fish. Additionally, the results showed chronic toxicity PNEC values for algae, compared to *Daphnia* and fish, with ribavirin (2.65×10^{5} ug/ℓ) and ritonavir (2.3×10^{-1} ug/ℓ) for algae. The computed risk quotient values also revealed that algae are the most sensitive to PCs, averaging 1.81×10^{4}, followed by *Daphnia* (Average: $1{:}28 \times 10^{4}$) and fish (Average: $1{,}028 \times 10^{3}$). in another study conducted in Portugal, Almeida et al. (2023) predicted the ecotoxicological risk of 22 antimicrobial pharmaceuticals in four different regions, using a risk assessment screening approach based on exposure and hazards in surface water. The study's outcome indicated that specific PCs predicted potential ecotoxicological risks for aquatic organisms and significant potential for antibiotic resistance (Almeida et al., 2023). Furthermore, chronic toxicity of selected PCs in some test species affected reproduction, resulting in decreased fecundity. The fertility and the reproduction rate also decreased from 0.33 mg/ℓ to 0.128 mg/ℓ (de Oliveira et al., 2016). On the other hand, Hayden et al. (2022) assessed the toxicity risk of dexamethasone on *Daphnia*, fish, and algae, and observed a low to moderate risk quotient ($RQ \leq 1$). Although the findings were not critical, the possibility of dexamethasone accumulating in water resources over time cannot be neglected. However, a more comprehensive risk assessment that considers low-level exposure over long periods and the combined risks of pharmaceuticals is needed to address this critical knowledge gap. This will provide an in-depth understanding of the pharmaceuticals interacting with water resources and aquatic life. Moreover, Nieto-Juárez et al. (2021) assessed the environmental risk of azithromycin concentrations in rivers and wastewater effluent in Peru. An $RQ > 1$ was observed, which demonstrated a high environmental risk. This finding showed the persistence of azithromycin in wastewater treatment facilities and the accumulation of trace amounts in rivers in Peru.

While risk assessment studies of pharmaceuticals have generally focused on parent compounds, there is growing concern that their transformation products, such as intermediates and metabolites, may exhibit equal or even greater toxicity to aquatic organisms and humans. These compounds may also persist in the environment and retain biological activity, contributing to cumulative toxic effects. However, risk assessments often overlook these derivatives due to limited toxicological data. Data on the risk assessment of the metabolites of prednisone, dexamethasone, and azithromycin are incredibly scarce. Emphasising these understudied metabolites is crucial, as they may evade detection and regulation while contributing to chronic toxicity. The limited inclusion of metabolites in risk assessments highlights a critical area for future research and regulatory consideration. Expanding toxicity profiling to include transformation products will significantly enhance the predictive accuracy and impact of environmental risk assessments for pharmaceutical contaminants.

2.12 Assessment of potential risks of pharmaceutical compounds on human health

Human health is under threat due to increasing levels of pharmaceutical residues in water resources. A scenario-based evaluation approach was used to provide an understanding of the potential risks arising from environmental exposure of humans to pharmaceuticals. Bercu et al. (2008) and Kumar and Xagoraraki (2010) employed the scenario evaluation-based approach to assess the risk of pharmaceuticals in water resources to human health. The findings from these studies revealed a low risk to human health. Similarly, Cunningham et al. (2009) also observed a low risk to human health from pharmaceuticals in water resources. Therefore, no specific human health risks could be attributed to the findings from these studies. The literature indicates that, in recent years, little research has been conducted on the risks to human health posed by pharmaceuticals from exposure to contaminants in water resources. Two of the research works found were reported in 2022 (Duarte et al., 2022) and in 2024 (Earl et al., 2024) and were done using estimated concentrations of selected PCs in conjunction with plant uptake, together with crop consumption data to estimate daily exposures to the PCs (Earl et al., 2024). Future work is encouraged to consider additional research options to evaluate human health risks, and even a mixture of PCs from different sources could pose a risk to humans. This could be driven primarily by the assumption that the risk is too low to be ignored. However, exposure to low traces over a long period and the combined risks of the pharmaceuticals need to be investigated. Moreover, a study that focuses solely on human health risks from PCs of interest through water resources remains a significant knowledge gap.

Furthermore, a few studies have reported the ecotoxicity of the three compounds of interest reviewed in this study – azithromycin, dexamethasone, and prednisone – as indicated in Table 2.3. It is essential to note that the ecotoxicity of these compounds, particularly prednisone, remains relatively underreported in the literature.

Table 2.3 Ecotoxicity studies of selected pharmaceuticals

Pharmaceutical	Tested organism/bioindicator	Adverse effect	Reference
Prednisone	*Thamnocephalus platyurus*	23% mortality recorded after 48 hours	(Dellagreca et al., 2003)
Azithromycin	*Raphidocelis subcapitata*	Oxidative damage	(Almeida et al., 2021)
Dexamethasone sodium phosphate	Zebrafish embryos	Rise in mortality in the zebrafish embryos during a 96-hour exposure	(Di Paola et al., 2022)

2.13 Assessment of potential risks of PCs on human health and aquatic ecosystems using biomarkers

Biomarkers could provide significant evidence of the risks associated with pharmaceutical exposure from water resources. This is because biomarkers demonstrate the effects of harmful substances, such as PCs, on measurable biological responses (Yusuf et al., 2021). However, biomarkers are limited to assessing the risk of PCs on aquatic ecosystems and environmental systems (Lomartire et al., 2021). An integrated biomarker response strengthens the quality of biomarker assessments by incorporating morphological, physiological, and biochemical features of the used bioindicators (Lomartire et al., 2021). For example, bivalves were used to determine traces of pharmaceutical drugs in water resources. This was observed by increased oxidative bioactivation activities (Antunes et al., 2013).

On the other hand, the cytotoxicity of amoxicillin was assessed using *Perna perna* mussels. The permeability of the lysosome membrane of the *Perna perna* mussels was found to be brittle due to exposure to amoxicillin, thus affecting the functionality of the cells in the lysosome (de Souza et al., 2016). This biomarker risk assessment approach can be used alongside other risk assessment methods to confirm the potential risks posed by pharmaceuticals and validate the findings. Therefore, an integrated risk assessment approach could be more precise and informative, incorporating at least three methods that validate one another.

2.14 Modelling techniques used to predict the risk of selected pharmaceuticals

The use of models to predict PNEC, as well as the predicted environmental concentrations used to determine the ecological risk of chemical substances, such as PCs, is gaining more popularity in the world of science (Backhaus and Faust, 2012; Kumari and Kumar, 2021). Because such tools are used to predict environmental concentrations and estimate the ecotoxicity risks of newly discovered pharmaceutical contaminants, models provide valid primary data that can be used to assess and manage ecological health risks (Almeida et al., 2023). The release of chemical contaminants across Europe and the United States is monitored by the European Chemicals Agency and the United States Environmental Protection Agency, respectively (Khan et al., 2023). Both regulatory agencies advocate alternative testing approaches. The modelling techniques used to predict the risk of selected pharmaceuticals involve a range of *in silico* approaches, primarily quantitative structure-activity relationships (QSAR) and various software platforms. This shift aligns with ethical considerations and technological advancements, emphasising the need for predictive models that do not rely

on animal testing. QSAR models predict the environmental concentrations and potential ecotoxicity of PCs by correlating chemical structures with biological activity.

The PNEC for PCs, such as dexamethasone and azithromycin, using QSAR models alongside other software platforms, has been estimated recently (Tarazona et al., 2021; Khan et al., 2023). Most existing *in silico* predictive models for ecotoxicity have been designed using QSAR techniques (Rajpoot et al., 2022). The extensive use of QSAR techniques due to their cost-effectiveness, speed, and ability to handle large datasets underscores their importance in environmental risk assessment. However, the accuracy of QSAR predictions depends on the quality of the input data, as well as on inherent model assumptions and simplifications, which lead to prediction uncertainties. QSAR models are valuable tools as long as they are interpreted cautiously and supported by empirical data.

Other models employed to predict the environmental concentrations of several pharmaceuticals include the ChemFate model, using hospitalisation data (Desgens-Martin and Keller, 2021). Such models consider real-world factors such as pharmaceutical usage patterns and waste treatment efficiencies. The authors also compared results to predicted ecotoxicity concentrations obtained using ecological structure-activity relationships to investigate the risk that dexamethasone potentially poses to exposed organisms (Desgens-Martin and Keller, 2021). Despite their utility, these models have limitations. For instance, variability in hospital data and the complexity of environmental dynamics can introduce uncertainty into predictions. Additionally, species selection and model endpoints influence the comparison of predicted concentrations with ecotoxicity thresholds derived from ecological structure–activity relationships. This was established in the research work conducted by Li et al. (2018), where they reported poor interspecies correlations in reactive chemical levels among selected bioindicators. The thresholds of chemical excess toxicity were also evaluated by comparing them with fish toxicity sensitivity. Li and colleagues reported that the threshold for excess toxicity in Tetrahymena pyriformis differs from that in fish, *Daphnia magna*, and *Vibrio fischeri*.

Although some risk assessment models have been successfully employed, a critical concern is the potential for predictive models to over- or underestimate the risks posed by pharmaceuticals. Overestimation can lead to excessive regulatory actions and financial burdens, while underestimation can result in insufficient protection for ecosystems and human health. Researchers should prioritise developing risk assessment tools that yield accurate, precise results comparable to real-time analyses. This involves integrating modelling approaches, ensuring robust input data, and validating and calibrating predictive models against real-world observations or empirical data.

2.15 Way forward and prospects

The presence of pharmaceuticals such as azithromycin, dexamethasone, and prednisone in aquatic environments presents an urgent need for multidisciplinary research and evidence-based policy action. Despite growing awareness, significant gaps remain in understanding the environmental behaviour, transformation, and long-term effects of these compounds and their metabolites. Based on this, future research should prioritise the ecotoxicological assessment of metabolites and transformation products, especially those of azithromycin, which remain poorly characterised in environmental contexts. Studies should investigate chronic exposure effects, mixture toxicity, and sub-lethal outcomes in non-target aquatic species to support robust environmental quality standards. Moreover, method development and standardisation are critical. Current detection methods vary widely in sensitivity and specificity. There is a pressing need for validated,

cost-effective, and high-throughput analytical techniques to detect parent compounds and their metabolites at environmentally relevant concentrations.

Furthermore, the integration of environmental monitoring and risk assessment needs improvement. Future work should adopt a "One Health" approach that links environmental contamination to public health risks, particularly through food chains and water reuse systems. This includes strengthening risk-based regulatory thresholds and post-pandemic monitoring frameworks. Also, future studies should explore nature-based and advanced remediation technologies, such as constructed wetlands, membrane bioreactors, and biochar applications, to effectively remove these compounds from wastewater and sediments. Most importantly, policy reforms and public awareness should be driven by scientific evidence. Collaborative efforts among researchers, regulators, and the pharmaceutical industry are vital to improving waste-disposal practices, designing greener drugs, and implementing pharmaceutical take-back schemes. Addressing these areas will be instrumental in mitigating environmental and public health risks associated with pharmaceutical residues and advancing sustainable water management globally.

CHAPTER 3
LIQUID CHROMATOGRAPHY-MASS SPECTROMETRIC (LC-MS) AND SOLID-PHASE EXTRACTION (SPE) METHOD DEVELOPMENT

3.1 Introduction

There is no disputing the fact that environments are continuously stressed because of the presence of pollutants such as dyes, metals, polyfluoroalkyl substances, and other persistent organic pollutants (Oladoye et al., 2023; Ogunbiyi et al., 2023). Among other environmental pollutants, pharmaceutical residues remain significant contaminants, as their use is increasing due to an expanding population. These pharmaceutical pollutants eventually end up in the immediate environment (matrices), such as water, soils, and sediments. These compounds have since attracted the attention of researchers due to their lethal impact on living organisms upon release into the environment (Diniz et al., 2021; Omotola et al., 2022; Omotola et al., 2023; Piedade et al., 2020). For this study, selected pharmaceutical compounds (PCs) were investigated. These include three active pharmaceutical compounds, namely, prednisone, dexamethasone, and azithromycin. The study also aimed to monitor the occurrence pattern of a prednisone metabolite (prednisolone). These pharmaceuticals were chosen based on their high usage during the COVID-19 pandemic (Liu et al., 2023; Wang et al., 2020; Xing et al., 2022; Khan et al., 2023). In the present study, a solid phase extraction-liquid chromatography mass spectrometric (SPE−LC−MS) method was developed to isolate, detect, and separate the analytes of interest in water and sediment samples. Thereafter, the method developed was validated in accordance with the International Conference on Harmonisation (2005) validation procedures.

It is essential to state that heparin has been excluded from this study. Although the earlier submitted proposal included heparin, its analysis was not feasible because the instrument used to detect and quantify the other PCs could not detect heparin in its form. However, the detection of heparin could be conducted using ion-pair reversed-phase UPLC coupled with electrospray quadrupole time-of-flight mass spectrometry (IPRP-UPLC / ESI-QTof MS) (Yates and Rudd, 2016). Meanwhile, since the development of a compromise method for investigating the four PCs was the focus of this study, team members substituted heparin with prednisolone, a metabolite of prednisone.

3.2 Materials and methods

Prednisone (PRD), the metabolite prednisolone (m-PRD), dexamethasone (DEX), and azithromycin (AZI) standards were purchased from Sigma-Aldrich, South Africa. Liquid chromatography−mass spectrometry LC−MS grade acetonitrile and LC grade methanol were also purchased from Sigma Aldrich, South Africa. Formic acid was purchased from Honeywell, New Germany, South Africa. Hydrophilic−lipophilic balance (HLB) SPE cartridges (6 mL, 200 mg) were purchased from Sigma-Aldrich, United States. All other chemicals, reagents, and solvents used were of analytical grade. MilliQ water employed in the LC analysis was prepared using an Elix Millipore Water Purifier (Reference number PR0G0T0S2).

3.2.1 Software and instrument

The Shimadzu LC−MS 2020 system, comprising a quaternary pump, was employed during the development of the analytical method used in this study. The Excel 2016 package was used for regression analysis and for other nonparametric analyses of the results. A Mettler Toledo analytical balance was used for all mass determinations of neat pharmaceutical standards. All pH measurements were taken with a pre-calibrated pH meter (Mettler Toledo, C044384209). A centrifuge (Hermle, 65170037) and an ultrasonicator (Emerson Branson, M5800H-E) were employed for the extraction of analytes from sediments.

3.2.2 Preparation of stock solutions of pharmaceutical compounds

Approximately 0.01 g each of PRD, m-PRD, DEX, and AZI were weighed and dissolved in methanol in different 10 mL volumetric flasks and made up to the mark to attain the equivalent of 1 000 µg/mL stock solution of each of the analytes. The solutions were stored in a refrigerator at approximately 4 °C until use. Working standards at concentrations ranging from 0.01 to 0.10 µg/mL were prepared by diluting a specific volume of each stock solution in methanol to achieve the required concentration range for developing calibration curves for each analyte.

3.2.3 Instrumental method development

A single run over a defined concentration range for each analyte was first conducted by elution on a Shimadzu C18 reversed-phase column using a Shimadzu 2020 Liquid Chromatograph coupled to a Mass Spectrometer 2020 (LC−MS) to develop a single method that could detect, separate, and quantify four PCs simultaneously. This process aimed to identify the optimal instrument parameters – mobile phase pH and composition, column oven temperature, injection volume, and flow rate – that would enable the detection of the chosen analytes. Additionally, the process would help determine the retention times of each compound under the optimal instrument parameter setup. To verify the resolution of the targeted analytes, a mixture of the three active PCs and one metabolite was injected onto the LC-MS column. Gradient elution was used to successfully separate the compounds of interest. The next step was to plot the calibration curves for each analyte to determine how the device responded to changes in concentration. The ICH (2005) standard validation procedures were followed in validating the developed method.

3.2.4 Suitability test and method validation

The ICH-prescribed guideline procedure was applied to data generated from instrument calibration and analyte recovery. This was carried out to validate and assess the system's suitability, and to determine whether the developed method met the minimal requirements for both system performance and usefulness. The approach was to ensure that the method met the acceptable conformance specifications for its intended use. Suitability tests were conducted similarly to those earlier reported by Omotola and Olatunji (2020). The tests included an assessment of the peak tailing factor, chromatographic peak resolution, and the number of theoretical plates. The validation parameters tested included specificity, sensitivity, selectivity, linear range, linearity, limits of detection (LoD), limits of quantitation (LoQ), and precision. Stability assessments were performed on stock solutions. The stability of the analytes was checked over five consecutive days at 4 °C using a quality control standard containing 0.10 µg/mℓ of the compound mixture. All stock dilutions in the methanol mixture remained stable at temperatures below 25 °C. Optimal chromatographic conditions were applied to all samples to determine the peak intensities and areas of the analytes.

3.2.5 Extraction and recovery efficiency of pharmaceutical compounds from water and sediment samples

Solid phase extraction columns consisting of hydrophilic-lipophilic cartridges (Supel-Select) (200 mg/6 mL) were conditioned by eluting with 5 mℓ of acetonitrile, followed by sequential elution with buffered water (pH 4), using a few drops of 6 M HCl, through the column at a flow rate of 1-2 mℓ/min. Subsequently, 300 mℓ of water spiked with the analytes at low, medium, and high concentrations was loaded onto the conditioned column and eluted at a flow rate of 7-9 mℓ/min. Before sample loading, 12.5 mℓ of 0.25 M disodium dihydrogen ethylenediaminetetraacetic acid (EDTA) was added to the water sample to increase the selectivity of the SPE adsorbent (HLB). The cartridges were later dried under vacuum for 60 minutes after loading. Analyte elution was finally recovered with 5 mℓ of 0.5% methanoic acid/Methanol at 1−2 mℓ/min flow rate, as earlier stated by Omotola and Olatunji (2020). Thereafter, eluates were concentrated under compressed air to near dryness, reconstituted to 1 mℓ in 0.5% buffered methanol, and analysed using the validated LC−MS method.

For sediments, 200 micron mesh-size sieves were used to sieve all sediment samples after being dried at room temperature in the laboratory. About 5.0 g of the sieved sediments was weighed in 50 mℓ centrifuge tubes. Thereafter, sequential extraction of the PCs was performed using 10 mℓ of 2% aqueous ammonia in methanol, followed by 10 mℓ of 2% methanoic acid in methanol, and finally 10 mℓ of pure methanol. For each addition of extractant, the mixture was vortexed for 30 seconds, sonicated for 5 minutes, and centrifuged for 5 minutes at 6 000 rpm. Subsequently, the supernatants were combined and diluted with water (300 mℓ) plus 12.5 mℓ 0.25 M EDTA. This sample mixture was thereafter extracted using the same SP extraction method as described earlier for the water sample. The efficiencies with which the analytes were recovered were validated by calculating each recovery percentage for the spiked concentrations using the following equation:

$$Percentage\ Recovery\ (\%) = \frac{W - X}{Y} \times 100$$

Where W is the peak area of each PC after extraction of the spiked sample, X is the peak area of the analyte after extraction of the non-spiked sample (water or sediment), and Y is the peak area of each analyte (for spiking).

3.3 Results and discussion

A good resolution is required to ascertain effective chromatographic separation. An excellent resolution reduces quantitative errors, qualitative retention time shifts, and ambiguities in peak purity to a greater extent. The suitability of a choice column and a number of optimisation steps, including column conditioning, mobile phase composition, temperature control, mobile phase pH control, the use of mobile phase modifiers, and the use of ion-pair reagents when needed, is part of an empirical procedure for achieving good separation and quantification of complex mixtures. These factors have varying effects depending on the system being used and the complexity of the mixture or compounds to be separated. The compounds investigated consisted of two different classes, including PRD, m-PRD, and DEX, which are all active corticosteroids, and AZI, which is an antibiotic.

3.3.1 Optimisation and instrument parameters for efficient separation

The mobile phase, consisting of different fractions of mixtures of acetonitrile and Milli Q water, both spiked with 0.1% methanoic acid, was tested as eluent for the detection and separation of PRD, m-PRD, DEX, and AZI mix. The separation of the four PCs was achieved by gradient elution, with the compound mixture eluted from a C18 column (2.1 × 100 mm, 2.6 µm) as the mobile phase composition was changed. The aqueous phase (A) comprised 0.1% methanoic acid in Millipore water, while the organic phase (B) comprised 0.1% methanoic acid in acetonitrile. The analysis took 10 min. The suitable gradient programme condition, which resolved the mixture of the four PCs, started at 10% B and increased to 35% B over 2 minutes, then decreased to 10% B over 10 minutes. The optimal instrument parameter settings for separating the PCs were a flow rate of 0.25 mℓ/min, a column temperature of 40 °C, and an injection volume of 5 µL. The MS chromatogram showing the optimal separation characteristics, retention times, and separation efficiency of the four compounds is shown in Figure 3.1.

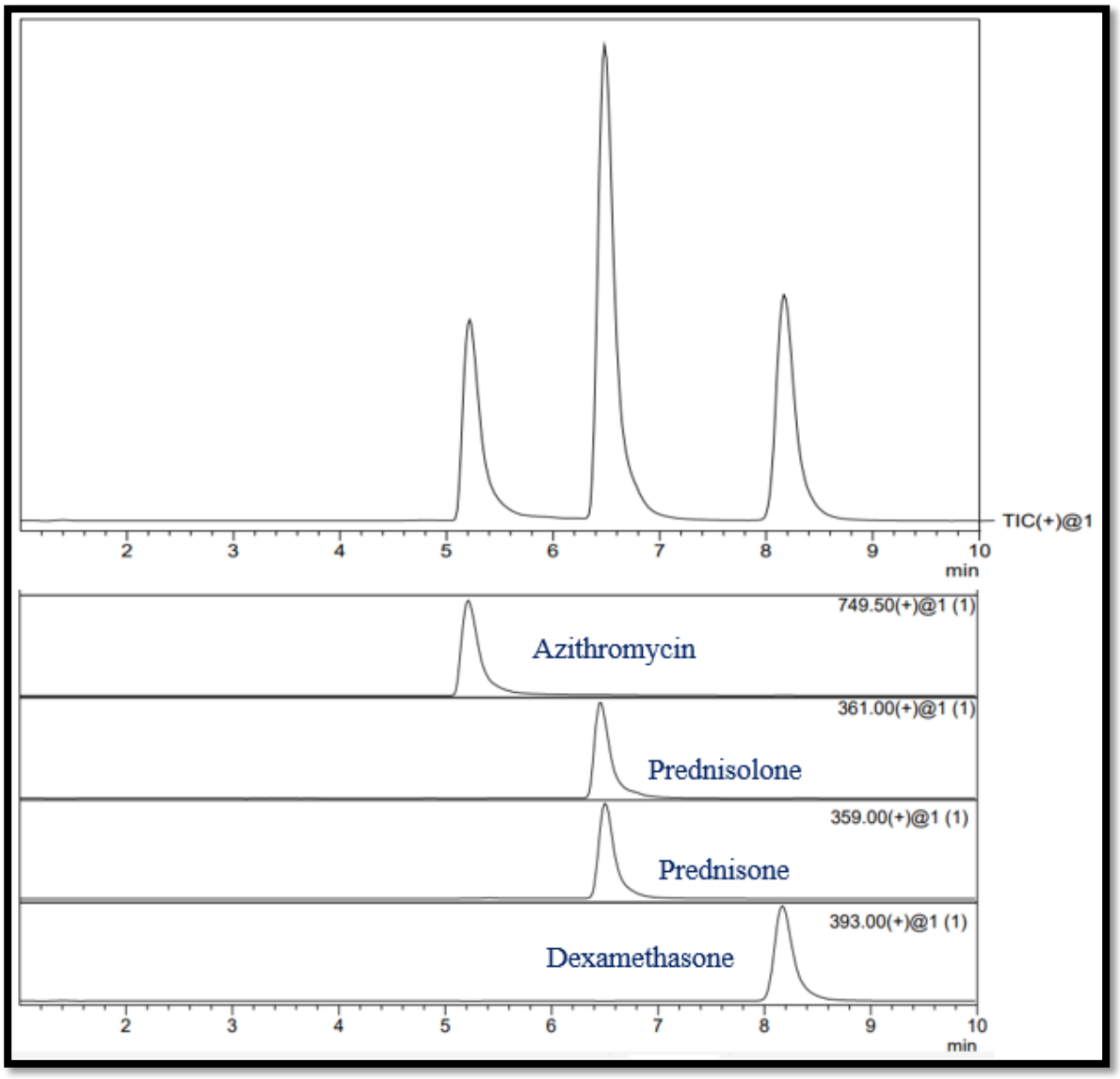

Figure 3.1 Mass spectrometry chromatogram of three active pharmaceutical compounds and one metabolite

The retention times (minutes) for AZI, m-PRD, PRD, and DEX were 5.25, 6.48, 6.51, and 8.21, respectively. The MS analysis was achieved in the positive electrospray ionisation mode for all PCs. The SRM m/z characteristic fragmentation patterns of the individual PCs were determined by five online injections at 749.50, 361.00, 359.00, and 393.00 for AZI, m-PRD, PRD, and DEX, respectively. The PCs were detected at a detector voltage of 1.45 kV. The chromatographic features of the PCs are given in Table 3.1.

Table 3.1 Chromatographic features of selected pharmaceutical compounds

S/N	Analyte	Retention Time (min) Mean ± S.D. (n=5)	Retention Time (min) % RSD*	Molecular formula	Molecular weight (g/mol)	$[M+H]^+$
1	AZI	5.25 ± 0.05	0.87	$C_{38}H_{72}N_2O_{12}$	749.0	749.5
2	m-PRD	6.48 ± 0.07	1.12	$C_{21}H_{28}O_5$	360.4	361
3	PRD	6.51 ± 0.07	1.12	$C_{21}H_{26}O_5$	358.4	359
4	DEX	8.21 ± 0.15	1.93	$C_{22}H_{29}FO_5$	392.5	393

*%RSD = Percentage relative standard deviation

3.3.2 System suitability

System suitability tests are predicated on the idea that tools and analytical processes constitute a fundamental framework for a methodical approach validation procedure. The precision and accuracy of the analyte peaks are used to assess the suitability of the chromatographic system. The evaluation of resolution, theoretical plates, system precision, and tailing factor – also referred to as symmetry factor – were the primary components of these tests. From sample injections, the tailing factor, resolution, theoretical plates, and system precision (%RSD of retention times of target analytes) were calculated for each PC as explained in the published article by Omotola and Olatunji (2020), with results shown in Table 3.2.

Table 3.2 Suitability tests of the chromatographic system

Pharmaceutical compounds	Resolution (R)	Theoretical plate number (N)	Tailing factor (S)	%RSD of retention times (n=5)
AZI	Btw AZI and m-PRD (2.66)	1 674.72	1.78	0.87
m-PRD	Btw m-PRD and PRD (0.10)	3 696.80	1.93	1.13
PRD	Btw PRD and DEX (2.85)	2 603.12	1.88	1.12
DEX		2 430.63	1.70	1.93

The acceptance criteria for the resolution between two consecutive chromatographic peaks should be >2, while the symmetry of a chromatographic peak should be ≤2, according to the Centre for Drug Evaluation and Research (Center for Drug Evaluation and Research, 1994). Furthermore, the system precision for target

analytes should be ≤5%, and the theoretical plate number, which depends on elution time, should generally be higher than 2 000. The average number of theoretical plates with respect to the chromatographic column used in this study is 2 601.32±834.23. The system precision, expressed as %RSD in Table 3.2, shows that the retention times of the analytes across five injections are ≤1.93 in all cases. Consequently, it can be safely concluded that the results from the suitability tests were all within the acceptable criteria, based on the Center for Drug Evaluation and Research (1994).

3.3.3 Method validation

Analytical results are frequently evaluated for quality, consistency, and reliability using the findings of method validation. To determine whether the deployment of the analytical method or procedure for the intended PC analytes is appropriate and sound for its intended application, the developed method was submitted to the ICH validation procedure. As a result, the specificity, linearity, range, accuracy, precision, LoD, and LoQ of the developed method were all validated in accordance with ICH guidelines.

3.3.3.1 Specificity

The ability of a technique to precisely measure the analyte response in the presence of every possible sample component is known as specificity. The specificity of the procedure was ascertained by comparing the chromatograms from samples of mixed PCs with those from a blank or methanol, which was used to prepare all analyte standards. To obtain the chromatographic peaks of all analytes, a solution mixture containing various concentrations of each analyte, ranging from 0.01 to 0.1 µg/mℓ, was injected into the column under ideal chromatographic conditions. The retention time of the compounds, as shown in Table 3.3, did not change across replicate injections (n=12) of the PC mix.

Table 3.3 Retention time, limits of detection, limits of quantitation, and linear range characteristics of the calibration curve for selected analytes

Analytes	Retention time (min)	Standard Error of the mean (S.E.) (n= 12)	Equation of the best fit	Slope	Coefficient of regression (R^2)	LoD (µg/ℓ)	LoQ (µg/ℓ)
AZI	5.25	3624.39	$y = 1735.3x + 275.43$	1735.30	0.9883	0.0209	0.0696
m-PRD	6.48	925.89	$y = 1179.7x - 675.36$	1179.70	0.9983	0.0079	0.0262
PRD	6.51	680.23	$y = 1288.1x - 724.36$	1288.10	0.9992	0.0053	0.0176
DEX	8.21	1171.29	$y = 1249.5x - 1478$	1249.50	0.9976	0.0094	0.0312

3.3.3.2 Linearity and linear range

An acceptable set of quantitative analytical results is firmly based on the quality of the six-point calibration curve. Six distinct levels of the PC's cocktail, ranging from 0.01 to 0.10 ng/µL, were prepared and injected twice for every concentration (n=12) on the LC−MS. Plotting the peak area of each analyte against its corresponding concentration allowed for the preparation of the calibration curves (Figure 3.2). For the

incremental concentration signals, the following coefficients of correlation (R^2) were obtained: AZI: R^2 = 0.9883; m-PRD: R^2 = 0.9983; PRD: R^2 = 0.9992; and DEX: R^2 = 0.9976 (Table 3.4). These values exceeded the acceptable value set by the International Conference on Harmonisation, which was 0.90. While individual compound responses differed from one another, the best-fit curve regression equation for each PC in the linear range of calibration concentrations 0.01−0.10 µg/mℓ (Table 3) indicated linear responses. As a result, the developed method was suitable for its intended application.

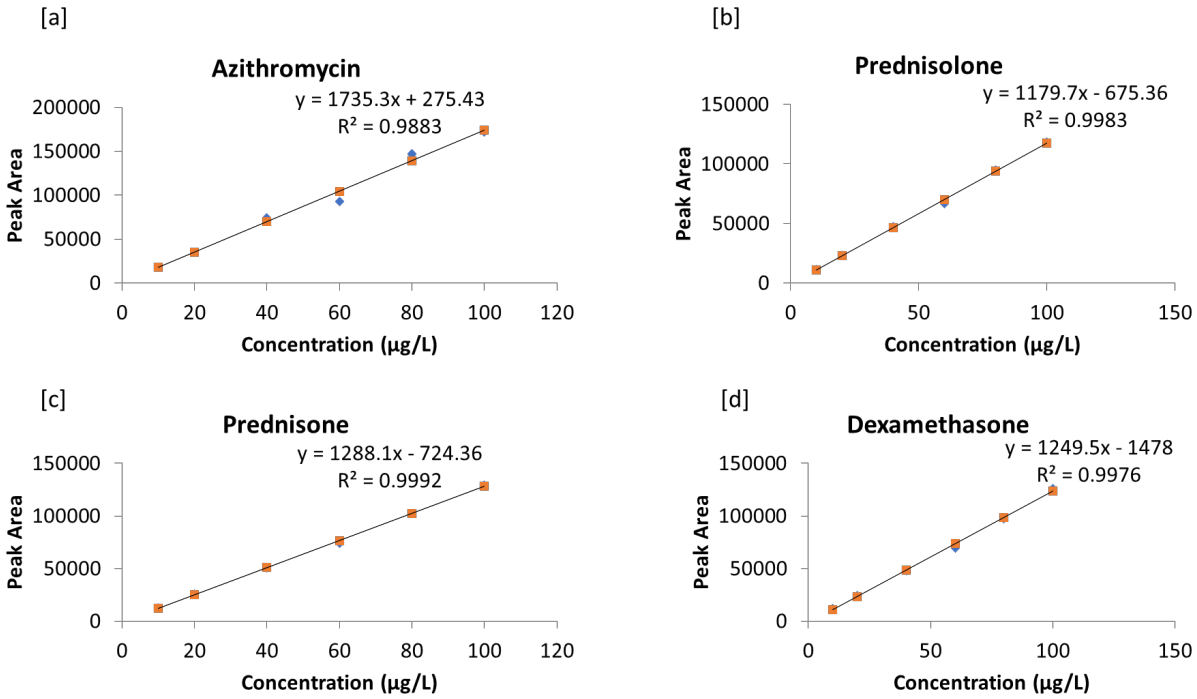

Figure 3.2 Calibration curves for the pharmaceutical compounds of interest

3.3.3.3 Limits of detection and quantification (limits of detection and limits of quantification)

The assessment of the LoD and LoQ precedes confirmation of the method's performance characteristics for the separation, detection, and quantitation of the PCs of interest in this study. According to Shrivastava and Gupta (2011) and Omotola and Olatunji (2020), LoD and LoQ indicate the lowest amount of an analyte that can be accurately measured with acceptable precision and accuracy using an analytical technique. By computing the signal-to-noise ratio obtained from a series of successive measurements of blank, which corresponded to three and ten times the noise levels, respectively, the LoD = $3s/_m$ and LoQ = $10s/_m$, of the developed method were ascertained, where *s* was taken as the standard error of the mean, and *m* as the slope. The LoDs (µg/ℓ) obtained for AZI, m-PRD, PRD, and DEX were 0.0209, 0.0079, 0.0053 and 0.0094, respectively, while the LoQs (µg/ℓ) were 0.0696, 0.0262, 0.0176, and 0.0312, respectively (Table 3.4).

3.3.3.4 Precision and accuracy

Precision and accuracy are among the crucial ICH validation parameters for evaluating the appropriateness and workability of a chromatographic test method. Precision gauges how closely individual measurements match one another, whereas accuracy gauges how closely an experimental value matches the true value (the actual amount of analyte in the matrix). In this study, repeatability and reproducibility analyses were used to

evaluate the precision of a set of results. The difference in data produced by subsequent measurements made with the same instrument, under the same conditions and parameter settings, was taken as a measure of repeatability. On the other hand, reproducibility quantifies the extent of fluctuations in the entire research or experimental data over a measurement period, typically spanning 5 days. This investigation included a 0.10 µg/mℓ quality control sample. For analysis, five injections of the designated quality control samples were performed five times over 24 hours for repeatability and five times over five days for reproducibility. The accuracy and precision of the method or instrument were predicted using the calculated mean and relative standard deviations of the measurements. The study's findings are summarised in Table 3.4. These findings of repeatability (intra-day) and reproducibility (inter-day) analyses were conducted to verify the precision of the measurements. Data on precision and accuracy showed that the components of the sample or instrument were not interfering. The inter- and intra-day replicates of the selected analytes yielded RSD values < 8.78%. The intra-day and inter-day values should not exceed 15% (Zhou and Wang, 2017). This value falls within the acceptable range for %RSD, as stated earlier. From these results, it was also established that the stock solutions of the PCs were stable, as the final assay values of the analytes were similar after five chromatographic injections over 5 days, as shown by the %RSD in Table 3.4.

Table 3.4 Intra-day and inter-day analyses of selected pharmaceutical compounds

S/N	Compounds	Class	Precision (%RSD, n=5) 0.10 µg/mℓ	
			Intra-day precision	Inter-day precision
1	AZI	Antibacterial	2.52	8.78
2	m-PRD	Glucocorticoids	2.06	5.87
3	PRD	Glucocorticoids	4.45	5.20
4	DEX	Glucocorticoids	4.09	4.67

3.4 Recoveries (%) of pharmaceutical compounds from water and sediments

Analyte recovery, typically expressed as a percentage, is a measurement of the extraction efficiency attained in the recovery of a known amount of an analyte. This was achieved by conditioning the cartridges (HLB), loading the samples (water and sediments), drying, and elution of analytes from the adsorbent column. Three different concentrations of PCs were evaluated for recovery: 0.01 µg/mℓ, 0.04 µg/mℓ, and 0.10 µg/mℓ. The per cent recoveries of the targeted analytes from aqueous solutions and sediments are reported in Tables 5a and 5b, respectively. The specific percentage recoveries in water samples ranged from 83.34 ± 1.01 to 98.30 ± 0.69, while in sediments, they ranged from 82.26 ± 0.47 to 95.20 ± 2.02. The results were within the acceptable range according to the ICH 2005 guidelines.

Table 3.5 Percentage recoveries of pharmaceutical compounds from water samples

S/N	PCs	% R1	% R2	Mean	SD	% RSD
			10 µg/ℓ			
1	AZI	88.03	90.91	89.47	2.04	2.28
2	m-PRD	94.21	95.09	94.65	0.62	0.66
3	PRD	96.10	95.00	95.55	0.77	0.81
4	DEX	93.39	90.78	92.08	1.85	2.01
			40 µg/ℓ			
1	AZI	82.62	84.05	83.34	1.01	1.22
2	m-PRD	94.35	95.81	95.08	1.04	1.09
3	PRD	89.39	89.90	89.65	0.36	0.41
4	DEX	99.63	97.34	98.49	1.62	1.64
			100 µg/ℓ			
1	AZI	84.77	84.66	84.71	0.08	0.09
2	m-PRD	89.65	91.28	90.46	1.15	1.27
3	PRD	86.52	85.27	85.90	0.88	1.03
4	DEX	97.82	98.79	98.30	0.69	0.70

Table 3.6 Percentage recoveries of pharmaceutical compounds from sediment samples

S/N	PCs	% R1	% R2	Mean	SD	% RSD
			10 µg/ℓ			
1	AZI	85.51	81.93	83.72	2.53	3.03
2	m-PRD	91.59	90.96	91.28	0.44	0.49
3	PRD	96.62	93.77	95.20	2.02	2.12
4	DEX	88.77	89.27	89.02	0.35	0.39
			40 µg/ℓ			
1	AZI	88.27	84.30	86.28	2.81	3.25
2	m-PRD	87.38	86.35	86.86	0.73	0.84
3	PRD	83.82	81.18	82.50	1.87	2.27
4	DEX	93.45	95.90	94.68	1.73	1.83

			100 µg/ℓ			
1	AZI	81.92	82.59	82.26	0.47	0.57
2	m-PRD	87.45	85.41	86.43	1.45	1.67
3	PRD	84.08	82.58	83.33	1.06	1.27
4	DEX	90.68	91.42	91.05	0.52	0.58

CHAPTER 4 [2]
OCCURRENCE AND SEASONAL VARIATION OF PHARMACEUTICAL RESIDUES OF EMERGING CONCERN IN SOUTH AFRICAN SURFACE WATERS

4.1 Introduction

There is no disputing that the environment is continually stressed by pollutants such as dyes, metals, polyfluoroalkyl substances, and other persistent organic pollutants. Among other environmental pollutants, pharmaceutical residues remain significant, as their use is likely to remain high due to population growth. The endpoint of these residues is the immediate environmental matrices, such as water, soils, and sediments. These compounds have since gained the attention of researchers because of their fatal impacts on living organisms upon their release into the environment (Omotola et al., 2023; Eapen et al., 2024). Pharmaceutical residues are reportedly released from diverse sources such as hospitals, manufacturing industries, animal rearing, domestic discharge and various research activities (Sharma et al., 2022). Because the unregulated discharge of these compounds from the mentioned multiple sources is relatively faster than their auto-degradation or remediation rates, pharmaceutical compounds (PCs) are regarded as pseudo-persistent organic contaminants (Sharma et al., 2022). As a result, the amount of pharmaceutical residues in the environment, especially in aqueous matrices, is increasing from ultra-trace to trace levels over time (about 1000 times escalation) (Patel et al., 2019; Sharma et al., 2022). Additionally, these pollutants are poorly removed by wastewater treatment plants, where most environmental waste ends up, leading to their discharge into surface waters and, subsequently, into ground and drinking water. Several authors have recently reported the presence or detection of PC residues in drinking water globally (Muambo et al., 2024; Guimarães et al., 2025). Over the years, PC residues have been detected primarily by chromatographic techniques, with high-performance liquid chromatography (HPLC) being the most commonly used. However, with the integration of cutting-edge technologies, such as hyphenated techniques and high-throughput analysis, into contemporary pharmaceutical research, the instrumental detection of pharmaceutical compounds has undergone significant evolution, moving from simple techniques with relatively low sensitivity to more sensitive methods, such as liquid chromatography-mass spectrometry (LC−MS). This has enabled the detection of even trace amounts of these compounds in complex matrices. Furthermore, LC-tandem MS has proven to be highly effective and more sensitive than LC-MS for detecting PC residues. However, this instrument (LC-tandem MS) is more expensive than LC−MS, making it less available in developing countries or semi-developed countries. In the present study, a relatively new LC−MS method was developed, validated and applied to real surface water samples within South Africa for the detection of four PCs.

Although analgesics such as paracetamol and ibuprofen, and antibiotics such as ciprofloxacin and sulfamethoxazole, are frequently studied as pharmaceutical contaminants, other compounds, including corticosteroids and macrolide antibiotics, remain under-investigated despite their environmental relevance. The pharmaceuticals selected in this study were widely used during the COVID-19 pandemic and may have entered aquatic systems through increased consumption and excretion. For example, dexamethasone was recommended during the pandemic as part of treatment protocols to reduce mortality, with studies reporting a

[2] Chapter 4: DOI: 10.1093/inteam/vjaf156.

one-third reduction in death for ventilated patients and a one-fifth reduction in those on oxygen therapy (Cârstea et al., 2023). Azithromycin was often co-administered to address secondary bacterial infections in early-stage COVID-19 cases (Butler et al., 2021). Given their widespread use and potential persistence, these pharmaceuticals merit investigation for their presence and seasonal variation in aquatic environments. Furthermore, current regulatory guidelines for these compounds in environmental waters are limited, highlighting the need for monitoring and risk evaluation.

It is envisaged that for some years to come, the presence of pharmaceutical residues in the environment will be a challenge due to the widespread use of medications, limitations in current wastewater treatment technologies, and the complex chemical nature of many pharmaceuticals. This makes them difficult to degrade, poses a potential for long-term environmental accumulation, and results from the lack of comprehensive regulations regarding pharmaceutical waste disposal; all of which contribute to the ongoing presence of these contaminants in water bodies and ecosystems. For this reason, the present study aimed to investigate the occurrence and seasonal variation of four PCs, namely prednisone, prednisolone (a metabolite of prednisone), dexamethasone, and azithromycin, in selected surface waters of the South African aquatic environment. Additionally, the study employed a newly developed and validated analytical method for their detection and quantification. The present study developed a solid phase extraction-liquid chromatography-mass spectrometric (SPE−LC−MS) method to isolate, detect, and separate the analytes of interest in water samples. Thereafter, the method developed was validated in accordance with the International Conference on Harmonisation (2005) validation procedures.

4.2 Materials and methods

Prednisone (PRD), the metabolite prednisolone (m-PRD), dexamethasone (DEX), and azithromycin (AZI) standards were purchased from Sigma Aldrich, South Africa. LC−MS-grade acetonitrile and LC-grade methanol were also purchased from Sigma Aldrich, South Africa. Formic acid was purchased from Honeywell, New Germany, South Africa. Hydrophilic-lipophilic balance (HLB, 6 mℓ, 200 mg) SPE cartridges were purchased from Sigma Aldrich, US. All other chemicals/reagents and solvents used were of analytical grade. MilliQ water employed in the LC analysis was prepared using an Elix Millipore Water Purifier (Reference number PR0G0T0S2).

4.2.1 Software and Instrument

The Shimadzu LC−MS 2020 system, comprising a quaternary pump, was employed during the development of the analytical method used in this study. The Excel 2016 package was used for regression analysis and for parametric analysis (t-test) of the results. A Mettler Toledo analytical balance was employed for all massing of neat pharmaceutical standards. All pH measurements were taken with a pre-calibrated pH meter.

4.2.2 Preparation of stock solution of pharmaceutical compounds

Approximately 0.01g each of PRD, m-PRD, DEX, and AZI was weighed and dissolved in MeOH in separate 10 mℓ volumetric flasks, made up to the mark to obtain a 1000 µg/mL stock solution of each analyte. The solutions were stored in a refrigerator at approximately 4°C until use. Working standards at concentrations ranging from 0.01 to 0.10 µg/mℓ were prepared by diluting a specific volume of each stock solution in methanol to achieve the required concentration range for developing calibration curves for each analyte. All analyses were carried out in duplicates.

4.2.3 Instrumental method development, suitability tests and method validation

A single run over a defined concentration range for each analyte was first conducted by elution on a Shimadzu C18 reversed-phase column using a Shimadzu 2020 Liquid Chromatograph coupled to a Mass Spectrometer (LC−MS) to develop a single method that could detect, separate, and quantify three APCs and one metabolite simultaneously. This process aimed to identify the ideal instrument parameters, such as mobile phase pH and composition, column oven temperature, injection volume, and flow rate, that would allow for the detection of the chosen analytes. Additionally, the process would help determine each compound's retention time under the optimal instrument parameter settings. A mixture of the three APCs and one metabolite was injected online into the LC−MS to verify the resolution of the targeted analytes. Gradient elution was used to successfully separate the compounds of interest. The next step was to plot the calibration curves for each analyte to determine how the device responded to changes in concentration. The International Conference on Harmonisation (2005) followed standard validation procedures for the validation of the developed method. Additionally, Suitability tests were conducted similarly to those earlier reported by Omotola and Olatunji (2020).

4.2.4 Extraction/recovery efficiency of PCs from water samples

Solid phase extraction columns consisting of hydrophilic-lipophilic cartridges (Supel-Select) (200 mg/6 mℓ) were conditioned by eluting with 5 mℓ of acetonitrile, followed by sequential elution with buffered water (pH 4), using a few drops of 6M HCl, through the column at a flow rate of 1-2 mℓ/min. Subsequently, 300 mℓ of water spiked with the analyte concentrations was loaded on the conditioned column and eluted at a flow rate of 7-9 mℓ/min. Before sample loading, 12.5 mℓ of 0.25 M disodium dihydrogen ethylenediaminetetraacetic acid (EDTA) was added to the water sample to increase the selectivity of the SPE adsorbent (HLB). The cartridges were later dried under vacuum for 60 minutes after loading. Analyte elution was finally recovered with 5 mℓ of 0.5 % methanoic acid/MeOH at a 1-2 mℓ/min flow rate, as earlier stated by Omotola and Olatunji (2020). Thereafter, eluates were concentrated under compressed air to near dryness, reconstituted to 1 mℓ in 0.5 % buffered methanol, and analysed using the validated LC−MS method.

The recovery efficiencies of the analytes were validated by calculating each recovery (%) at the spiked concentrations using the equation below.

$$Percent\ Recovery\ (\%) = \frac{W - X}{Y} \times 100$$

Where W is the peak area of each APC after extraction of the spiked sample, X is the peak area of the analyte after extraction of the non-spiked sample, $and\ Y$ is the peak area of each analyte (for spiking).

4.2.5 Description of study sites

Water samples were collected from eight sampling points from surface water sources, within sections of the Upper Orange River basin, South Africa, including two sampling points in the Gariep area (30°35'7.56"S and 25°30'54.62"E; 30°35'11.41"S and 25°29'11.45"E, sites 1 and 2, respectively), Aliwal North (30°41'12.71"S and 26°41'39.43"E, site 3), Zastron (30°17'57.91"S and 27° 6'43.95"E, site 4), Ladybrand (29°11'12.81"S and 27°29'7.82"E, site 5), Maseru (29°18'53.23"S and 27°28'2.11"E, site 6), Ficksburg (28°53'26.53"S and 27°53'45.73"E, site 7), and Clarens (28°31'17.23"S and 28°25'50.80"E, site 8). All samples were collected from the point of discharge (Point of discharge) of the surface water, where WWTPs discharge their effluents, as well as upstream (Upstream) and downstream (Downstream) regions of each sampling site. These zones were

selected to evaluate the distribution and dynamics of pharmaceutical pollution relative to potential contaminant entry points, such as wastewater discharge locations. The map containing all the sampling sites is shown in Figure 4.1.

The Gariep Dam is an important source of approximately 455 million litres of water for some dry regions around the Free State, South Africa. Therefore, it was important to investigate levels of PC residues in the water system and in the water surrounding it. The water samples were collected in 0.1 % nitric acid pre-cleaned 0.5 L amber and transparent glass bottles, respectively. The samples were labelled and kept in ice-chest coolers to preserve their integrity during transfer to the laboratory. All the samples were kept in the laboratory refrigerator at 4 ℃ and analysed within five days of collection. The physicochemical properties of the water samples were measured in situ at different sampling points.

4.3 Results

4.3.1 Optimisation and instrument parameters for efficient separation

The mobile phase, consisting of different fractions of mixtures of acetonitrile and Milli-Q water, both spiked with 0.1% methanoic acid, was tested as the eluent for the detection and separation of PRD, m-PRD, DEX, and the AZI mix. The separation of the four PCs was achieved by gradient elution, with the compound mixture eluted from a C18 column (2.1×100 mm, 2.6 µm) as the mobile phase composition was changed. The aqueous phase (A) comprised 0.1 % methanoic acid in Millipore water, while the organic phase (B) comprised 0.1% methanoic acid in acetonitrile. The analysis run time was 10 min. The suitable gradient programme condition that resolved the mixture of the four PCs started at 10% B and increased to 35% B in 2 min, then decreased to 10% B in 10 min. The optimal instrument parameter settings for separating the PCs were flow rate, 0.25 mℓ/min; column temperature, 40 ℃; and an injection volume of 5 µL. The MS chromatogram showing the optimal separation characteristics, retention times, and separation efficiency of the four compounds is shown in Figure 4.2.

The retention times (min) for AZI, m-PRD, PRD, and DEX were 5.25, 6.48, 6.51 and 8.21, respectively. The MS analyses were achieved in the positive electrospray ionisation mode for all PCs. The SRM m/z characteristic fragmentation pattern of each individual PC was determined through five online injections at 749.50, 361.00, 359.00, and 393.00 for AZI, m-PRD, PRD, and DEX, respectively. The chromatographic features of the PCs are given in Table 4.1.

4.4 System suitability

System suitability tests are predicated on the idea that tools and analytical processes constitute a fundamental framework for a methodical approach validation procedure. The precision and accuracy of the analyte's peak characteristics are used to assess the suitability of the chromatographic system. The evaluation of resolution, theoretical plates, system precision, and the tailing factor (also referred to as the symmetry factor) is the primary component of these tests. from sample injections, the tailing factor, resolution, theoretical plates, and system precision (%RSD of retention times of target analytes) were calculated for each PC as explained in the published article by Omotola and Olatunji (2020), with results shown in supplementary material Table S5.

The acceptance criteria for the resolution between two consecutive chromatographic peaks should be >2, while the symmetry of a chromatographic peak should be ≤2, according to the Centre for Drug Evaluation and

Research (CDER, 1994). Furthermore, the system precision for target analytes should be ≤5%, and the theoretical plate number, which depends on the elution time, should generally be >2000. Meanwhile, the average number of theoretical plates for the chromatographic column used in this study is 2601.32 ± 834.23. The system precision, presented as %RSD in supplementary material Table S5, shows that the retention times of the analytes across five injections are ≤ 1.93 in all cases. Consequently, it can be safely concluded that the results of the suitability tests were all within the acceptable criteria set by the CDER (1994).

4.5 Method validation

4.5.1 Specificity

The ability of a technique to precisely measure the analyte response in the presence of every possible sample component is known as specificity. By comparing the chromatograms of the mixed PCs samples with those of the blank or methanol used to prepare all analyte standards, the procedure's specificity was confirmed. To obtain the chromatographic peaks of all analytes, a solution mixture containing various concentrations of each analyte, ranging from 0.01 to 0.1 µg/mℓ, was injected into the column under ideal chromatographic conditions. The compounds' retention time, as shown in supplementary material Table S6, does not change across replicate injections (n=12) of the PC mix.

4.5.2 Linearity and linear range

An acceptable set of quantitative analytical results is firmly based on the quality of the six-point calibration curve. Six distinct levels of the PC cocktail, ranging from 0.01 to 0.10 ng/µL, were prepared and injected twice for every concentration (n = 12) on the LC−MS. Plotting each analyte's peak area against its corresponding concentration allowed for the preparation of the calibration curves (online supplementary material figures S1-S4). For the incremental concentration signals, the following coefficients of correlation (R^2) were obtained: AZI, R^2 = 0.9883; m-PRD, R^2 = 0.9983; PRD, R^2 = 0.9992; and DEX, R^2 = 0.9976 (supplementary material Table S6). These values exceeded the acceptable value set by the International Conference on Harmonisation, which is 0.90. While individual compound responses varied, the best-fit regression equation for each PC in the calibration concentration range 0.01–0.10 µg/mℓ indicated linear responses. As a result, this method is deemed suitable for its intended application.

4.5.3 Limits of detection and quantification (LoD and LoQ)

The assessment of the limits of detection (LoD) and quantification (LoQ) precedes the confirmation of the performance characteristics of the method designed for the separation, detection, and quantification of the PCs of interest in this study. According to Shrivastava and Gupta (2011) and Omotola and Olatunji (2020), LoD and LoQ indicate the lowest amount of an analyte that can be accurately measured with acceptable precision and accuracy using an analytical technique. By computing the signal-to-noise ratio obtained from a series of successive measurements of blank, which corresponded to three and ten times the noise levels, respectively, the LoD ($LoD = {}^{3s}/_{m}$) and LoQ ($LoQ = {}^{10s}/_{m}$) of the developed method were ascertained; taken s as the standard error of the mean, and m as the slope. The LoDs (µg/ℓ) obtained for AZI, m-PRD, PRD, and DEX were 0.0209, 0.0079, 0.0053 and 0.0094, while the LoQs (µg/ℓ) were 0.0696, 0.0262, 0.0176, and 0.0312, respectively.

4.6 Recoveries (%) of pcs from water

Analyte recovery, typically expressed as a percentage, is a measurement of the extraction efficiency attained in the recovery of a known amount of an analyte. This was achieved by conditioning the cartridges (HLB), loading the water samples, drying the adsorbent, and eluting the analytes from the adsorbent column. Three different concentrations of PCs were evaluated for their recoveries, including 0.01 µg/mℓ, 0.04 µg/mℓ, and 0.10 µg/mℓ. Specific percentage recoveries in water samples range from 83.34±1.01 to 98.30±0.69. The results were within the acceptable range according to the ICH 2005 guidelines.

4.6.1 Physicochemical properties of the sampled water

The pH of the water samples ranged from 7 to 9, while the temperature ranged from 19.3 ℃ to 37.4 ℃. Furthermore, the EC of the water samples ranged from 184.7 µS/cm to 1414.0 µS/cm. Although measuring the physicochemical properties of water samples is insufficient to identify pollutants in the environment, it provides information on pollution levels. A relatively high EC suggests that the water sampled is polluted. In the present study, based on EC values, the farm dam is the most polluted, with the highest EC value of 1414.0 µS/cm.

4.7 Analysis of PC residues in water samples during summer and winter

A total of eight sampling sites, subdivided into the point of discharge (P), upstream (U), and downstream (D), were investigated for the presence of four PC residues in water, yielding 16 water samples during summer and 17 during winter. The disparity in the number of samples collected could be due to higher water levels during winter rather than summer. For instance, there was no ‘point of discharge’ water sample at site four during summer, whereas it was available at the same site during winter. Consequently, the soil became more tender, and more water samples were available in winter. Conversely, some water samples available during the summer were not accessible during winter, and vice versa, because some sites had experienced higher water levels due to high water or runoff. Moreover, some water surfaces were covered with many reeds, making them inaccessible. The results showing the variations in the concentrations of the analyte PCs during summer and winter are shown in Tables 2a and 2b, respectively. Part of the data obtained in this study, including concentrations and respective standard deviations, is presented in the online supplementary material Tables S1a-S4b.

The mean concentrations of the PCs ranged from <LoD to >40 µg/ℓ and <LoD to <20 µg/ℓ in the water samples during summer and winter, respectively. Some pharmaceutical residues were not detected at the sampling sites, perhaps due to dilution processes as the pollutants move downstream along the river, thus reducing their concentrations. It is worth noting that the dilution phenomenon depends to some extent on the nature of the analytes of interest, including their hydrophobicity or hydrophilicity. Another essential feature of the analyte that corroborates this process is its octanol-water partition coefficient (Amézqueta et al., 2020). This feature explains the distribution of a compound between the octanol and water phases, thereby substantiating its lipophilicity and hydrophilicity. Moreover, it is essential to state that rivers possess self-purification ability (Vaideliene and Michailov), thereby leading to variations in the quantification pattern of targeted pollutants at different river sections. This reason, among others, could be part of why the concentrations of PCs in summer (<LoD to >40 µg/ℓ) were reduced by almost half in winter (<LoD to <20 µg/ℓ).

4.8 Discussion

4.8.1 Azithromycin residues in the studied surface water during summer and winter

The levels of AZI residues in the sampled surface waters during the summer and winter seasons varied significantly across the eight sampled sites, as shown in Figure 4.3. In general, AZI was detected across all three zones (P, U, D) during both seasons. Several sampling sites showed higher AZI concentrations in winter than in summer. For instance, sites such as P7 and P8 exhibited significantly elevated levels during winter. Conversely, some sites, such as P1, P4, U6 and D6, recorded high concentrations in the summer, but not as high as those obtained during winter. This may reflect seasonal patterns of pharmaceutical use, particularly the increased antibiotic prescription and consumption of antibiotics during colder months, when infection rates can be higher (Serletti et al., 2023). Moreover, this trend could be attributed to several environmental factors, including reduced microbial degradation due to cooler temperatures, lower water volumes leading to less dilution, and the continuous discharge of untreated or poorly treated wastewater, which is a known challenge in many South African municipalities (Horel and Schiewer, 2020).

When evaluating the zone-based distribution, the point-of-discharge sites generally showed the highest concentrations of AZI, highlighting the role of direct anthropogenic inputs, such as effluents from WWTPs and hospital waste. Sites P7 and P8, in particular, recorded the highest mean concentrations, slightly exceeding 19 µg/ℓ in the winter, which strongly points to WWTPs as major contributors to environmental pharmaceutical contamination (Bonnière et al., 2024). However, exceptions were observed; for example, U6 showed a relatively high level of 16.47 µg/ℓ in summer, suggesting possible non-point source pollution, perhaps from informal settlements or agricultural activity upstream. Downstream sites also revealed notable trends. For instance, sites D7 and D8 had higher concentrations in winter, indicating cumulative pollution from both upstream and discharge areas as water flows downstream.

To further analyse the seasonal differences, a paired comparison of the shared sites across both seasons was performed. Some sites showed significant seasonal variation, such as U6 and D6, with >16 µg/ℓ observed in summer. The differences between summer and winter mean values at sites P1, D4, U6, D6, P7, D7, P8, and D8 were evaluated using a paired t-test. A Pearson correlation coefficient of -0.135 was obtained, indicating a very weak negative correlation between the AZI concentrations reported across both seasons. However, t-test analysis showed that the difference between the results was not statistically significant at the 0.05 level (P calculated = 0.854). This reveals that while there are meaningful site-specific variations, there is no strong seasonal trend when considering the dataset. The environmental and policy implications of these findings are substantial. The consistently high concentrations of AZI, particularly around discharge zones, reinforce concerns about the effectiveness of South Africa's existing WWTP infrastructure. Most conventional WWTPs are not equipped to remove pharmaceutical residues (Neth et al., 2023), leading to their persistence in aquatic environments (Kayode-Afolayan et al., 2022). This poses environmental risks, notably the promotion of antimicrobial resistance among aquatic microorganisms (Okeke et al., 2022). Furthermore, climatic factors specific to the South African context, such as intense flooding and seasonal droughts, complicate sampling efforts and may influence the distribution of pollutants, including pharmaceutical residues. For instance, specific sites were inaccessible during either summer or winter due to dry riverbeds or excessive flooding. These hydrological extremes are increasingly common due to climate variability and underscore the need for adaptive water quality monitoring programmes.

4.8.2 Prednisolone residues in the studied surface water during summer and winter

The distribution of m-PRD residues in the studied water samples reveals distinct spatial and seasonal patterns that reflect underlying sources of pollution, infrastructural inefficiencies, and environmental processes. As shown in Figure 4.4, m-PRD was detected at only a limited number of sites, with significant differences between the summer and winter seasons. Most striking in the data is the exceptionally high summer concentration at site U6, which recorded a m-PRD level of nearly 40 µg/ℓ. This is the highest recorded value across both seasons and sites. Site U6 should, in theory, show lower pollution levels due to the distance from a point source. However, such a spike suggests the possibility of significant non-point source contamination, possibly from agricultural runoffs, informal settlements or illicit dumping upstream. It also raises questions about the designation of U6 along the Bloemfontein region. Furthermore, the results for m-PRD show that upstream areas can still be influenced by anthropogenic activities when the site is near dense or unregulated residential areas.

Another site showing elevated summer values is D6, with m-PRD levels nearing 18 µg/ℓ. This supports the notion of cumulative loading, where residues from both upstream and discharge zones accumulate and persist downstream, potentially due to reduced dilution during dry months and the low biodegradability of synthetic corticosteroids like m-PRD (Shen et al., 2020). During the winter season, m-PRD detections were more modest but still notable. Sites D4, D5, and P8 showed measurable levels of m-PRD ranging from approximately 8 to 12 µg/ℓ. These levels are still environmentally significant, given that m-PRD is an active pharmaceutical compound that can disrupt endocrine and immune functions in aquatic organisms at low concentrations (Islam et al., 2024).

The levels of m-PRD were not reported at many sites, especially across some zones, suggesting either true non-detection or inaccessibility due to seasonal extremes, such as flooding or dry riverbeds. The lack of winter detection at high-summer sites such as U6 and D6 may imply episodic discharges during dry periods when flow is low (Lentz et al., 2024), and concentrations spike due to reduced dilution (Du et al., 2014). The seasonal trends revealed a strong summer dominance in concentration magnitudes, particularly due to the anomalous value at U6. From an environmental policy perspective, these findings are highly concerning. m-PRD, like other corticosteroids, is not routinely removed by conventional wastewater treatment processes, which are common across South Africa (Munzhelele et al., 2024). These processes typically target organic matter and nutrients, but are not effective enough to handle emerging contaminants like pharmaceutical residues (Molinari et al., 2024). The detection of such high concentrations in surface waters, particularly at locations not designated as discharge zones, indicates potential regulatory gaps and infrastructure deficiencies, all of which contribute to environmental contamination.

The results reveal that m-PRD residues in South African aquatic systems exhibit spatial and seasonal variation, highlighting point and nonpoint pollution sources. The highest concentrations occurred in summer, notably at an upstream site, raising concerns about unregulated environmental inputs. The persistence of m-PRD at several downstream locations suggests cumulative loading. These results underscore the need for improved wastewater infrastructure, urgent pharmaceutical pollution control policies, and seasonally adaptive monitoring programmes that reflect the dynamic hydrology typical of South African catchments.

4.8.3 Dexamethasone residues in the studied surface water during summer and winter

The results obtained for DEX in this study (Figure 4.5) revealed a distinct seasonal and spatial pattern in its occurrence, with detectable concentrations observed during the summer season, while none were detected in winter. Residues of DEX were detected at four out of all site-season combinations, specifically at P4 (17.80 µg/ℓ), D4 (41.79 µg/ℓ), U8 (10.12 µg/ℓ), and D8 (19.17 µg/ℓ). However, the residues of DEX were not detected at the remaining sampling points, either because of dry riverbeds that prevented the collection of water samples or because DEX concentrations were below the analytical limit of detection.

The highest concentration (41.79 µg/ℓ) was observed at D4, a downstream site, suggesting accumulation of DEX residues or input from secondary pollution sources between the upstream and downstream segments of the sites. The elevated DEX level at D4, compared to its source, P4 (17.80 µg/ℓ), suggests potential contributions from point sources, particularly during dry seasonal conditions when dilution capacity and flow velocity are reduced. A similar pattern was observed at site 8, where the concentration increased from 10.12 µg/ℓ at U8 to 19.17 µg/ℓ at D8, further corroborating the possibility of downstream accumulation or additional contamination. The absence of DEX residues during the winter season across all sites is notable and may be attributed to several factors, such as reduced discharge from WWTPs due to seasonal flow adjustments (Indranil et al., 2024).

The environmental implications of these findings are significant. DEX, a potent glucocorticoid with endocrine-disrupting properties, can have detrimental effects on aquatic organisms (Guiloski et al., 2015). Its presence in surface water can interfere with fish reproduction, stress response mechanisms, and microbial community dynamics, especially when co-occurring with antibiotics or other pharmaceuticals (Guiloski et al., 2015). These observations align with broader concerns in South Africa about emerging contaminants, particularly pharmaceutical residues in aquatic environments. Recent studies and reports have documented the persistent presence of active pharmaceutical ingredients in treated and untreated wastewater across the country (Waleng and Nomngongo, 2022; Munzhelele et al., 2024). This is aggravated by challenges stemming mainly from inadequate public awareness of proper disposal methods and the limited capacity of WWTPs to remove pharmaceutical compounds effectively.

4.8.4 Prednisone residues in the studied surface water during summer and winter

The PRD concentrations in the water samples studied in both summer and winter, with several sites showing no detectable levels, are shown in Figure 4.6. This Figure indicates an apparent seasonal disparity in PRD levels across the sampled sites. Winter concentrations were generally higher, particularly at the point of discharge site (P6). Site P6 recorded the highest winter concentration at 13.49 µg/ℓ. In contrast, site U5 (upstream zone) showed a higher summer concentration (11.19 µg/ℓ), whereas site D5 (downstream zone) showed a lower concentration (7.14 µg/ℓ).

These results further highlight the variability driven by hydrological conditions. The elevated PRD concentration at the discharge point indicates that these residues enter South African water bodies primarily through WWTP effluents. The absence of detectable PRD at upstream sites such as U4 and U6 suggests limited diffuse sources in those areas, though runoff from agricultural or urban activities may contribute to downstream concentrations, as observed in D5 during summer. The ageing WWTP infrastructure in South Africa and limited treatment capacity worsen pharmaceutical pollution, particularly in urban catchments like those along the uMngeni River, with high concentrations of antibiotics and analgesics (Omotola and Olatunji, 2020).

4.8.5 Distribution of the total concentration of the four studied analytes in both seasons

The concentrations of the four studied analytes in water samples exhibited spatial and seasonal variations, as illustrated in Figure 4.7. The most consistently detected pharmaceutical across all sampling sites and in both seasons was AZI, with remarkably elevated concentrations observed at specific sites (S) S1, S4, S6, S7, and S8. The highest AZI concentration was recorded at site four during winter, while relatively lower concentrations were observed at sites 2 and 5.

Residues of m-PRD showed a highly variable seasonal and spatial pattern, with its most prominent occurrence at S6 during the winter season. This site also displayed elevated levels of AZI and PRD in summer and winter, respectively, suggesting a potential hotspot of pharmaceutical contamination. At a few sites, DEX was detected, with the highest concentration recorded at S5 in summer, with moderate levels at S4 and S8. This trend in its detection may indicate selective usage patterns or differences in its persistence in the environment. While PRD levels were lower than those of AZI, m-PRD, and DEX overall, their presence alongside other pharmaceuticals highlights the accumulated contamination burden at specific sites.

Furthermore, AZI concentrations tended to be higher in the summer months across most sites, signifying greater pharmaceutical input or enhanced persistence during this period. The data obtained in this study suggest seasonal differences, perhaps driven by environmental factors such as temperature and rainfall, or by changes in pharmaceutical use, which might have influenced the distribution and concentration of these compounds. Conclusively, the elevated concentrations of targeted pharmaceutical residues observed at S5, S6, and S8 point out the potential risks of pharmaceutical pollution in the studied aquatic matrices and highlight the need for continuous monitoring and mitigation strategies.

4.8.6 Comparison of the levels of pharmaceuticals detected in this study with other studies

Studies investigating the occurrence of PRD, m-PRD, DEX, and AZI in African surface waters remain scarce. While a limited number of studies have reported their presence in surface waters globally, comparable data from Africa are largely absent. This underscores a substantial continental knowledge gap and highlights the need for targeted regional monitoring. Consequently, the results of the present study are compared with the available global data on the targeted analytes, without a specific focus on Africa.

Although AZI has been stated to be among the least studied antibiotic groups, called macrolides (Loos et al., 2018; Sousa et al., 2018; Pizzini et al., 2024), it was an important PC examined for its presence in the environment due to its usage before and during the COVID-19 pandemic. The AZI levels in water during winter ranged from 3.23 ± 0.06 µg/ℓ to 19.32± 0.26 µg/ℓ. These values are higher than those reported in the water samples obtained from the northern part of the Persian Gulf around Bushehr port (Mirzaie et al., 2022). Also in this study, 1.10 µg/ℓ AZI, which is slightly lower than the maximum concentration (1.27 µg/ℓ) of AZI reported along the El Albujon water course in Southern Spain (Moreno-González et al., 2014). During summer, the PC with the highest concentration was DEX, with 41.79 ± 1.92 µg/ℓ. This value obtained for DEX is higher than those reported for flush water from a swine farm in Bohai, China (Liu et al., 2012) and Lake Michigan's water (Boy-Roura et al., 2018), respectively. A larger percentage of the sampling sites investigated showed traces of PRD, with most concentrations below the limit of detection. However, its traces were only found in summer water samples, ranging from 7.14 µg/ℓ to 11.19 µg/ℓ. These values of PRD obtained in this study are higher than those recently reported in the water samples from an agricultural area of Guangzhou City, China (Lin et al., 2024). Residues of m-PRD were only detected both in the downstream zones (7.70 ± 0.13 µg/ℓ and

12.14 ± 0.52 µg/ℓ) and at the point of discharge (11.86 ± 0.22 µg/ℓ). These values fall within or slightly below the range (8.78 µg/ℓ – 448.20 µg/ℓ) of m-PRD levels detected in the influent samples investigated within the KwaZulu-Natal region of Durban, South Africa (Inarmal and Moodley, 2023). Conversely, the m-PRD values reported in Chinese flush water (1.39 µg/ℓ) by Liu et al. (2012) are below those reported in this study.

Residues of PRD showed the lowest occurrence and concentration patterns compared to m-PRD during summer, while this trend was similar for DEX residues during winter. This could be traced to the fact that PRD is usually converted to m-PRD in the liver, and that 20% of it is released through the excretion of urine (Chun et al., 2007; Bashar et al., 2018). As such, the presence of PRD residues in the environment can be safely linked to non-point sources, such as the inappropriate disposal of expired or unused medications into the ecosystem.

CHAPTER 5 [3]
DEPTH-RESOLVED SEASONAL INVESTIGATION OF PHARMACEUTICAL RESIDUES IN SEDIMENTS FROM BLOEMFONTEIN, SOUTH AFRICA

5.1 Introduction

Pharmaceutical residues have become increasingly prevalent contaminants of emerging concern in aquatic environments worldwide. Their presence in sediment samples has drawn the attention of environmental scientists and policymakers due to the persistence, bioactivity, and potential risks these substances pose to aquatic ecosystems and public health (Dankwa et al., 2024; Tegegne, Mekasha, Ayu, Hasen, and Suleman, 2024; Adedipe et al., 2024; Korkmaz, Caglar, and Aksu, 2022). These compounds originate from a variety of anthropogenic sources, primarily including human and veterinary medicinal usage. Once administered, pharmaceuticals can be excreted in unmetabolized or partially metabolised forms, eventually entering the environment via wastewater treatment effluents, surface runoff from agricultural lands, leaching from landfills, and improper disposal of unused medications (Nand et al., 2025; Sanganyado, 2025; Byers et al., 2025; Sardar et al., 2025; Choudhury et al., 2025; Okeke, Nwankwo, Ezeorba, Ogugofor, and Nwuche, 2025; Pappas et al., 2025; Royano, Navarro, Torre, and Martínez, 2024). A major environmental concern is the ability of sediments in aquatic environments to act as both reservoirs (sinks) and sources for pharmaceutical contaminants (Dulsat-Masvidal et al., 2025). Due to their hydrophobic nature, many pharmaceutical residues have a strong affinity for particulate matter, facilitating their accumulation in bottom sediments (Samal, Mahapatra, and Hibzur Ali, 2022; Gworek, Kijeńska, Wrzosek, and Graniewska, 2021). Over time, these sediments can release contaminants back into the overlying water column under changing environmental conditions, thus serving as secondary sources of pollution (Lv, Luo, Zhao, Wang, and Mai, 2021). This persistence leads to long-term ecological risks, including the disruption of microbial communities, toxicity to benthic organisms, bioaccumulation through the food chain, and the propagation of antimicrobial resistance genes—a public health threat of global concern (Chauhan et al., 2023; Kayode-Afolayan, Ahuekwe, and Nwinyi, 2022; Pinto, Simões, and Gomes, 2022; Zenker, Cicero, Prestinaci, Bottoni, and Carere, 2014).

Recent studies conducted across various regions of Africa have demonstrated the widespread detection of pharmaceutical residues in environmental compartments, including surface waters, sediments, and biota (Thavarayan andMoodley, 2025; Okeke et al., 2025; Hossein andRipanda, 2025; Munzhelele, Ayinde, Gitari, Pindihama, and Mudzielwana, 2025; Omotola andOlatunji, 2020). However, data specific to sediment contamination in Southern Africa remains relatively limited (as compared to their detection in water samples), thereby necessitating more localised studies to inform mitigation strategies. Given the importance of sediments in environmental fate and transport processes, their analysis is crucial for comprehensive environmental monitoring programmes. The present study was therefore designed to assess the occurrence and concentration of selected pharmaceutical residues in surface sediment samples collected within South Africa. Specifically, four pharmaceutical compounds were targeted: prednisone, prednisolone, dexamethasone, and azithromycin. These compounds were selected due to their widespread use in both human and veterinary medicine and, most importantly, their usage during the COVID-19 pandemic (Liu et al., 2023; Khan, Kar, and

[3] Chapter 5: https://doi.org/10.1016/j.envc.2025.101267.

Roy, 2023; Xing et al., 2022; Wang et al., 2020). As such, this study presents a comprehensive assessment of the seasonal occurrence and distribution of azithromycin, prednisolone, dexamethasone, and prednisone in selected surface sediment samples collected from the Bloemfontein Environs, South Africa. While several studies have investigated pharmaceutical residues in sediments globally, our study provides insights by focusing on the depth- and seasonally resolved distribution of selected pharmaceuticals, including dexamethasone, in sediments from Bloemfontein, South Africa.

Recent studies have reported the occurrence of dexamethasone in wastewater and aquatic systems (Piazza et al., 2025; Tanui et al., 2025; Arumugam et al., 2025; Xiang et al., 2024), but data on its occurrence pattern in sediment matrices remain limited, particularly in African environments. Our study seeks to bridge this gap by assessing dexamethasone alongside other pharmaceuticals in a spatiotemporal sediment context. To our knowledge, this is among the few studies in Southern Africa that integrates both vertical sediment profiling and seasonal variation of these compounds.

The analytical approach employed in this investigation used liquid chromatography coupled with mass spectrometry (LC−MS), a sensitive and selective technique for detecting trace levels of pharmaceutical contaminants in complex matrices, such as sediment. The use of LC tandem MS is on the rise, especially in developed countries, for the detection of trace pollutants. One limitation of this study is the use of a single-quadrupole LC−MS (Shimadzu 2020), which, while sufficient for quantitative screening and detection of pharmaceutical residues at moderate levels, offers lower sensitivity compared to tandem MS (LC−MS/MS) systems. This may have affected the detection limits of certain compounds of interest, especially those present at ultra-trace levels. Moreover, LC tandem MS utilises two stages of mass spectrometry, offering better selectivity and reduced background noise, thereby improving detection limits. The additional stage allows for the isolation and fragmentation of specific ions, followed by the detection of their daughter ions, which enhances the signal-to-noise ratio and allows for the detection of very low concentrations of analytes (Antoniewicz, 2013). The findings from this study are expected to contribute significantly to the growing body of literature on pharmaceutical pollution in African aquatic and terrestrial environments.

5.2 Materials and methods

In this study, analytical-grade reagents and high-purity solvents were used to ensure accurate and reliable detection and quantification of pharmaceutical residues in sediment samples. Neat standards of prednisone, prednisolone, dexamethasone, and azithromycin were procured from Sigma-Aldrich, South Africa. These standards were used to prepare calibration curves and quality-control samples for quantitative analysis. LC−MS grade acetonitrile and liquid chromatography (LC) grade methanol were also obtained from Sigma-Aldrich, South Africa, and served as the primary organic solvents for sample extraction and instrumental analysis. Additionally, HPLC-grade methanoic acid was sourced from Honeywell (New Germany, South Africa) and utilised in the mobile phase to enhance ionisation efficiency during mass spectrometric detection. Solid-phase extraction (SPE) was employed for sample cleanup and preconcentration. Hydrophilic-lipophilic balance (HLB) SPE cartridges (6 mℓ, 200 mg) were purchased from Sigma-Aldrich, US. These cartridges are widely recognised for their efficiency in extracting a broad range of polar and non-polar compounds from aqueous and solid matrices, making them suitable for the pharmaceutical residues under investigation. MilliQ water, used in all aqueous preparations and LC−MS analysis, was produced using an Elix Millipore Water Purification System (Model Reference: PR0G0T0S2). This ensured the elimination of ionic, organic, and microbial

impurities that could otherwise compromise analytical performance. All other chemicals, reagents, and solvents utilised throughout the study were of analytical grade and were used without further purification. Careful attention was paid to reagent handling and storage conditions to prevent contamination and maintain the integrity of the analytical procedures.

5.2.1 Software and Instrumentation

The analytical method employed in this study was developed and executed on a single-quadrupole Shimadzu LC−MS 2020 system, which features a quaternary pump for precise solvent delivery and gradient elution. Due to equipment availability and resource constraints typical in many African analytical laboratories, the single-quadrupole system was utilised for this study. Despite its limitations, the instrument provided sufficient resolution to quantify the target pharmaceuticals within the observed concentration range. This system enabled the accurate detection and quantification of the selected pharmaceutical compounds in the sediment matrices. Data processing, regression analysis, and other nonparametric statistical evaluations were conducted using Microsoft Excel 2016. This software package was utilised to construct calibration curves, determine correlation coefficients, and perform basic descriptive analyses on the measured concentrations. All weighing of pharmaceutical standards was performed using a Mettler Toledo analytical balance, known for its high precision and reliability in laboratory measurements. pH measurements for various sample preparation steps were performed using a pre-calibrated digital pH meter to ensure consistent, accurate pH control throughout the analytical workflow. Sample preparation steps also included using a Hermle centrifuge to separate supernatants from solid residues during the extraction process. Furthermore, an Emerson Branson ultrasonic bath was used to enhance analyte extraction from sediment samples by promoting particle dispersion and solvent penetration. The OpenStreetMap application was used to visually represent the geographic distribution of sediment sampling sites and to generate a detailed map of the study area. This facilitated a spatial understanding of sampling locations and helped interpret the environmental context of the findings.

5.2.2 Preparation of stock solutions of pharmaceutical compounds

Approximately 0.01 g of each pharmaceutical compound was accurately weighed and dissolved in methanol (MeOH) using separate 10 mℓ volumetric flasks. Each solution was diluted to the mark to yield a stock concentration of 1000 µg/mℓ for the respective analyte. The stock solutions were stored at approximately 4 °C in a refrigerator until required. Working standard solutions ranging from 0.01 to 0.10 µg/mℓ were prepared by serial dilution of the stock solutions in methanol to obtain the desired concentration range for calibration curve development.

5.2.3 Instrumental method development

Method development began with a single run for each analyte over a specified concentration range. This was achieved using a Shimadzu C18 reversed-phase column on a Shimadzu 2020 Liquid Chromatograph coupled to a Mass Spectrometer. The objective was to establish a unified method capable of simultaneously detecting, separating, and quantifying the selected pharmaceutical compounds. The method development process involved optimising key instrument parameters, including mobile phase composition and pH, column oven temperature, injection volume, and flow rate, to achieve optimal analyte detection. Retention times for each compound were determined under these optimised conditions. To evaluate the resolution of the analytes, a mixed solution containing all four compounds was analysed using gradient elution, which effectively separated

the target compounds. Subsequently, calibration curves were constructed for each analyte to assess the instrument's response to varying concentrations. The method was validated in accordance with the International Conference on Harmonisation (International Conference on Harmonisation, 2005) guidelines. Analyses were carried out in duplicates.

5.2.4 Suitability testing and method validation

System suitability and method validation were carried out in accordance with ICH guidelines, using data from instrument calibration and analyte recovery studies. The purpose was to verify that the developed method met the required performance and applicability standards. Suitability tests, modelled after the procedures described by Omotola and Olatunji (2020), included evaluations of peak tailing factors, chromatographic resolution, and theoretical plate numbers. Validation parameters assessed included specificity, sensitivity, selectivity, linearity, linear range, limit of detection (LoD), limit of quantification (LoQ), and precision. The stability of the analytes was tested over five consecutive days at 4 °C using a quality control standard containing 0.10 µg/mℓ of the compound mixture. All methanol-based stock dilutions remained stable at temperatures below 25 °C. The optimised chromatographic conditions were applied across all sample analyses to consistently determine peak intensities and areas for each analyte.

5.2.5 Extraction/recovery efficiency of analytes from sediment samples

Sediment samples were sieved using a 200-micron mesh-size sieve after being dried at room temperature in the laboratory. About 5.0 g of the sieved sediments was weighed in 50 mℓ centrifuge tubes. Thereafter, sequential extraction of the pharmaceutical compounds (PCs) was achieved using 10 mℓ of 2% aqueous ammonia in methanol, followed by 10 mℓ of 2% methanoic acid in methanol, and finally with 10 mℓ of pure methanol. For each addition of extractant, the mixture was vortexed for 30 seconds, sonicated for 5 minutes, and centrifuged for 5 minutes at 6 000 rpm. Subsequently, the supernatants were combined and diluted with a water sample (300 mℓ) plus 12.5 mℓ 0.25 M EDTA. This sample mixture was thereafter subjected to a solid-phase extraction process using extraction columns consisting of hydrophilic-lipophilic cartridges (Supel-Select) (200 mg/6 mℓ). These cartridges were conditioned by eluting with 5 mℓ of acetonitrile, followed by sequential elution with buffered water (pH 4), using a few drops of 6 M HCl, through the column at a flow rate of 1-2 mℓ/min. Subsequently, the sample was loaded on the conditioned column and eluted at a flow rate of 7-9 mℓ/min. The efficiencies of analyte recovery were validated by calculating each recovery (%) for the spiked concentrations using equation I.

$$Percent\ Recovery\ (\%) = \frac{A-B}{C} \times 100 \ldots\ldots \text{eq I}$$

Where A is the peak area of each PC after extraction of the spiked sample, B is the peak area of the analyte after extraction of the non-spiked sample (water or sediment), $and\ C$ is the peak area of each analyte that was used for spiking. The concentrations of analyte PCs in sediments were calculated using equation II.

$$Concentration\ of\ PCs\ in\ sediments\ (ng/g) = \frac{X_{(\mu g/L)} \times Y_{(L)}}{mass\ of\ sediment\ used\ (g)} \times 1000 \ldots\ldots \text{eq II}$$

Where $X_{(\mu g/L)}$ is the concentration of each PC residue obtained from the respective equation of the graph; $Y_{(L)}$ is the volume (1 mℓ ~ 0.001 L) of the concentrated analytes extracted after SPE, while the mass of the sediment sample used was 5.0 g.

5.2.6 Sample collection

Sediment samples were collected from eight different sampling points within Bloemfontein, South Africa, during the summer and winter periods of 2024. Sediment samples were collected between 0 and 15 cm depth using an auger. These locations include the Gariep area (where two samples were obtained from different spots, Sites 1 and 2), Aliwal North (Site 3), Zastron (Site 4), Ladybrand (Site 5), Maseru (Site 6), Ficksburg (Site 7), and Clarens (Site 8). The samples were collected in 5cm depth increments, such as (0−5 cm; 5−10 cm and 10−15 cm). All samples were collected from the point of discharge (P), the upstream (U) and downstream (D) regions of each sampling site, except when samples were not accessible or too rocky for collection. The map of all sampling sites is shown in Figure 5.1.

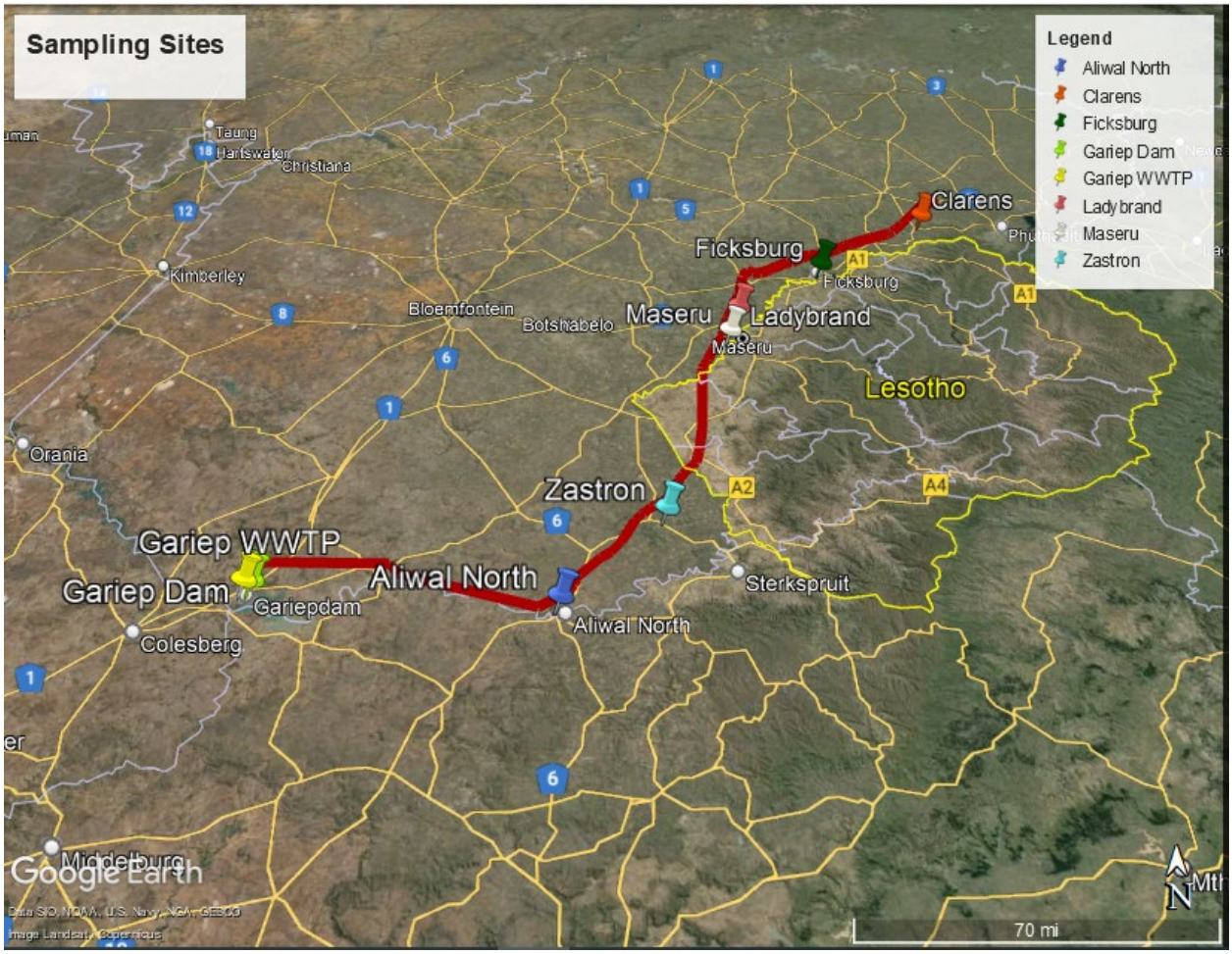

Figure 5.1 Map of sampling points

The sediment samples were collected in 0.5ℓ glass bottles, pre-cleaned with 0.1% nitric acid. Samples were labelled, stored in ice-chest coolers to maintain integrity during transport, and refrigerated at 4 °C in the laboratory. Analysis was performed within 5 days of collection. Before analysis, samples were air-dried, ground, and sieved.

5.3 Results and discussion

5.3.1 Optimisation of instrument parameters for effective separation

To achieve effective separation and detection of prednisone, prednisolone, dexamethasone, and azithromycin, various mixtures of acetonitrile and Milli-Q water, each containing 0.1% methanoic acid, were evaluated as the mobile phase. Separation was achieved by gradient elution on a C18 column (2.1 × 100 mm, 2.6 µm), with the mobile phase composition adjusted during the run. The aqueous phase (A) consisted of 0.1% methanoic acid in Millipore water, while the organic phase (B) contained 0.1% methanoic acid in acetonitrile. The optimal gradient programme began with 10% B, increased to 35% B within 2 minutes, and then returned to 10% B by the end of the 10-minute run. Instrument conditions were optimised as follows: a flow rate of 0.25 mℓ/min, a column temperature of 40 °C, and an injection volume of 5 µℓ. The resulting MS chromatogram, presented in Figure 5.2, illustrates the successful separation, retention times, and efficiency for all four compounds.

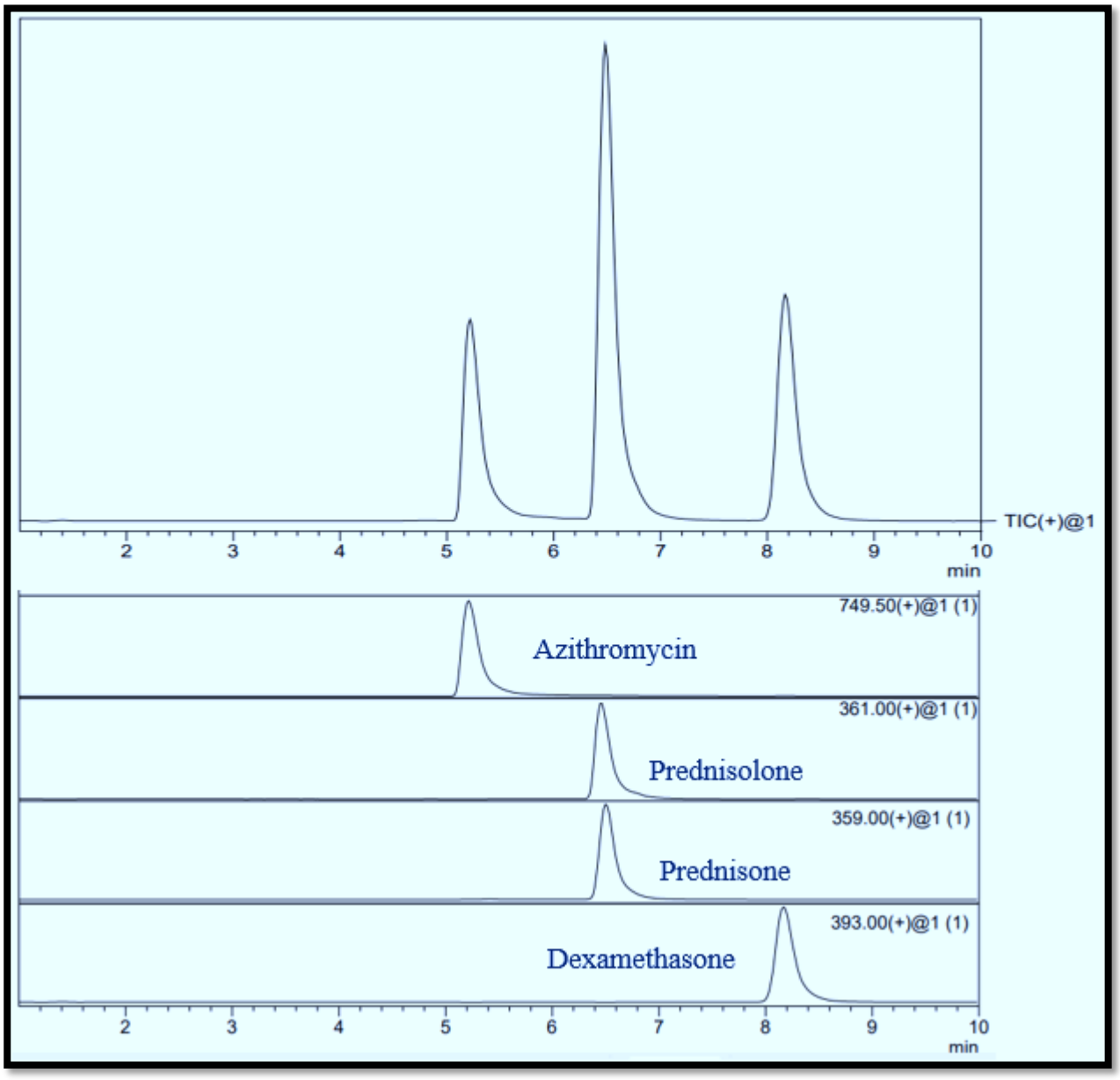

Figure 5.2 MS chromatogram of the four PC analytes studied

The retention times for azithromycin, prednisolone, prednisone, and dexamethasone were 5.25, 6.48, 6.51, and 8.21 minutes, respectively. Mass spectrometric analysis was performed using positive electrospray ionisation (ESI) mode for all compounds. Preliminary optimisation trials during method development indicated better ionisation efficiency in the positive mode for the analytes studied.

Generally, compounds with acidic functional groups (like carboxylic acids) tend to ionise more readily in negative mode (ESI-). In contrast, those with basic functional groups (such as amines) ionise more readily in positive mode (ESI+). This is because negative mode involves deprotonation, and positive mode involves protonation. However, the best mode for ionisation can depend on the specific molecule and experimental conditions (Saitman, 2019; Paull andNesterenko, 2013). The use of positive ESI mode was supported by recovery studies conducted during method validation, which showed acceptable recovery rates above 80% for all analytes. The characteristic fragmentation patterns for each compound were identified using selected reaction monitoring (SRM), with m/z values of 749.50 for azithromycin, 361.00 for prednisolone, 359.00 for prednisone, and 393.00 for dexamethasone. These values were confirmed through five replicate online injections for each analyte.

5.3.2 System suitability

System suitability tests are essential to method validation, ensuring that analytical instruments and processes function reliably. These tests assess the chromatographic system's performance based on the precision and accuracy of the analyte peak characteristics. Key parameters include resolution, theoretical plate count, system precision (expressed as %RSD of retention times), and tailing factor (also known as the symmetry factor). For each pharmaceutical compound (PC), these parameters were calculated from repeated sample injections, following the approach described by Omotola and Olatunji (2020). According to the Cder (1994), acceptable system suitability criteria are as follows: resolution between two adjacent peaks should be greater than 2; tailing factor should not exceed 2; system precision should be ≤5% RSD; and the number of theoretical plates should typically be above 2000. In this study, the average theoretical plate number for the chromatographic column was 2601.32 ± 834.23, indicating sufficient column efficiency. System precision showed %RSD values for retention times of all analytes to be ≤1.98 across five injections. These results confirm that all system suitability parameters fall within the acceptable limits, validating the reliability and robustness of the analytical method.

5.3.3 Method validation

A mixture containing varying concentrations of each analyte (ranging from 0.01 to 0.1 µg/mℓ) was injected into the chromatographic column under optimal conditions to obtain distinct chromatographic peaks for all analytes. The retention times of the compounds remained consistent across twelve replicate injections (n = 12) of the PCs mixture, confirming the method’s specificity. Moreover, six concentration levels of the analyte mixture, ranging from 0.01 to 0.10 ng/µℓ, were prepared, and each level was injected into the LC−MS system (n = 12). Calibration curves were constructed by plotting the peak area of each analyte against its corresponding concentration. The following correlation coefficients (R^2) were obtained: azithromycin, R^2 = 0.9883; prednisolone, R^2 = 0.9983; prednisone, R^2 = 0.9992; and dexamethasone, R^2 = 0.9976. These values exceed the minimum acceptable threshold of 0.90 set by the International Council for Harmonisation (ICH), indicating strong linearity. Although the responses varied slightly across compounds, the linear regression equations within the 0.01–0.10 µg/mℓ range confirmed linearity, supporting the method’s validity for quantitative analysis.

According to Shrivastava and Gupta (2011) and Omotola and Olatunji (2020), limit of detection (LoD) and limit of quantification (LoQ) represent the minimum detectable and quantifiable concentrations of an analyte with acceptable precision and accuracy. These values were calculated based on the signal-to-noise ratio from repeated blank measurements, corresponding to three times the noise level for LoD and ten times for LoQ. The calculations used the formulas: LoD = 3s/m and LoQ = 10s/m, where **s** is the standard error of the mean and **m** is the slope of the calibration curve. The resulting LoDs (µg/ℓ) for azithromycin, prednisolone, prednisone, and dexamethasone were 0.0209, 0.0079, 0.0053, and 0.0094, respectively. Corresponding LoQs were 0.0696, 0.0262, 0.0176, and 0.0312.

In this study, both intra-day (repeatability) and inter-day (reproducibility) precision were examined. Repeatability was evaluated by performing five injections of a 0.10 µg/mℓ quality control sample within 24 hours. Reproducibility was assessed by repeating the procedure daily for 5 consecutive days. The method's accuracy and precision were determined by calculating the mean and relative standard deviation (RSD) of the results. The %RSD for both intra-day and inter-day measurements was ≤8.78%, which falls well within the acceptable limit of 15% as recommended by Zhou and Wang (2017) and reaffirmed by Omotola and Olatunji (2020). Furthermore, the consistency in assay values over five days confirms the stability of the stock solutions and analytes, validating the reliability of the developed method.

5.3.4 Recoveries (%) of PCs from aqueous solutions and sediments

Analyte recovery, expressed as a percentage, reflects the efficiency of an extraction method in retrieving a known quantity of analyte from a sample matrix. In this study, recovery was assessed by conditioning HLB cartridges, loading isolated analyte mixtures from sediment samples, and drying and eluting the analytes from the adsorbent column. Three concentrations of the analyte PCs; 0.01 µg/mℓ, 0.04 µg/mℓ, and 0.10 µg/mℓ, were tested for recovery efficiency. The percentage recoveries for each analyte in aqueous solutions ranged from 83.34 ± 1.01% to 98.30 ± 0.69%, while those in sediment samples ranged from 82.26 ± 0.47% to 95.20 ± 2.02%. All recovery values fell within the acceptable limits set by ICH guidelines (2005), indicating satisfactory method performance for both matrices.

5.4 Analysis of PC residues in sediment samples during summer and winter

Residues of four pharmaceutical compounds were detected at varying concentrations across eight sediment sampling sites within Bloemfontein, Free State, South Africa. These sites were categorised into three hydrological zones, namely point of discharge (P), upstream (U), and downstream (D). The sediment samples were collected during both seasons from three depth intervals: 0–5 cm, 5–10 cm, and 10–15 cm, for a total of 44 sediment samples during summer and 45 during winter. In the sediment samples, the levels of the four analyte PCs varied greatly among the three different depths of sediments under study for both seasons, while others were either not detected or below the instrument's limit of detection (**Supplementary information 1a to 4b**). The mean concentrations of the targeted analytes are shown in Tables 5.1 and 5.2. All the standard deviation data are presented in Supplementary Information 1a-4b**.**

Table 5.1 Mean levels (ng/g) of targeted PCs in sediments during summer (n=88)

Compound	Zone	Depth	S1	S2	S3	S4	S5	S6	S7	S8	∑PC
Azithromycin	P	5 cm	<LoD	1.12	<LoD	<LoD	<LoD	—	<LoD	<LoD	
		10 cm	0.49	<LoD	<LoD	—	<LoD	—	<LoD	<LoD	
		15 cm	0.40	<LoD	<LoD	—	<LoD	—	1.08	—	
	U	5 cm	—	—	—	<LoD	0.87	0.91	0.23	<LoD	
		10 cm	—	—	—	—	<LoD	0.68	0.63	<LoD	
		15 cm	—	—	—	—	<LoD	<LoD	1.49	<LoD	
	D	5 cm	—	—	—	<LoD	<LoD	<LoD	<LoD	3.39	
		10 cm	—	—	—	—	<LoD	<LoD	<LoD	<LoD	
		15 cm	—	—	—	—	<LoD	<LoD	<LoD	<LoD	
											11.30
Prednisolone	P	5 cm	<LoD	<LoD	1.28	<LoD	2.49	—	0.62	4.41	
		10 cm	<LoD	<LoD	<LoD	—	1.01	—	0.53	<LoD	
		15 cm	<LoD	<LoD	<LoD	—	1.58	—	<LoD	—	
	U	5 cm	—	—	—	1.98	<LoD	<LoD	<LoD	2.40	
		10 cm	—	—	—	—	0.77	0.42	<LoD	1.20	
		15 cm	—	—	—	—	0.69	<LoD	<LoD	<LoD	
	D	5 cm	—	—	—	<LoD	<LoD	<LoD	1.25	<LoD	
		10 cm	—	—	—	—	<LoD	0.67	0.50	<LoD	
		15 cm	—	—	—	—	0.74	<LoD	0.47	<LoD	
											23.01
Dexamethasone	P	5 cm	<LoD	<LoD	<LoD	<LoD	<LoD	—	<LoD	4.69	
		10 cm	0.79	<LoD	<LoD	—	<LoD	—	<LoD	1.47	
		15 cm	<LoD	<LoD	<LoD	—	<LoD	—	3.77	—	
	U	5 cm	—	—	—	<LoD	1.13	<LoD	<LoD	6.33	
		10 cm	—	—	—	—	1.62	<LoD	<LoD	<LoD	
		15 cm	—	—	—	—	<LoD	<LoD	1.29	<LoD	
	D	5 cm	—	—	—	<LoD	12.18	<LoD	1.21	<LoD	
		10 cm	—	—	—	—	1.36	<LoD	<LoD	<LoD	
		15 cm	—	—	—	—	4.29	<LoD	<LoD	<LoD	
											40.13
Prednisone	P	5 cm	1.02	<LoD	0.59	<LoD	<LoD	—	<LoD	<LoD	
		10 cm	<LoD	<LoD	<LoD	—	<LoD	—	<LoD	<LoD	
		15 cm	<LoD	1.22	<LoD	—	<LoD	—	0.41	—	
	U	5 cm	—	—	—	<LoD	0.85	<LoD	1.44	2.16	
		10 cm	—	—	—	—	<LoD	<LoD	<LoD	<LoD	
		15 cm	—	—	—	—	<LoD	<LoD	<LoD	<LoD	
	D	5 cm	—	—	—	<LoD	<LoD	<LoD	<LoD	<LoD	
		10 cm	—	—	—	—	<LoD	<LoD	<LoD	<LoD	

Compound	Zone	Depth	S1	S2	S3	S4	S5	S6	S7	S8	∑PC
		15 cm	—	—	—	—	<LoD	<LoD	<LoD	<LoD	
											7.69
Total Σ PCs											82.12

Values below the limit of detection are denoted as "<LoD". Sampling depths: 5, 10, and 15 cm. Zones: P = Point of discharge, U = Upstream, D = Downstream. Sites: S1–S8. '_' represents sites where samples were not obtained due to dry beds or flooding during summer and winter, respectively.

Table 5.2 Mean Levels (ng/g) of targeted PCs in sediments during winter (n=90)

Compound	Zone	Depth	S1	S2	S3	S4	S5	S6	S7	S8	∑PC
Azithromycin	P	5 cm	1.74	<LoD	<LoD	2.05	0.80	0.69	<LoD	<LoD	
		10 cm	<LoD	<LoD	<LoD	2.67	<LoD	0.94	<LoD	—	
		15 cm	<LoD	<LoD	0.72	—	<LoD	0.66	<LoD	—	
	U	5 cm	—	—	—	1.12	0.88	1.07	<LoD	0.70	
		10 cm	—	—	—	1.10	<LoD	0.83	0.69	<LoD	
		15 cm	—	—	—	—	<LoD	0.55	1.03	<LoD	
	D	5 cm	—	—	—	0.73	—	<LoD	0.71	2.46	
		10 cm	—	—	—	—	—	1.05	<LoD	0.70	
		15 cm	—	—	—	—	—	1.03	<LoD	<LoD	
											24.92
Prednisolone	P	5 cm	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	
		10 cm	<LoD	<LoD	<LoD	<LoD	<LoD	2.29	<LoD	—	
		15 cm	<LoD	<LoD	<LoD	—	<LoD	<LoD	<LoD	—	
	U	5 cm	—	—	—	<LoD	0.96	<LoD	<LoD	<LoD	
		10 cm	—	—	—	<LoD	<LoD	<LoD	<LoD	<LoD	
		15 cm	—	—	—	—	<LoD	<LoD	<LoD	<LoD	
	D	5 cm	—	—	—	<LoD	—	<LoD	<LoD	<LoD	
		10 cm	—	—	—	—	—	<LoD	<LoD	0.56	
		15 cm	—	—	—	—	—	<LoD	<LoD	<LoD	
											3.81
Dexamethasone	P	5 cm	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	
		10 cm	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	—	
		15 cm	<LoD	<LoD	<LoD	—	<LoD	<LoD	<LoD	—	
	U	5 cm	—	—	—	<LoD	<LoD	<LoD	<LoD	4.91	
		10 cm	—	—	—	<LoD	<LoD	<LoD	<LoD	<LoD	
		15 cm	—	—	—	—	<LoD	<LoD	<LoD	<LoD	
	D	5 cm	—	—	—	<LoD	—	<LoD	<LoD	<LoD	
		10 cm	—	—	—	—	—	<LoD	<LoD	<LoD	
		15 cm	—	—	—	—	—	<LoD	<LoD	<LoD	
											4.91
Prednisone	P	5 cm	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	

Compound	Zone	Depth	S1	S2	S3	S4	S5	S6	S7	S8	∑PC
		10 cm	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	—	
		15 cm	<LoD	<LoD	<LoD	—	<LoD	<LoD	<LoD	—	
	U	5 cm	—	—	—	<LoD	<LoD	<LoD	<LoD	<LoD	
		10 cm	—	—	—	<LoD	<LoD	<LoD	<LoD	<LoD	
		15 cm	—	—	—	—	<LoD	<LoD	<LoD	<LoD	
	D	5 cm	—	—	—	<LoD	—	<LoD	<LoD	<LoD	
		10 cm	—	—	—	—	—	<LoD	<LoD	<LoD	
		15 cm	—	—	—	—	—	<LoD	<LoD	<LoD	
											0.00
Total Σ PCs											33.64

The average concentration of azithromycin during summer ranged between 0.23 ±0.01 ng/g in the 5 cm sediment layer found at the upstream of site 7 and a relatively high level of 3.39 ± 0.07 ng/g in the 5 cm section of the downstream region of site 8. During winter, the mean concentration of azithromycin ranged between 0.55 ±0.00 ng/g in the 15 cm sediment layer found at the upstream of site 6 and a relatively high level of 2.67 ± 0.07 ng/g in the 10 cm section of the point of discharge region of site 4. Notably, the second-highest mean value (2.46 ± 0.02 ng/g) of azithromycin was found at the 5 cm portion of the downstream region of site 8. For prednisolone, mean levels ranged from 0.42 ± 0.01 ng/g in the upstream 10 cm layer of site 6 sediment samples to 4.41 ± 0.27 ng/g in the 5 cm layer of the point of discharge of site 8, while others were <LoD during summer. In winter, prednisolone residues were not detected in the eight sites except at sites 5, 6, and 8 at levels ranging from 0.56 ± 0.02 ng/g at the 10 cm downstream subset of site 8 to 2.29 ± 0.01 ng/g at the 10 cm point of the discharge zone of site 6. The mean concentrations of dexamethasone during summer ranged from 0.79 ±0.00 ng/g in the 10 cm layer of the point of discharge of site 1 to 12.18 ± 0.28 ng/g in the 5 cm portion of the sediment samples obtained from the downstream zone of site 5. Conversely, dexamethasone was not detected at any of the eight sites during winter, except at the 5 cm section of the upstream at site 8, where it was detected at 4.91 ± 0.05 ng/g. Prednisone residues were not detected at all in the winter sediments; their levels ranged from 0.41 ±0.02 ng/g in the point of discharge (15 cm) in site 7 to 2.16 ± 0.25 ng/g in the upstream 5 cm section of site 8.

5.4.1 Seasonal azithromycin levels in sediment samples

The azithromycin levels ranged from below the detection limit to 3.4 ng/g, observed in the downstream zone of site 8 during summer at a sediment depth of 15 cm (Figure 5.3). Moreso, higher concentrations of azithromycin were generally recorded at the point of discharge and downstream regions of the sampling sites, indicating that anthropogenic sources, particularly wastewater effluents, have a greater impact in these areas. Site 8 consistently showed the highest azithromycin concentrations in both winter and summer, with a marked peak at the deepest sediment layer (15 cm) in summer. On the other hand, sites 2 and 3 exhibited slight contamination, with azithromycin residues either undetected or <1 ng/g across all seasons and depths. It is worth noting that the absence of values at multiple sites and depths is attributed either to sample-collection challenges, such as very soft sediment beds unsuitable for sampling, or to concentrations below the limit of detection, reflecting low or negligible environmental loading at those locations. This applies to other pharmaceutical residues detected in this study.

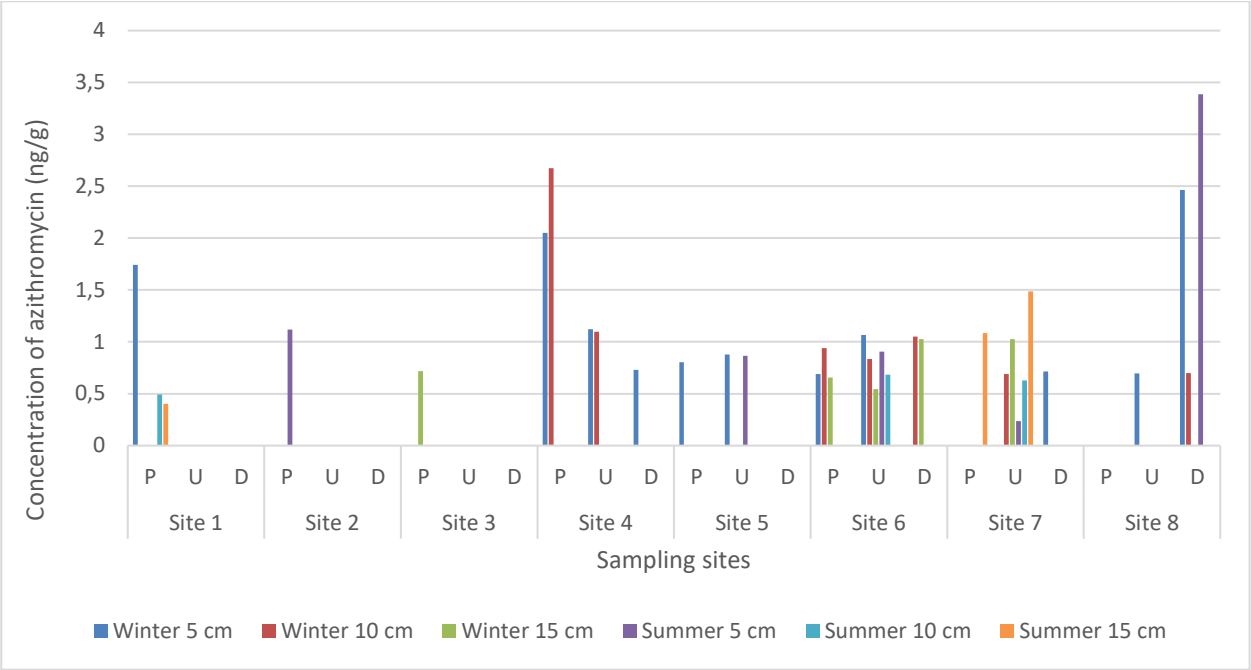

Figure 5.3 Mean concentrations (ng/g) of azithromycin during both seasons

For the most part, the (0–5 cm) surface sediment layer had a higher frequency of detection for azithromycin residues than deeper layers across both seasons. However, Site 8 downstream presented an exception, with its 15 cm depth exhibiting the highest concentration recorded in the study (3.4 ng/g). This may suggest vertical migration or persistent deposition of azithromycin residues over time. Vertical migration in sediment can result from a combination of porewater diffusion, advection, and bioturbation, wherein benthic organisms disturb sediment layers, causing mixing and redistribution of contaminants (Loskotová, Straka, and Pařil, 2019; Morys, Powilleit, and Forster, 2017; Outridge andWang, 2015). Furthermore, the persistence of azithromycin in deeper layers may be explained by its resistance to degradation under anaerobic conditions, a known limitation of some macrolide antibiotics (Martínez-Polanco et al., 2022; Vermillion Maier andTjeerdema, 2018). A two-way analysis of variance (ANOVA) showed that the difference among azithromycin levels across the three depths and seasons is statistically significant ($P<0.05$). Prior to performing two-way ANOVA, assumptions of normality and homogeneity of variances were assessed using Levene's test (University, 2025). Levene's test indicates that the variances are equal ($P<0.05$), so it was appropriate to proceed with a two-way ANOVA. When assumptions were met, a two-way ANOVA was used to assess the effects of season and zone, and their interaction, on pharmaceutical concentrations.

5.4.2 Seasonal prednisolone levels in sediment samples

The concentration of prednisolone in the studied sediment samples varied significantly across sites, seasons, depths, and zones, as shown in Figure 5.4. Detected levels ranged from below the detectable limit to 4.41 ng/g, with remarkable seasonal and zonal differences. At sites 1-3, prednisolone was either not detected or was present at very low concentrations (<1 ng/g), with slightly higher values in winter at 5 cm depth, suggesting limited contamination sources or better degradation. Residues of prednisolone were not detected at site 4 except during summer at the 5 cm depth (1.98 ng/g). This value (at the topmost surface, 5 cm) suggests recent seasonal runoff from diverse sources.

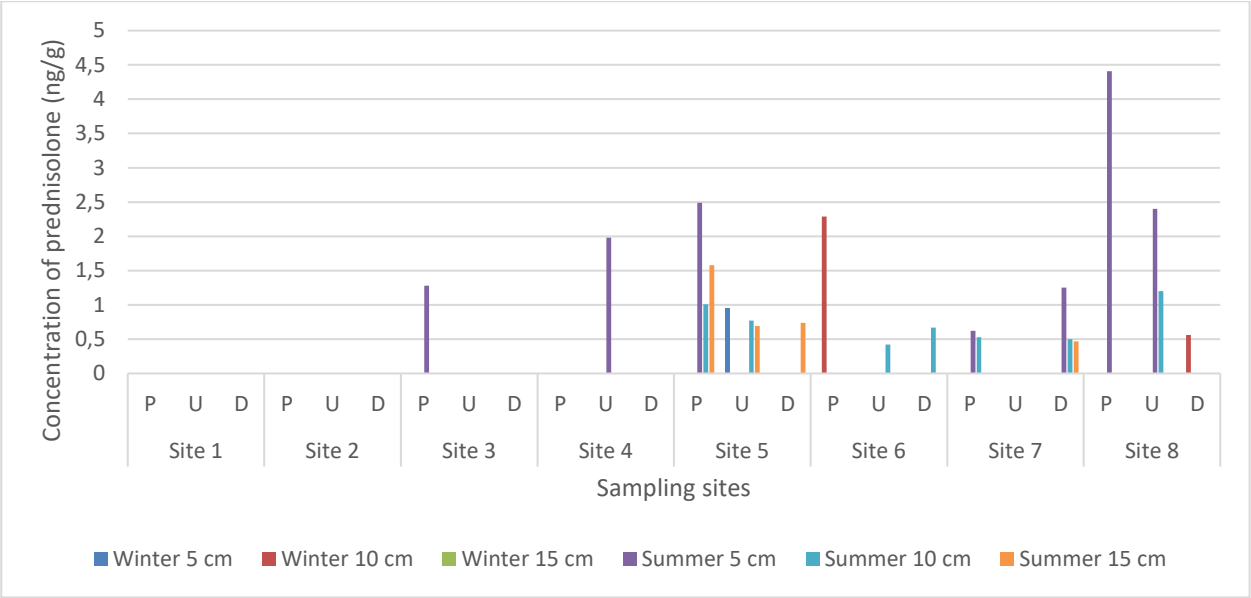

Figure 5.4 Mean concentrations (ng/g) of prednisolone during both seasons

Furthermore, prednisolone shows high variability at site 5, with notable peaks during summer, reaching the highest concentrations, particularly in the point of discharge zone, where the 5 cm summer depth reached 2.49 ng/g. Like most of the sites, lower prednisolone levels were observed at sites 6 and 7, with the highest levels (2.29 ng/g) at 10 cm during winter in the point of discharge zone, and conversely, during summer in the downstream zone at 5 cm (1.25 ng/g), respectively. Overall, site 8 exhibited the highest prednisolone level, especially at the point of discharge during summer, at 5 cm (4.41 ng/g). This result strongly indicates site 8 as a hotspot for prednisolone pollution. The findings of this study show that the discharge zones consistently contained some level of prednisolone, with the highest concentrations, particularly during summer, indicating direct input from hospital, industrial, or municipal effluents. It is worth noting that hospital effluents can significantly affect upstream water sources by discharging pollutants and pathogens. (Silvester et al., 2025). This contamination can lead to a range of problems, including the spread of antibiotic resistance, the introduction of harmful chemicals, and the disruption of aquatic ecosystems.

Moreover, the upstream zones generally showed lower concentrations, while the downstream regions often had residual contamination, indicating downstream transport and persistence via discharge points. Summer concentrations of prednisolone were generally higher than winter, particularly at shallow depths. This may be attributed to continuous anthropogenic activities or enhanced runoff during summer storms (Müller, Österlund, Marsalek, and Viklander, 2020). In both seasons, 5 cm zones consistently recorded higher concentrations than 10 cm and 15 cm layers, suggesting recent contamination episodes accumulating in top sediments, lower vertical mobility of the residues of prednisolone, and possibly, some level of biodegradation in deeper layers (Kunkel and Radke, 2008). A two-way ANOVA showed that the effects of season and depth on prednisolone concentration were statistically significant ($p < 0.05$). Contrarily, the interaction effect between season and depth is not significant ($p > 0.05$), suggesting that the effect of depth does not depend on the season with respect to the levels of concentration of prednisolone detected. In all, deeper sediment layers showing lower levels of analyte may serve as long-term pollutant sinks- a major environmental concern.

5.4.3 Seasonal dexamethasone levels in sediment samples

Dexamethasone residues were detected in a limited number of samples, with most values reported during the summer season (Figure 5.5). Concentrations ranged from non-detected to 12.18 ng/g, with the highest levels detected in the downstream zone at Site 5 (5 cm summer). Similarly, in the same season, zone, and site, but at a different depth (15 cm), 4.29 ng/g of dexamethasone was detected. This result indicates that site 5 may receive direct pharmaceutical discharges, possibly from municipal, medical, or industrial effluents. The accumulation of dexamethasone in the downstream zones may be due to sediment backflow or settling. In sites 1 to 4 and site 6, dexamethasone residues were not detected in most depths or zones, indicating either effective natural attenuation or negligible inputs from pollutant sources.

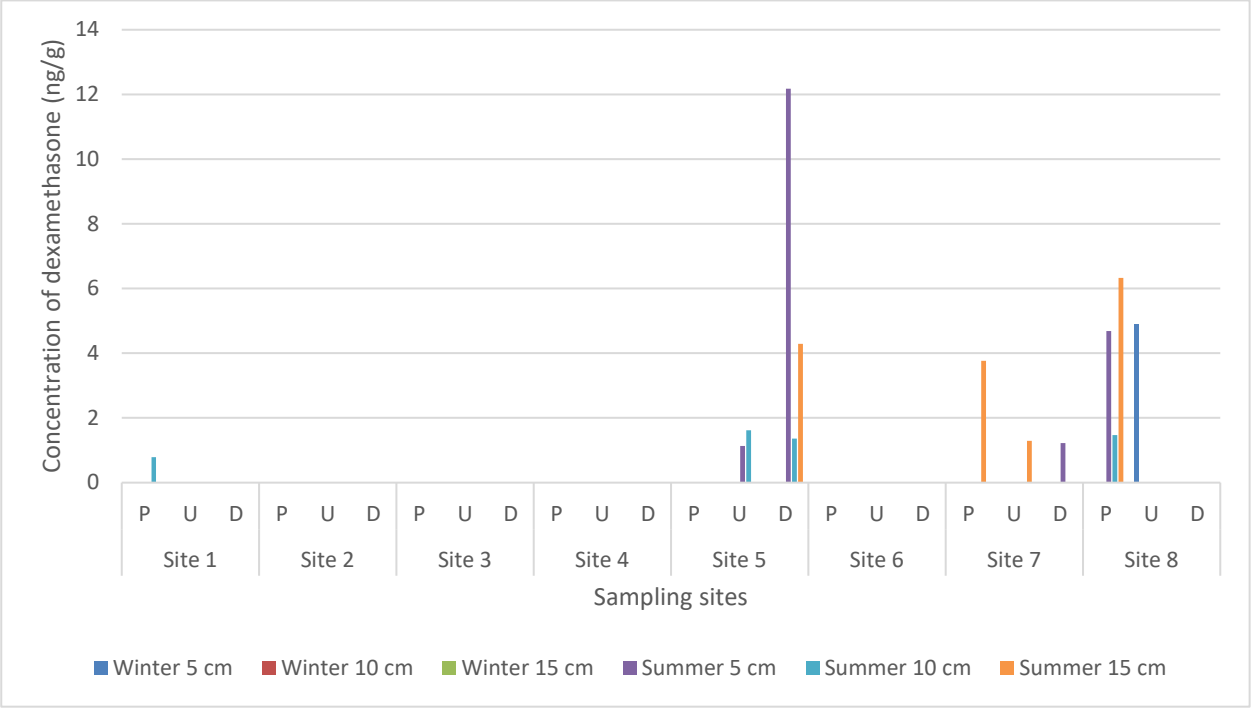

Figure 5.5 Mean concentrations (ng/g) of dexamethasone during both seasons

However, a trace amount of dexamethasone (0.79 ng/g) was detected at site 1 (summer, 10 cm, discharge zone). This suggests that pollution has emerged from intermittent inputs from likely sources. Notable at site 7 are the values recorded at the summer point of discharge and upstream (15 cm), not at the downstream zone, suggesting significant, possibly recent discharges that infiltrated into deeper layers, consistent with pulsed or persistent contamination episodes. Of all the sites studied, site 8 remains the only site where dexamethasone was detected during winter (4.91 ng/g) at the upstream zone, indicating ongoing transport and sedimentation of the pollutant. The sporadic but elevated levels of dexamethasone in deeper sediments indicate poor attenuation and accumulative pollution. From the literature, the pharmacological activity of dexamethasone reveals that it poses ecotoxicological risks to living entities (Efrain Merma Chacca, Maldonado, and Vilca, 2022). As such, its residues must be eliminated from the environment to a large extent.

5.4.4 Seasonal prednisone levels in sediment samples

A distinct seasonal variation in prednisone levels was observed across the sediment zones and depths investigated in this study. Prednisone residues were detected exclusively during the summer season, with no

detectable concentrations in any of the winter samples at any depth or zone. This implies potential seasonal influences, such as reduced degradation rates during warmer months, increased pharmaceutical use, and increased runoff, all of which lead to the accumulation of prednisone in sediment samples during summer. During the summer, prednisone levels were most prevalent in the sediment surface layer (0–5 cm depth), with detections recorded at multiple sites, including 1, 3, 5, 7, and 8, as shown in Figure 5.6.

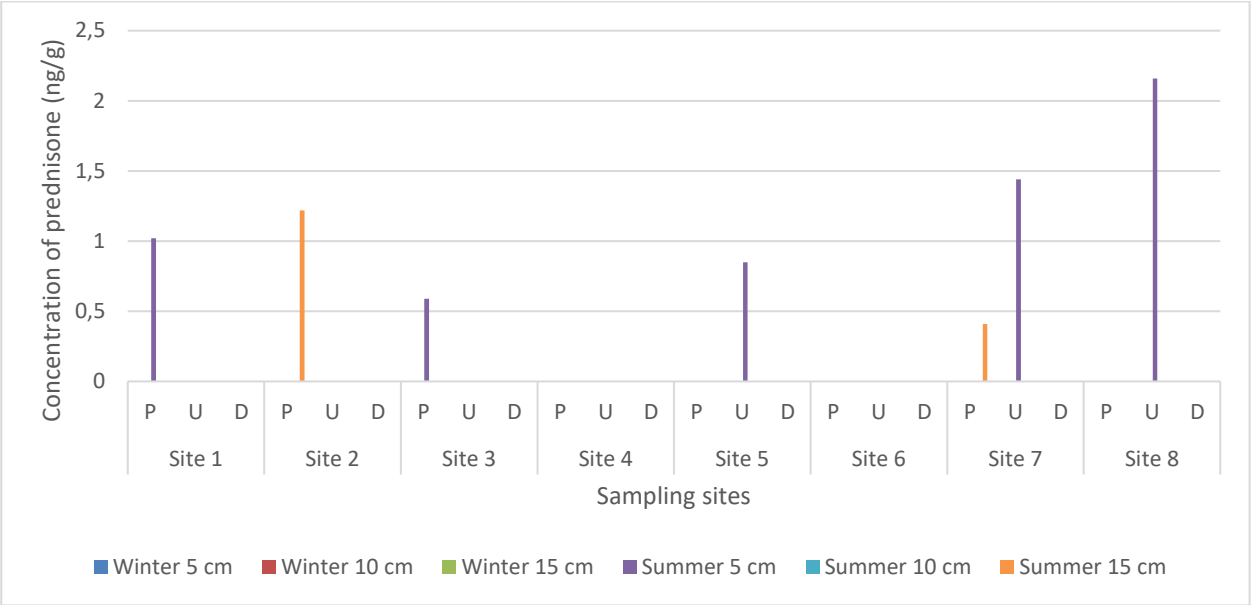

Figure 5.6 Mean concentrations (ng/g) of prednisone during both seasons

These concentrations ranged from 0.59 ng/g to 2.16 ng/g, with the highest, 2.16 ng/g, observed at site 8, upstream zone, at a depth of 5 cm. These values agree with the literature, which indicates that the point of discharge serves as a direct input route for pharmaceutical residues. (Munzhelele, Mudzielwana, Ayinde, and Gitari, 2024; Kumar et al., 2023). Conversely, detections at 10–15 cm depth were sparse, recorded only at the discharge points of sites 2 and 7, with concentrations of 1.22 ng/g and 0.41 ng/g, respectively. However, the highest concentrations were observed in the upstream zone, particularly at site 8 (2.16 ng/g), followed by site 7 (1.44 ng/g), suggesting potential pharmaceutical-residue dispersal dynamics or upstream contamination sources not primarily linked to downstream effluent. Based on the zonal distribution, this study's findings indicate that the point of discharge and the upstream zones are critical sites for monitoring pharmaceutical pollution associated with prednisone detection. These results highlight the need to continue mitigating the residues of pharmaceutical compounds in ecosystems.

5.4.5 Total mean concentrations of analyte PCs in the sediment samples

Seasonal comparison of the total mean concentrations for the four analytes revealed that dexamethasone consistently exhibited the highest concentration across both sampling periods (Figure 5.7), underscoring site 5, where it was detected, as a potential hotspot for persistent pharmaceutical contamination. The total average concentrations of azithromycin, prednisolone, dexamethasone, and prednisone are 11.30 ng/g, 23.01 ng/g, 40.13 ng/g, and 7.69 ng/g, respectively, during summer. Except for prednisone, whose concentration was not

detected at all sites during winter, the average total concentrations of azithromycin, prednisolone, and dexamethasone were 24.92 ng/g, 3.81 ng/g, and 4.91 ng/g, respectively.

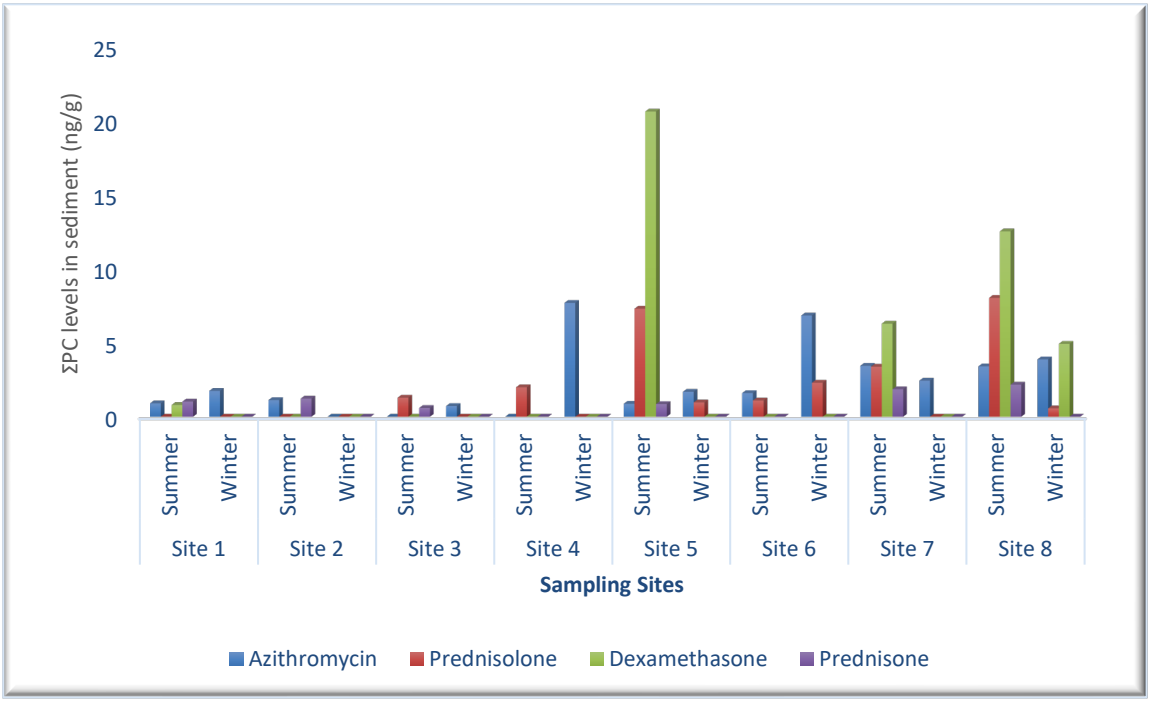

Figure 5.7 Total mean concentration (ng/g) of analyte PCs in sediments during summer and winter

Consequently, the total concentrations (ng/g) of the four analyte PCs at all sampling sites during both seasons are 82.12 ng/g and 33.64 ng/g, respectively. In this study, the levels of PCs in sediments during winter are more than twice those recorded during summer. Aside from the reasons mentioned earlier, including the dilution of PC concentrations by continuous rainfall, PCs can undergo diverse processes, thus yielding new transformation products in the environment (Kot-Wasik, Jakimska, and Śliwka-Kaszyńska, 2016). It is worth noting that this phenomenon occurs over time and may yield more toxic products than the parent compounds. As a result, the concentrations of some parent compounds may vary greatly across seasons. This is an important gap that toxicologists should address when assessing the risk posed by PC residues to living organisms. Moreover, Ogunbanwo et al. (2022) reported that pollution from pharmaceuticals varied with the season. Ogunbanwo and colleagues reported that certain compounds were detected at high concentrations during the dry season, while others were detected at high concentrations during the wet season. Additionally, seasonal usage and alterations in environmental factors, including temperature and river flow, have been linked to variations in levels of contaminants detected in the environment during different periods (Fekadu et al., 2019; Ogunbanwo et al., 2022; Tewari et al., 2013). Furthermore, the complexity of the spatial patterns observed in this study may be attributed to multiple sources of pharmaceuticals at the sampling sites, some of which may experience continuous effluent discharges.

In all, the detection of the PCs, especially in the 15 cm layer of the sediment samples, indicated historical use of these compounds. In contrast, those detected in the 0-5 cm region/upstream indicate more recent use. Comparatively, the levels of PC residues detected during summer are higher than those in winter, except for azithromycin, which was relatively higher in winter. Noteworthy in South Africa is that many people experience

flu and colds in winter, which could contribute to higher antibiotic use during that season. This result indicates continuous use of azithromycin compared to other PCs under study. It suggests a strong adsorption tendency of azithromycin to sediments, so it does not leach easily in the presence of rain or runoff. A recent article explained that the sorption mechanism of azithromycin could be linked to the cation exchange capacity and not the easily degradable organic carbon (Pan et al., 2024) content of the sediment, thus making it sorbed mostly to the mineral components of the sediments (Hanamoto and Ogawa, 2019). Therefore, it is unlikely that azithromycin would degrade readily, leading to increased levels in the environment.

5.4.6 Comparison between this study's results and previous studies

The azithromycin values obtained in this study are below those obtained in the sediment samples (6.00 ng/g) from the Northern part of the Persian Gulf around the Bushehr port (Mirzaie et al., 2022). Additionally, azithromycin concentrations were below the LoD at other sampling sites, except for sediment samples, which were not obtained due to inaccessibility. Similarly, the prednisolone values obtained in this study are far below the relatively high concentration of 176.00 ng/g reported in sediments from Beihai Bay, Guangxi, China (Ren et al., 2022). Additionally, the 9.35 ng/g level of prednisolone reported in Chinese sediments [60] is also higher than that reported in this study. This study's dexamethasone values are higher than those reported in the mariculture sediment (0.14 ng/g) of Pulau Kukup, Johor, Malaysia (Ismail et al., 2020), except for the sediment samples whose dexamethasone concentrations were below the LoD. Moreover, the levels of prednisone reported in this study are lower than those reported in sediments (2.23 ng/g) from Guangzhou, China (Lin et al., 2024).

Compared with other analyte PCs in both seasons, the detection frequency of azithromycin in sediment samples (38%) is half that in water samples (79%). This trend had earlier been discussed by Kondor et al. (2022), where some PC residues in sediments were reported in approximately 10% of those found in the analysed water samples at the same sampling location. Also, in the report by Kondor and team members, it was observed that some PCs were found in sediments but not detected in water (Kondor et al., 2022). The observable variations of the analyte PCs between the water and sediments can be linked to the diverse logarithmic partition coefficient (log K_D) variation usually exhibited by specific pollutants (Kondor et al., 2022; Čelić, Gros, Farré, Barceló, and Petrović, 2019; Fairbairn et al., 2015). Also, other characteristics of pollutants that determine their distribution between the solid (sediment) and liquid (water) phases include, but are not limited to, their nature (e.g., hydrophobicity or hydrophilicity) and solubility. However, the presence of imbalanced local effects, such as fluctuations in contamination, might limit the applicability of equilibrium partition coefficients in the samples analysed. (Kondor et al., 2022).

CHAPTER 6
TOXICOLOGY OF COVID-19 DRUGS ON AQUATIC ORGANISMS AND HUMAN HEALTH

6.1 Background

The release of a wide range of chemical contaminants into the environment is a significant concern, as these substances, including COVID-19 pharmaceuticals, can potentially pose adverse ecological risks even at low concentrations. Assessing the ecotoxicity of these contaminant residues is essential for understanding their risks to living organisms. Such assessments provide valuable insights into the risks posed by the chemical contaminants, including the pharmaceutical compounds (PCs) under investigation.

Chemical analysis is essential for evaluating contamination levels in surface water caused by various chemical pollutants. It also plays a significant role in assessing the potential risks posed by these contaminants to aquatic life and human health. However, relying solely on chemical analysis means the focus is only on the risks associated with individual chemicals, without considering the combined effects of different contaminant mixtures (Truter et al., 2016). In contrast, ecotoxicological studies offer several advantages over traditional chemical analysis, as they detect a broader range of contaminants, reveal their interactions, and are generally more sensitive (Neale et al., 2023). These tests can help identify health risks from certain water sources and indicate the presence of various contaminants, reflecting water quality. However, bioassays alone cannot determine the origin of contamination. Combining chemical analyses and bioassays provides a comprehensive understanding of the extent of contamination in water sources and the risks these contaminants pose to aquatic organisms and human health (Neale et al., 2023). The chemical analysis of selected COVID-19 drugs in water within the Orange−Senqu River basin was presented in Deliverable 3, submitted to the WRC, while the current Deliverable 4 focuses on ecotoxicity.

This study used bioassays representing various biomarkers, including estrogen receptor agonism (estrogenicity), androgen receptor (AR) antagonism (anti-androgenicity), and aryl hydrocarbon receptor (AhR) activation, to assess the potential health risks associated with exposure to water samples collected from eight sites within the Orange−Senqu River basin. Specifically, three in vitro test assays were employed to detect estrogenic and androgenic activities: the Yeast Estrogen Screen (YES), the Yeast Anti-Androgen Screen (YAAS), and the Arxula Yeast Dioxin Screen.

Anti-androgenicity and estrogen receptor agonism, commonly referred to as estrogenicity, are significant mechanisms by which certain chemicals can disrupt endocrine function in living organisms. These endocrine-disrupting chemicals (EDCs) can mimic or interfere with the body's natural hormones, potentially causing a range of health and environmental issues.

To effectively screen for estrogenic activity in water samples, researchers can employ both in vitro (test-tube or cell-based) and in vivo (live-organism-based) reporter gene assays. These assays are designed to detect and quantify the estrogenic effects of substances present in the samples. Genetically engineered yeast has emerged as a reliable and validated approach for screening for estrogenic activity, enabling scientists to assess the presence of estrogenic compounds with high accuracy. Over the years, several specific assays utilising genetically engineered yeast have been developed to enhance the screening process (Bovee et al., 2004; Pham et al., 2012; Routledge and Sumpter, 1996).

A wide range of environmental chemicals has been identified as having estrogenic properties. These include not only natural and synthetic hormones but also specific pharmaceuticals that can enter water systems through various disposal and runoff pathways. Additionally, industrial chemicals such as plasticisers, which are commonly used to enhance the flexibility and durability of plastics, and certain pesticides used in agriculture have also been found to exhibit estrogenic activity (Pamplona-Silva et al., 2018). The presence of these chemicals in the environment raises concerns about their potential impacts on wildlife and human health, emphasising the need for ongoing monitoring and regulation of EDCs in our ecosystems.

As is the case with estrogenicity, screening for androgenicity and anti-androgenicity was also performed using yeast-engineered bioassays to represent human hormone signalling (Sohoni and Sumpter, 1998). Examples of the best-known sources of androgenic and anti-androgenic chemicals in the environment are agriculture (Archer and Van Wyk, 2015; Orton et al., 2011), and wastewater treatment works (Chen et al., 2007; Jobling et al., 2009).

Furthermore, activation of the aryl hydrocarbon receptor (AhR) is a biomarker of xenobiotic activity. This transcription factor is known to regulate various enzymes involved with xenobiotic metabolism, including certain cytochrome p450's, and evidence exists linking AhR action and various cancers, autoimmune disorders, inflammatory bowel diseases, multiple sclerosis, and arthritis (Granados et al., 2022; Lin et al., 2022). The ligands of AhR include hydrocarbons, brominated compounds, phytochemicals, and certain pharmaceuticals, among others (Lin et al., 2022) and are known to be present in environmental waterbodies (Gouukon et al., 2020). The Global Water Research Coalition (2023) included AhR activation in the framework for drinking water quality testing. AhR activation was identified as a priority biomarker by the European Commission-funded solutions project (Brack et al., 2019).

On a different note, embryotoxicity and teratogenicity were evaluated in zebrafish embryos to assess the potential risks posed by drugs, chemicals, and other substances. This assessment involved exposing zebrafish embryos to water samples collected from four sites within the Orange Senqu River basin, which exhibited the highest endocrine-disrupting activity. Since the Zebrafish is a vertebrate, the embryotoxic and teratogenic effects observed can be extrapolated to higher vertebrates, including humans (Halii and Quilang, 2011), making Zebrafish an important organism for toxicological studies and the assessment of developmental risks.

Embryotoxicity refers to the harmful effects that can impact developing embryos from conception through the fetal stage. These adverse effects may lead to complications such as growth restrictions or biochemical disruptions, which can affect the health of the offspring (Sogoorb et al., 2014). In contrast, teratogenicity involves structural malformations or defects in the offspring that occur after the embryonic development phase has concluded. These malformations can involve significant physical abnormalities in organs or body structures, potentially resulting in lasting implications for the individual’s health and development (Sogoorb et al., 2014).

The broader term "toxicity to reproduction" encompasses a wide range of effects that not only target the developing embryo or fetus but also affect overall reproductive health. This can include disruptions to fertility, alterations in reproductive behaviour, or complications in gestation. For instance, chemical exposures can reduce hatching rates in certain species, suggesting potential issues with embryonic viability.

Hormonal imbalances caused by environmental toxins may influence reproductive cycles or hormone production, further compromising fertility. Additionally, exposure to harmful chemicals can result in reduced

sperm quality, which can adversely affect male reproductive health. The implications of these reproductive toxicities are profound, as they can have lasting effects not only on individual reproductive success but also on the health and well-being of future generations. It is crucial to consider these risks when assessing the safety of chemicals and their potential reproductive health impacts across species.

Therefore, this investigation aimed to assess the human and aquatic ecosystem risks from exposure to contaminant loads in water samples using key biomarkers representing three key modes of action (estrogenicity, anti-androgenicity, and aryl hydrocarbon receptor binding). To achieve this, selected in vitro bioassays were applied, including the Yeast Estrogen Screen (YES), Yeast Anti-Androgen Screen (YAAS), Arxula Yeast Dioxin Screen. On the other hand, embryotoxicity, teratogenicity, and hatching rate were assessed in zebrafish embryos to test for the toxicity and teratogenicity of drugs, chemicals, and other contaminants in water samples collected from the Orange Senqu River basin.

6.2 Methodology

6.2.1 Study area

The Orange River basin is the largest south of the Zambezi, covering over one million square kilometres and spanning across Lesotho, Botswana, Namibia, and South Africa. It is also the most established transboundary river basin in the Southern African area, with various water transfer schemes providing water to municipalities, industries, and farms in and outside the basin (Earle et al. 2005). The Orange–Senqu basin can be partitioned into three major sub-basins: the Vaal, Upper Orange–Senqu, and Lower Orange. Sampling in this study was conducted in the Orange−Senqu River and the Mohokare-Caledon River, both of which belong to the Upper-Orange sub-basin.

The transboundary water resources of the Orange−Senqu River basin are overseen by the Orange−Senqu River Basin Commission (ORASECOM), the river basin organisation established in 2000 by the governments of the four riparian nations involved. ORASECOM's mission is to advance the beneficial development of the basin for socio-economic well-being and ensure its environmental safeguarding (Africa Water Facility, 2016).

The Orange−Senqu River begins in the Lesotho Highlands and travels westward for 2 200 km, eventually emptying into the Atlantic Ocean on the west coast of South Africa (Mare, 2007). The Caledon River is among its main tributaries (Kranz et al. 2005) and forms the western boundary of Lesotho. Since the Caledon flows into the Orange−Senqu River, which extends over Lesotho, South Africa, and Namibia, its water quality is significant, especially for the riparian countries that rely on it for their water resources (Chatanga et al. 2019). South Africa withdraws approximately 63 per cent of the water, making it the largest consumer among the four riparian states, while upstream Lesotho, with its abundant water supply, withdraws a mere 0.2 per cent (Mahlakeng, 2020).

6.2.2 Sampling site description and sample collection

This study focused on the detailed analysis of water and sediment from eight distinct sites along the winding courses of the Caledon (Mohokare) River and the Orange−Senqu River, shown in Figure 7.1. Each site was carefully selected for its accessibility, which makes it easier to collect samples, as well as for its strategic proximity to wastewater treatment plants (WWTPs). At each location, samples were collected from three key points: upstream, downstream, and directly at the WWTP discharge point. This targeted sampling approach

allows researchers to examine the characteristics of the effluent released into the river, providing valuable insights into its effects on the river's delicate ecosystem and overall health.

Two sampling campaigns were conducted: one in February to represent the summer season and another in April to represent the winter season. These campaigns took place at various geographical points along the Orange−Senqu River, specifically at Zastron, Gariep Dam, a farm dam in the Gariep, Aliwal North, and Clarens. Additionally, three sites along the Mohokare (Caledon) River were included: Ha Thetsane, located in Maseru, Lesotho, and Ladybrand and Ficksburg in South Africa. The Mohokare River is a major tributary of the Orange−Senqu River (Chatanga et al., 2019), which is why it was included in this study.

Water samples were collected in methanol-rinsed glass and stored at 4 °C. An ultrapure water field blank/control was also included in the experiment. Sample processing, including solid-phase extraction, was performed within 8 days of collection.

Figure 6.1 shows the Mohokare-Caledon and Orange−Senqu Rivers sampling sites.

Figure 6.1 Mohokare-Caledon and Orange−Senqu Rivers sampling sites

Legend: Sampling sites along the Caledon River; Sampling sites along the Orange−Senqu River; Sampling Route

S1: Site 1(Farm Dam); S2: Site 2 (Gariep Dam); S3: Site 3 (Aliwal North); S4: Site 4 (Zastron); S5: Site 5 (Ladybrand)

S6: Site 6 (Ha Thetsane, Lesotho); S7: Site 7 (Ficksburg); S8: Site 8 (Clarens)

6.2.3 Solid phase extraction

A total of 800 mℓ of water was passed through a 1.2 µm glass fibre filter to remove particulate matter. The filtered samples were subsequently passed through 200 mg, 6cc hydrophilic-lipophilic balanced (HLB) columns (Oasis, Waters, US) at a flow rate of approximately ±5 mℓ/min using a vacuum manifold. The columns were

preconditioned using 6 mℓ of methanol and 6 mℓ of ultrapure water. The columns were subsequently dried under vacuum and sealed for shipping to Stellenbosch University. The columns were stored at -20 °C before elution. Extracts were eluted from the columns using 6 mℓ of methanol, subsequently evaporated to dryness using a gentle stream of nitrogen and reconstituted in 1 mℓ of ethanol (1 000x concentrated state).

6.2.4 Yeast estrogen screen (YES) and yeast anti-androgen screen (YAAS)

Estrogenicity and anti-androgenicity were screened for using the YES and YAAS recombinant yeast assays as previously described (Routledge and Sumpter, 1996; Truter et al., 2017a). The yeast strains constitutively express human hormone receptors (ERα and AR) and contain response elements upstream of a β-galactosidase reporter gene, which serves as a biomarker for estrogenic or anti-androgenic compounds. Sample extracts were tested in duplicate as a dilution series (i.e., 25x, 12.5x, and 6.25x relative to the environmental concentration) to increase assay sensitivity and pre-empt toxicity, since poor-quality samples are known to be cytotoxic to the yeast. Each assay plate included a 10-point two-fold serial dilution of 17-β-estradiol for YES (2.76 µg/ℓ to 53.95 ng/ℓ) and of flutamide for YAAS (2762.1 to 53.95 µg/ℓ), serving as reference standards. An ultrapure H_2O method blank representing the filtration and SPE workflows was also tested to correct for possible background activity. Assay plates were incubated for 48h at 30°C, followed by 12h to 48h at room temperature. Absorbance was then measured at nm and 625 nm, respectively, as indicators of reporter gene expression levels and cell density (iMark, Biorad, US). Turbidity-normalised absorbance at 570 nm was used to calculate β-estradiol equivalents (EEQ) and flutamide equivalents (FEQ) using linear regression equations derived from the reference standard curves.

6.2.5 Aryl hydrocarbon receptor activation screen

A commercially available yeast assay was applied to test extracts for AhR agonists (A-YDS, New Diagnostics, DE). The kits feature engineered yeast expressing the human AhR protein as well as an alkaline phosphatase reporter gene induced through AhR binding to xenobiotic response elements (XREs). The kit was used according to the manufacturer's instructions. In short, lyophilised yeast was reactivated, and samples were exposed at a 10x enrichment factor to increase assay sensitivity. A seven-point standard curve of the AhR agonist β-naphthoflavone (βNF) was included as the reference standard (20 to 0.73 µg/ℓ a serial dilution). An ultrapure H_2O method blank representing the filtration and SPE workflows was also tested to correct for possible background activity. All samples and standards were tested in duplicate. Samples were exposed to activated yeast cells for 20h at 30°C and 700 rpm, after which the cells were pelleted by centrifugation, the supernatants removed, and the samples supplemented with a developer solution and incubated for a further 1h. Reactions were then stopped with 5 M NaOH, and absorbance was measured at 415nm (iMark, Bio-Rad, US). Yeast pellets were resuspended, and absorbance was measured at 625nm as an indicator of turbidity and, therefore, cell numbers. Turbidity-normalised normalised $absorbance_{415nm}$ values were used to calculate βNF equivalents (βNF-EQ) using a linear regression equation of the βNF standard curve.

6.2.6 OECD 236 FET Test

Water extracts were tested for embryotoxicity and teratogenicity using the OECD 236 fish embryo toxicity method with slight modifications. E3 medium was used as the negative control and was prepared in a 60X concentration by adding 4.8 g NaCl, 1.6 g KCl, 5.8 g $CaCl_2 \cdot 2H_2O$, 9.78 g $MgCl_2 \cdot 6H_2O$ to 2l ddH_2O. To prepare 1X medium,16.5 mℓ of the 60X stock was diluted to 1 ℓ, and 3 mℓ of 1% methylene blue was added. The positive

control consisted of 4mg dichloroaniline in 1 ℓ of ddH_2O. Analysis was done with 5 times concentrated HLB extracts (5µℓ extracts added to 1ml of E3 media). Medium replacement was performed at 72h, and exposures terminated at 96h.

Embryos were collected at the Tygerberg Medical Facility and promptly transferred to the Stellenbosch University Natural Science Building. Ten embryos were applied per sample, with 2 sites performed in a 24-well plate and the remaining 4 wells used as the internal negative control. Observations were done at 24 hours post-fertilisation (hpf), 48 hpf, 72 hpf, and 96 hpf. Coagulation rate, malformation rate, and hatching rate were all recorded at 24-hour intervals. Malformations included oedemata (pericardial or yolk), lack of somite formation, and lack of tail detachment.

6.3 Results and discussion

The Estrogenic activity, expressed as 17-β estradiol equivalents (EEQ) and illustrated in Figure 6.2 and Table 6.1, was observed at various sampling sites within the transboundary river basin. Estradiol equivalent concentrations ranged from less than the limit of detection (LoDLoD) to 35 ng/ℓ during the summer season and from less than the LoD to 58 ng/ℓ during winter. No estrogenic activity was observed at Site 6 during both summer and winter, as well as at the point of discharge of Sites 2 and 3, and upstream of Site 7. This suggests that the concentrations of compounds responsible for estrogenic activity at these sampling points were likely too low to elicit estrogenic activity.

Estrogenic activity was more prevalent during the winter season, with a greater number of sampling points exhibiting detectable activity than in summer. More observations of estrogenic activity during winter could be explained by increased use of estrogenic compounds, such as pharmaceuticals, during that season. Moreover, the river level is low in winter; therefore, with lower flows, there is less dilution of contaminants, such as WWTP effluents entering rivers.

During the summer season, Sites 4 and 5 exhibited higher EEQ concentrations upstream of the point of discharge than downstream, suggesting distinct sources of estrogenic compounds within the wastewater treatment plants serving these sites. The elevated EEQ concentration at Site 4 upstream, in contrast to the site's lower EEQ discharge point value, may be attributable to runoff from a nearby informal settlement lacking proper sanitation, as well as to cattle grazing in the surrounding fields.

Seasonal variation was observed at Sites 1, 4, and 5, where summer EEQ concentrations were higher than winter values, and at Site 8, where winter values exceeded those recorded in summer. Comparisons at other sampling points were not possible because no values were recorded, indicating the absence of estrogenic activity during either the summer or winter seasons, or both.

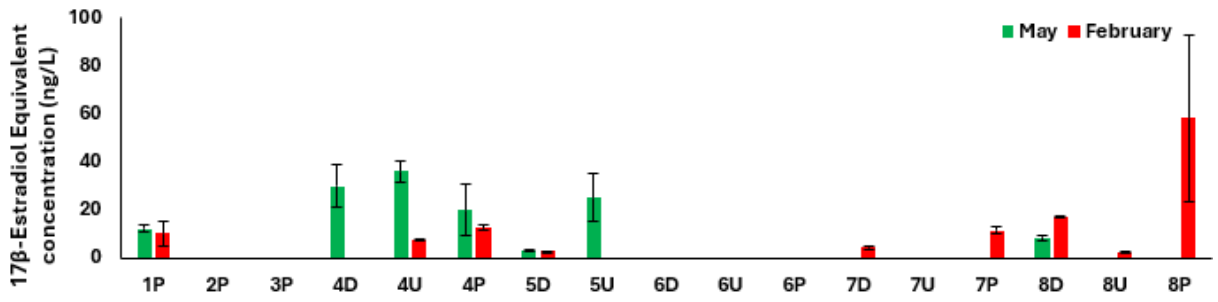

Figure 6.2 Estrogenic activity at sampling sites during summer and winter

Note: Summer is denoted by the red bar, and winter is shown as the green bar. P refers to the point source where effluent directly enters the river, U refers to water upstream of the discharge point, and D is the water downstream of the point of discharge.

Tang et al. (2021) reported that estrogen-equivalent levels ranged from 0 to 33 ng/ℓ in surface waters across 32 Asian countries. These values are lower than the values observed in the current study, indicating higher estrogenic activity in the waters of the current study. Based on the work of Williams et al. (2009), the study's EEQ levels indicate high risk, as they exceed 10 ng/ℓ. Other categories in Williams' work are no risk, denoted by levels < 1 ng E2/ℓ, and at risk, which range from 1-10 ng E2/ℓ.

Tang's study is not the only one showing EEQ levels lower than those in the current study. The estradiol equivalent concentrations of surface water samples from the Maracana River and Mangue Channel ranged from below the LoD to a maximum of 1,6ng/ℓ (do Nascimento et al. 2022), while Gea et al. (2024) reported EEQ levels of less than the LoD to 3.8 ng/ℓ for wastewater samples in northern Italy. The EEQ concentrations in the study by Gea et al. (2022) are lower than all discharge-point EEQ concentrations in the current study, again indicating higher estrogenic activity in the samples from the current study's sites.

Olifants River catchment by Genthe et al. (2013), where EEQ levels ranged from 0,04-8,88 ng/ℓ with the discharge point value of site 8 being the highest, are much closer to the EEQ values of raw influent wastewater documented by Archer et al. (2020), where the summer EEQ values ranged from 4.4 to 45.4 ng/ℓ, while the winter values ranged from 6.6 to 31.5 ng/ℓ. This similarity suggests that the wastewater treatment plants at the sampling sites are ineffective at removing estrogenic compounds from wastewater influents.

Hof et al. (2024) regard concentrations of 0.1 to 0.4 ng EEQ/ℓ in municipal WWTP effluents to be safe. All the sampling sites in the present study had markedly higher EEQ/ℓ values for point-of-discharge samples. Notably, the discharge point of Site 8 had an EEQ concentration of 60 ng/ℓ, which is 150 times larger than the threshold value considered safe by Hof et al. (2024). None of the samples tested exhibited androgenic activity, suggesting limited risk of masculinization to vertebrates inhabiting the system.

Table 6.1 Equivalent concentrations (mean ± standard deviation) of all sites expressed as EEQ (Estrogenicity), Flu (Anti-androgenicity), and β-NFEQ (Aryl hydrocarbon activity)

	EEQ (ng/ℓ)		Flu (µg/ℓ)		β-NFEQ (ng/ℓ)	
	Feb	**May**	**Feb**	**May**	**Feb**	**May**
1P	10.05 ± 5.43	12.14 ± 1.23	0	41.14 ± 10.94	969.66 ± 37.76	999.4 ± 22.38
2P	0	0	0	15.24 ± 1.3	0	98.82 ± 21.25
3P	0	0	0	16.43 ± 1.16	0	0
4D	0	29.77 ± 8.74	0	25.21 ± 1.35	977.97 ± 130.01	1118.66 ± 59.46
4U	7.26 ± 0.21	35.99 ± 4.54	0	0	904.59 ± 31.65	999.4 ± 36.85
4P	12.34 ± 1.18	20 ± 10.47	0	24.9 ± 1	852.63 ± 99.26	984.53 ± 66.92
5D	2.68 ± 0.11	2.9 ± 0.46	22.65 ± 6.29	18.82 ± 1.05	351.42 ± 14.47	671.81 ± 20.8
5U	0	24.85 ± 9.97	17.51 ± 0.76	0	139.59 ± 2.94	1032.33 ± 16.96
6D	0	0	20.3 ± 0.63	0	371.41 ± 53.13	207.54 ± 49.74
6U	0	0	47.09 ± 7.36	29.76 ± 2.33	451.19 ± 10.4	1037.77 ± 106.94
6P	0	0	27.28 ± 5.56	0	408.18 ± 12.89	1046.24 ± 150.58
7D	4.13 ± 0.7	0	0	0	816.34 ± 384.82	403.54 ± 197.61
7U	0	0	18.93 ± 1.76	12.96 ± 0.08	0	151.58 ± 18.09
7P	11.21 ± 1.55	0	0	0	901.55 ± 70.32	827.85 ± 32.56
8D	17.27 ± 0.05	8.45 ± 1.04	0	0	975.26 ± 60.14	975.26 ± 8.14
8U	2.38 ± 0.17	0	0	0	232.16 ± 19.44	416.17 ± 13.79
8P	58.23 ± 34.78	0	0	0	1042.4 ± 0.9	942.48 ± 13.34

The EEQs of the current study are higher still than those observed in a study conducted in 9 localities of the Regarding anti-androgenic activity, flutamide equivalent (FEQ) concentrations ranged between less than the LoD and 47.09 µg/ℓ in summer (Site 6 upstream) and less than the detection limit to 41.14 µg/ℓ (Site 1 Point of discharge) in the winter (Figure 6.3).

In addition, temporal variation is only observed in Sites 6 and 7 upstream, as well as in Site 5 downstream, as these locations are the only ones with flutamide-equivalent concentrations in both summer and winter seasons. Across these sites, winter anti-androgenic activity surpasses summer activity. The upstream of Site 6 shows higher flutamide equivalent concentrations than the point of discharge, suggesting the presence of alternative sources of anti-androgenic compounds other than the site's WWTP.

Anti-androgenic activity and estrogenicity in the current study have an inverse relationship: sites with estrogenicity values below the detection limit or low depict high anti-androgenic activity. Sites with high FEQ and low EEQ may be influenced by industrial discharges rich in anti-androgenic substances, whereas sites with high EEQ and low FEQ may reflect contamination from pharmaceutical or personal care products.

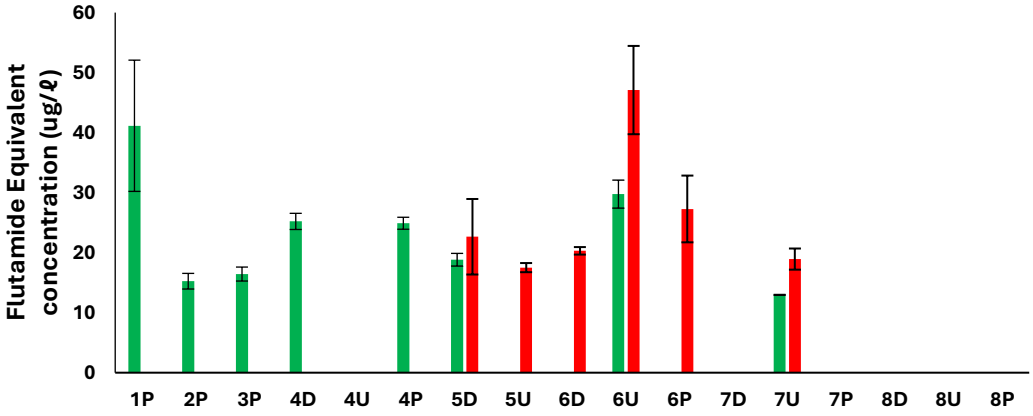

Figure 6.3 Anti-androgenic activity in water samples during summer and winter

Note: Summer is denoted by the red bar, and winter is shown as the green bar. P refers to the point source where effluent directly enters the river, U refers to water upstream of the discharge point, and D is the water downstream of the point of discharge

Aryl hydrocarbon activity was detected at all sites during both summer and winter, except Site 3P, where activity was absent, and Sites 2P and 7U, where activity was not observed during summer. β-Naphthoflavone Equivalent concentrations ranged from below the detection limit to 1 050 ng/ℓ in summer and below the LoD to 1130 ng/ℓ in winter, as illustrated in Figure 6.4.

In a study by Hof et al. (2024), the β-Naphthoflavone Equivalent concentrations ranged from below the limit of detection to 0,05 µg/ℓ at sites not influenced by WWTPs across all seasons, and from 0,01 to 0,29 µg/ℓ at sites affected by WWTPs. This indicates much higher dioxin-like activity in the current study, indicating the presence of more AhR agonists at the study's sampling sites. Similarly, Kuschik-Maczollek et al. (2024) reported β-Naphthoflavone Equivalent concentrations of 0,29 µg/ℓ in water samples from the tributary of the Main River, emphasising the significantly higher dioxin-like activity in the current research.

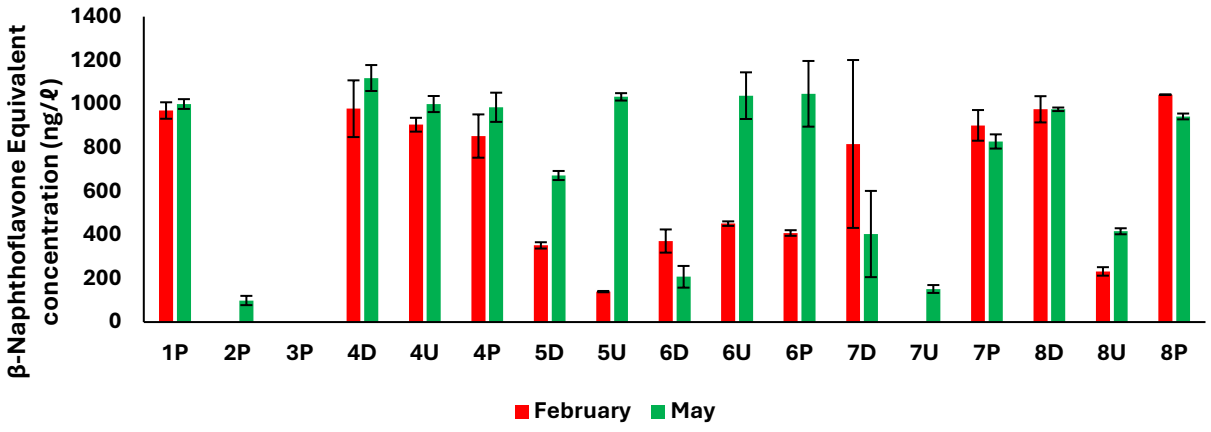

Figure 6.4 Aryl hydrocarbon activity at sampling sites

Legend: Summer, denoted by the red bar and winter shown as the green bar. P refers to the point source where effluent directly enters the river, U refers to water upstream of the discharge point, and D is the water downstream of the point of discharge.

In contrast, embryotoxicity and teratogenicity were analysed at only four of the studied sites, which showed high levels of contamination during chemical analysis. Additionally, the sites were selected based on the current in vitro data and historical chemistry data. Samples from three sites (1P, 7P, and 8P) caused 100% mortality; as shown in Figure 6.5A, this was observed exclusively during the February sampling. This acute lethality emphasises the potential severity of azithromycin, which previous research has shown to cause a variety of adverse impacts in zebrafish, such as morphological malformations, impaired swimming and feeding activities, decreased acetylcholinesterase (AChE) behaviour, increased dopamine levels and unusual expression of genes related to neurodevelopment (Chen et al., 2023). A consistent decrease in mortality was observed across all locations in May, with 1P showing 0% mortality (but high oedema occurrence), 8P at 60% mortality, and 7P closely resembling the negative control. The only site that showed minimal seasonal differences was 4P, where mortality only varied by 10%, as illustrated in Figure 6.5A.

Hatching was strongly inhibited in both 1P and 8P during both seasons and to a lesser extent at 4P (Figure 6.5B). The 7P May sample again resembled the negative control, with the 80% hatching rate most closely resembling 100% in the E3 media. Finally, the positive control acted as intended, as evidenced by the high incidence of oedema. A clear increase in mortality was observed at 96 hpf (just after a water change). Further malformations in the back and tail could also be noted owing to the teratogen exposure.

Figure 6.5 Embryotoxicity and teratogenicity of selected discharge points

Legend: Mortality and oedema formation (A) and hatching rate (B) are shown for each site over both seasons, with negative and positive control shown on the right two bars. Each treatment is represented by ten embryos.

CHAPTER 7
RISK ASSESSMENT OF COVID-19 DRUGS ON AQUATIC ORGANISMS AND HUMAN HEALTH

7.1 Introduction

Water pollution significantly threatens global water resources (Papagiannaki et al., 2022). One key concern is contamination from pharmaceuticals, which can negatively impact aquatic ecosystems and human health (Marques et al., 2023). The environmental presence of pharmaceutical substances is increasingly recognised as a global issue, with these compounds categorised as emerging contaminants in water and soil systems (Freitas and Radis Baptista, 2021). The ecological repercussions of pharmaceutical production and use remain poorly understood, underscoring the need for further research (Samal et al., 2022). Pharmaceutical residues raise concerns as their prevalence continues to rise due to population growth and increased pharmaceutical consumption. These residues can accumulate in environmental media, including water, soil, and sediments, presenting significant health and ecological risks (Omotola et al., 2023).

This study focuses on four pharmaceuticals: the glucocorticoids prednisone and its metabolite prednisolone, and dexamethasone; and the macrolide antibiotic azithromycin. These compounds were chosen for their widespread use during the COVID-19 pandemic, where they demonstrated effectiveness in reducing mortality (Horby et al., 2021). Glucocorticoids are commonly used steroid hormones that can disrupt endocrine systems. They may enter aquatic ecosystems through sewage discharge, surface runoff, and stormwater, as these pharmaceuticals may not be effectively removed during wastewater treatment plant (WWTP) treatment (Archer et al., 2017; Swartz et al., 2018). As a result, the effluent from WWTPs has become a significant source of pharmaceutical contamination in water resources (Archer et al., 2017; Swartz et al., 2018; Gwenzi et al., 2022). Once present, they can bioaccumulate, adversely affecting both human health and aquatic environments (Ying et al., 2002). Moreover, azithromycin saw increased use during the pandemic, further stressing ecological systems.

Pharmaceutical pollutants in surface water pose a significant risk to public health and the environment (Ebele et al., 2017). The toxic effects of these substances on human health, along with their persistence and tendency to accumulate across different environmental media, raise serious public health concerns (Ebele et al., 2017; Jamil, 2019; Oluwole et al., 2020; Reyes et al., 2020). Additionally, the health and environmental hazards linked to exposure to these pharmaceuticals in water resources remain poorly understood. Researchers, including O'Flynn et al. (2021), have investigated the origins and pathways of pharmaceuticals in aquatic ecosystems through life-cycle assessments. Studies by Gwenzi et al. (2022) and Kumari and Kumar (2021, 2022) examined the environmental and health risks associated with COVID-19 antiviral drugs such as ribavirin, ritonavir, and lopinavir, focusing on their impact across three levels of aquatic organisms: algae, Daphnia, and fish. Their research utilised half-maximal effective concentration (EC_{50}) or lethal concentration (LC_{50}) values from the Ecological Structure Activity Relationships Class Program (ECOSAR database) from the U. S. Environmental Protection Agency (2008), which has been known to sometimes underestimate toxic effects (Raimondo et al., 2010). Additionally, Tarazona et al. (2021) reviewed the environmental consequences of therapeutic approaches for COVID-19 drugs. Their prospective analysis leveraged various models and available data to predict the environmental risks associated with different COVID-19 medications. Almeida

et al. (2023), evaluated the ecotoxicological risks of 22 antimicrobial pharmaceuticals, finding that rifaximin and atovaquone could present significant risks to aquatic life. Conversely, flucloxacillin, piperacillin/tazobactam, meropenem, ceftriaxone, fosfomycin, and metronidazole showed considerable potential to foster antibiotic resistance (Almeida et al., 2023). Likewise, Desgens-Martin and Keller (2021) modelled the fate and toxicity risks to aquatic ecosystems posed by remdesivir and dexamethasone, both used in COVID-19 treatments, based on predicted environmental concentrations generated by the ChemFate model.

Despite existing research, there is a significant gap in the literature regarding the ecological and human health risks posed by repurposed corticosteroids (such as prednisone and dexamethasone), anticoagulants (such as heparin), and antivirals (such as azithromycin) used for COVID-19. Studies that clarify their contamination in water resources and the ecological and human health dangers associated with these drugs are lacking. This deficiency of comprehensive data hinders a practical assessment and mitigation of related risks. Filling these knowledge gaps is crucial for guiding post-pandemic water quality monitoring efforts in South Africa and globally, thereby protecting aquatic environments and public health.

This research study embarks on a pioneering effort to apply experimental data from field studies to assess the spatial distribution of four COVID-19 pharmaceuticals and their ecological risks, as well as the human health risks in a transboundary water basin. The presence of pharmaceutical residues in aquatic ecosystems can lead to prolonged ecological and health consequences. The effects of these drugs on aquatic organisms are particularly concerning due to the risk of extended exposure to contaminated water.

To address these issues, various researchers have developed risk assessment methodologies to classify risks as acute or chronic and rate them as high, medium, or low. These frameworks provide crucial insights into the lethality of pharmaceuticals on aquatic life. A commonly used approach is the Risk Quotient (RQ) method, which measures the risks posed by pharmaceuticals across different environmental compartments, including soil, water, fauna, and flora (Hayden et al., 2022). Furthermore, other effective strategies, such as exposure-based scenario evaluation methods, have successfully highlighted the risks these pharmaceuticals pose to human health in environmental settings (Bercu et al., 2008; Kumar et al., 2010). Researchers utilise ecological risk assessment techniques, including the RQ, to quantify these risks, categorising them as low, moderate, or high based on measured environmental concentrations (MEC) and predicted no-effect concentrations (PNEC - the environmental concentration at which no adverse effect on aquatic ecosystem function is to be expected) (Zhang et al., 2022).

7.2 Materials and methods

7.2.1 Study area

The focus of this research is the Orange-Sengu transboundary river basin, located in Southern Africa. As one of the region's largest river basins, it covers approximately 1000000 km² and spans four countries: Lesotho, South Africa, Botswana, and Namibia (Brown, 2013). The basin starts in the Lesotho highlands and flows as the Sengu River through the lowlands, receiving input from several minor tributaries, including the Malibamatso, Mashai, and Sequnyane Rivers (Brown, 2013). The Caledon River serves as one of its major tributaries (Kranz et al., 2005) and marks the western border of Lesotho. As the Caledon merges into the Orange−Senqu River, which traverses Lesotho, South Africa, and Namibia, its water quality becomes increasingly crucial, especially for the riparian nations that rely on it for their water needs (Chatanga et al.,

2019). South Africa accounts for about 63% of the total water withdrawal, making it the largest user among the four riparian countries. In contrast, upstream Lesotho, with its ample water resources, only withdraws around 0.2% (Mahlakeng 2020). The study area commences at the Thetsane River in Maseru and extends to the Gariep Dam, just before the upper Orange River joins the Vaal River.

Safeguarding water resources in the Orange−Sengu River Basin is crucial for ensuring sustainable socio-economic and environmental advancement in the region. The primary goal is to achieve a thorough understanding of pharmaceutical pollution in the area and to inform the formulation of effective monitoring and mitigation strategies.

7.2.2 Sampling site description and sample collection

This study involved collecting water samples from eight locations along the Caledon (Mohokare) River and the Orange−Senqu River. The sites were chosen based on their accessibility for sampling and proximity to wastewater treatment plants (WWTPs). Samples were collected upstream, downstream, and at the WWTP discharge point at each location. Focusing on sampling at these discharge points aims to determine the composition of the effluent entering the river, thus highlighting its impact on the river's ecosystem.

Two sampling campaigns were carried out: one in February, representing the summer season, and the other in May, representing the winter season. These operations spanned various geographical points along the Orange−Senqu River, including Site 1: Gariep WWTP (30°35'7.56"S and 25°30'54.62"E); Site 2: Gariep Dam (30°35'11.41"S and 25°29'11.45"E); Site 3: Aliwal North (30°41'12.71"S and 26°41'39.43"E); Site 4: Zastron (30°17'57.91"S and 27° 6'43.95"E); Site 5: Ladybrand (29°11'12.81"S and 27°29'7.82"E); Site 6: Maseru (29°18'53.23"S and 27°28'2.11"E); Site 7: Ficksburg (28°53'26.53"S and 27°53'45.73"E); and Site 8: Clarens (28°31'17.23"S and 28°25'50.80"E). Three sites were added along the Mohokare (Caledon) River, including Lesotho (Maseru), Ladybrand, and Ficksburg. The Mohokare River is a major tributary of the Orange-Senqu River (Chatanga et al., 2019), which justifies its inclusion in this study. Figure 7.1 illustrates the locations of the sampling sites.

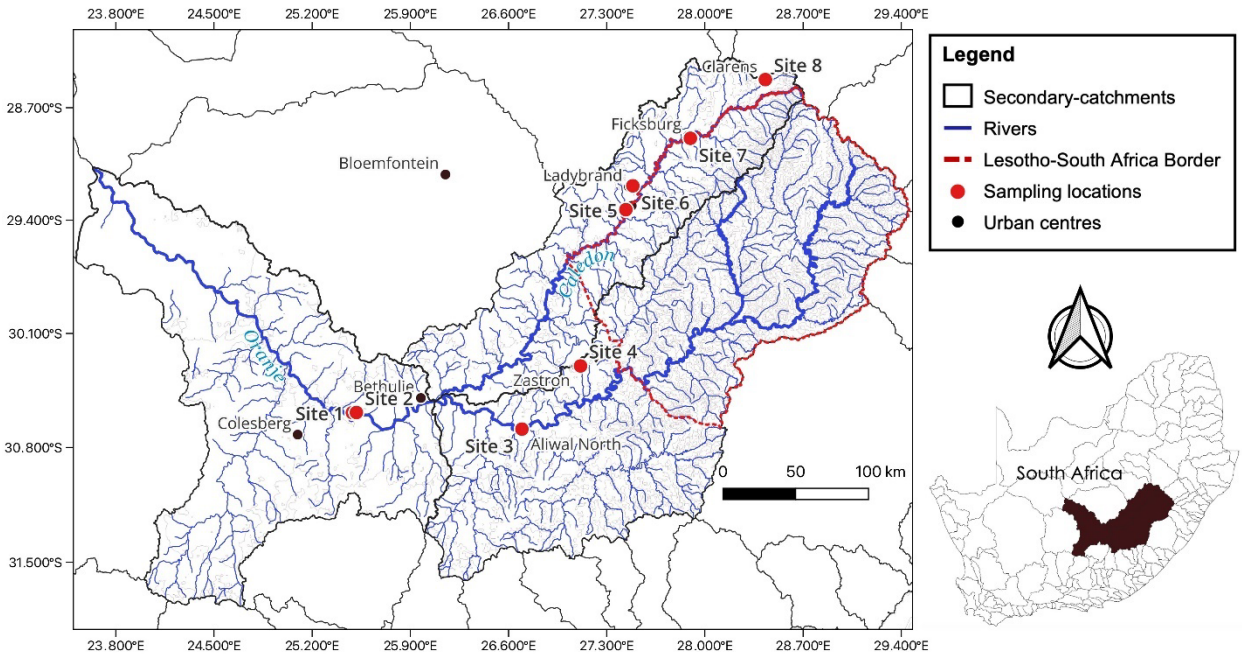

Figure 7.1 Location of sampling sites along the Orange−Senqu River

Site 1: Gariep WWTP (30°35'7.56"S and 25°30'54.62"E); Site 2: Gariep Dam (30°35'11.41"S and 25°29'11.45"E); Site 3: Aliwal North (30°41'12.71"S and 26°41'39.43"E); Site 4: Zastron (30°17'57.91"S and 27° 6'43.95"E); Site 5: Ladybrand (29°11'12.81"S and 27°29'7.82"E); Site 6: Maseru (29°18'53.23"S and 27°28'2.11"E); Site 7: Ficksburg (28°53'26.53"S and 27°53'45.73"E); Site 8: Clarens (28°31'17.23"S and 28°25'50.80"E).

7.2.3 Sample preparation

The water samples were filtered using 0.45 µm glass fibre membranes (Millipore), with a filtering unit thoroughly cleaned with methanol and deionised water. After filtration, the water samples were concentrated using solid-phase extraction (SPE). The SPE process for the samples included sequentially conditioning HLB cartridges with 10 mℓ of MeOH followed by 10 mℓ of water. The 400 mℓ sample was loaded at a rate of 3–5 mℓ/min, and the columns were dried under vacuum for 30 minutes. Analytes were then eluted using 10 mℓ of MeOH in salinised vials. The eluates were concentrated with compressed air and reconstituted in 1 mℓ of MeOH before analysis via LC-ESI-Q-MS. The recovery efficiency of the analytes was assessed and validated (Omotola et al. 2024).

7.2.4 Quality control and quality assurance

All sampling equipment, including amber bottles for water and glass jars for sediment, was pre-cleaned with deionised water and rinsed with dilute nitric acid to minimise contamination. Surface water samples were collected in sterile amber glass bottles to prevent photodegradation and immediately stored at 4 °C in ice chest coolers. Neat analyte standards, acetonitrile (LC-MS grade), and methanol (LC grade) were obtained from Sigma-Aldrich (South Africa), while solid-phase extraction cartridges (HLB, 6 mℓ, 200 mg) were purchased from Sigma-Aldrich (US). All other chemicals and solvents were of analytical grade. Milli-Q water for LC analysis was produced using a Millipore Elix Water Purifier, and all glassware was cleaned with laboratory detergent, followed by sequential rinsing with acetonitrile and Milli-Q water.

Analyte concentrations were determined using the method of Omotola et al. (2024). Working standards, prepared from 1 000 mg L^{-1} stock solutions, were analysed under gradient conditions on a Kinetex phenyl-hexyl column (150 × 4.60 mm, 2.6 µm) coupled to an LC–ESI–Q–MS 2020 system. A limited range of mobile-phase compositions was tested to optimise separation, and method validation followed the ICH Harmonised Tripartite Guideline (1994). Calibration sensitivity was assessed from the slope of a calibration curve (peak area vs. azithromycin concentration) prepared in Microsoft Excel. The limits of detection (26.57 µg L^{-1}) and quantification (80.52 µg L^{-1}) were calculated as 3.3 and 10 times, respectively, the standard error of the mean divided by the slope.

7.2.5 Environmental risk assessment

The environmental risk assessment associated with the presence of azithromycin in the water was determined by the risk quotient (RQ) and hazard index (HI) for azithromycin and the glucocorticoids (prednisone, prednisolone, and dexamethasone) using the measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC) (Equation 1) following the European Union's ecological risk assessment approach (Zhang et al., 2022).

$$Risk\ Quotient\ (RQ) = MEC/PNEC \qquad (1)$$

To account for variability in reported thresholds, the PNEC value of 84 µg L^{-1} for azithromycin in fish (Pereira et al., 2020; European Commission, 2003) and 50 µg L^{-1} (Stanton et al., 2020) were applied in the analysis. PNEC values (ng L^{-1}) for target glucocorticoids (prednisone (100), prednisolone (89.6), and dexamethasone (13.74)) were sourced from the literature (Tan et al., 2024). The RQ is typically used to characterise the degree of ecological risk based on the value obtained with classification as follows: RQ < 0.01- minimal risk; 0.01 ≤ RQ < 0.1 − low risk; 0.1 ≤ RQ < 1 − medium risk; and RQ ≥ 1 − high risk (Chen et al., 2022).

A region's hazard index (HI) is the sum of the potential environmental risks posed by various pharmaceutical compounds, calculated using Equation 2 (Xiang et al., 2024).

$$HI = \Sigma RQi \qquad (2)$$

7.2.6 Human health risk assessment

Although direct fish tissue data were unavailable, human health risks associated with pharmaceutical contaminants could be estimated using predicted fish tissue concentrations. This approach involves calculating the estimated daily intake (EDI) from fish consumption and comparing it to acceptable daily intakes (ADIs).

Predicted fish concentrations (C_{fish}, µg g^{-1}, ww) were estimated by applying bioaccumulation or bioconcentration factors (BAFs/BCFs) sourced from the literature (BAF = 6.3 × 10^4 L kg^{-1}, BCF = 4.4 L kg^{-1}) (Zu et al, 2022; Terzic et al., 2024) to the MECs (µg L^{-1}) of azithromycin (Equation 3) (Terzic et al., 2024).

$$C_{fish} = MEC \times BAF\ or\ BCF \qquad (3)$$

The EDI was calculated using Equation 4, which considers the predicted fish concentration (µg g-1), the adult fish ingestion rate (IR, 0.02 kg per day), and the estimated adult body weight (BW, 70 kg) (Moodley et al, 2021).

$$EDI = (C_{fish} \times IR) / BW \qquad (4)$$

The hazard quotient (HQ) of long-term potential health effects was evaluated using Equation 5 and classified as follows: HQ < 1 – acceptable risk; HQ ≥ 1 - unacceptable risk (Chen et al., 2011), where ADI (mg kg^{-1} bw) is the acceptable daily intake level. An authoritative microbiological ADI for azithromycin of 1.7 µg kg^{-1} per day with no clear toxicological NOAEL/NOAEC (no-observed-adverse-effect level/concentration) for human health derivatives was used (Leung et al., 2013).

$$HQ = EDI / ADI \qquad (5)$$

7.3 Results and discussion

Pharmaceutical concentrations of three glucocorticoids (prednisolone, dexamethasone, prednisone and the antibiotic azithromycin) were quantified in surface water samples from eight sites classified as upstream from wastewater treatment plants (WWTPs), (U), point of discharge (P), and downstream (D) during summer (n = 16) and winter (n = 17) 2024 sampling campaigns. Due to dense riparian vegetation that limited access, winter sampling was partially constrained (Table 7.1).

Table 7.1 Mean concentrations (µg L^{-1}, (SD), n=3) of pharmaceutical residues in water at the 17 sampling points (U - upstream, P - point of discharge and D – downstream)

	Mean Concentrations (µg L^{-1})						
	Summer	Winter	Summer	Summer	Winter	Summer	Winter
Site	Azithromycin		Dexamethasone	Prednisolone		Prednisone	
P1	18.04 (0.69)	13.35 (0.02)					
P2	6.71 (0.43)	3.23 (0.06)					
U4	16.67 (0.34)	13.94 (0.35)	17.8 (0.25)				
P4		13.67 (0.1)					
D4	7 (0.24)	13.48 (0.1)			7.7 (0.13)		
U5	1.87 (0.38)					11.19 (1.34)	
P5	12.48 (0.58)		41.79 (1.92)			6.44 (0.61)	
D5		3.42 (0.03)			12.14 (0.52)	7.14 (0.74)	
U6	16.47 (0.01)	4.09 (0.06)		39.94 (0.94)			
P6		4.23 (0.02)					13.49 (0.96)
D6	17.2 (0.16)	5.18 (0.06)		17.54 (0.8)			
U7						9.07 (0.67)	
P7	8.04 (0.39)	19.29 (0.27)					
D7	1.1 (0.02)	8.49 (0.4)					
U8	1.86 (0.01)		10.12 (0.89)				
P8	14.76 (0.16)	19.32 (0.26)			11.86 (0.22)		
D8	11 (0.71)	11.26 (0.02)	19.17 (0.41)				

Sites: 1 Gariep Dam WWTP*, 2 - Gariep Dam, 3 - Aliwal North, 4 - Zastron WWTP, 5 - Ladybrand WWTP, 6 – Maseru Thetsane River, 7 - Ficksburg Sewage Work Final Effluent and 8 - Clarens WWTP. *WWTP – wastewater treatment plant.

Across all sites, concentrations ranged from below the limit of detection (<LoD) to 41.79 µg L^{-1} in summer and <LoD to 19.32 µg L^{-1} in winter (Table 7.1). Dexamethasone recorded the highest individual value during summer at Site P5 (Ladybrand WWTP), whereas azithromycin peaked in winter at Site P8 (Clarens WWTP). Azithromycin was also the most frequently detected analyte, particularly at discharge points.

A paired t-test was conducted for azithromycin using the 10 sites with complete summer and winter data. The mean summer concentration was 11.7 (0.3) ng L^{-1}, compared with 11.2 (0.2) ng L^{-1} in winter. The calculated t-statistic was 0.211 ($p = 0.838$), indicating no statistically significant seasonal difference ($p > 0.05$). While some sites exhibited pronounced shifts, such as decreases at U6 and D6 from summer to winter versus increases at P7 and D7, the magnitude and direction of these changes varied among locations, preventing the emergence of a uniform seasonal trend. Prednisolone and prednisone were generally not detected at the same sites, except at D5, and their detection was sporadic. The highest prednisolone concentration was recorded at Site P6 during summer, while prednisone concentrations were typically close to the limit of detection (LoD). Dexamethasone occurrence was comparatively low, although occasional high spikes suggest episodic discharge events.

Compared with previous studies, the concentrations observed in this study are generally low. Yazdan et al. (2021) reported prednisone concentrations in surface water ranging from 0.05 to 433 ng L^{-1}, and Gong et al. (2019) observed 0.03–29 ng L^{-1} across multiple countries, with the highest value reaching 230 ng L^{-1}. In contrast, our measured prednisone concentrations were near the LoD. Similarly, dexamethasone concentrations in this study were mostly low, while He et al. (2022) reported 0.02 - 0.09 ng L^{-1} in Chinese wastewater effluents, and Musee et al. (2021) recorded much higher surface water concentrations of 147 ng L^{-1} in Switzerland, 353 ng L^{-1} in Portugal, and 360 ng L^{-1} in Spain. These comparisons suggest that, although occasional spikes occur, overall corticosteroid contamination in the studied sites is lower than that reported in several other international locations. The sporadic detection of dexamethasone, coupled with occasional high peaks, suggests episodic inputs, potentially linked to hospital or seasonal veterinary usage.

The frequent detection of azithromycin, especially at WWTP discharge points, reflects its high prescription rate and environmental persistence. Usage patterns likely remain elevated in the aftermath of the COVID-19 pandemic, when azithromycin was widely prescribed as a prophylactic or adjunct therapy (Maremane et al., 2025). Its chemical stability and resistance to biodegradation (Michael et al., 2013) contribute to prolonged environmental residence, with concentrations persisting through both summer and winter.

The lack of a statistically significant seasonal difference in mean azithromycin concentrations contrasts with studies reporting higher summer values, often attributed to reduced river flow, lower dilution capacity, and increased pharmaceutical consumption in warmer months (Zhang et al., 2022). In this case, the relatively close seasonal means may indicate that high baseline inputs from continuous WWTP discharge have overridden seasonal dilution and degradation effects. This pattern has also been documented during COVID-19, where winter and summer values (~7 ng L^{-1}) were nearly identical (Mirzaie et al., 2022). Nevertheless, seasonal hydrological factors remain relevant: in winter, increased river discharge from

rainfall and snowmelt enhances dilution and facilitates removal via sediment sorption and biodegradation (Kruć-Fijałkowska et al., 2025), while in summer, elevated sunlight and temperature promote photodegradation and microbial activity (Mirzaie et al., 2022).

Spatial patterns were also evident. Sites with consistently low or non-detectable concentrations, such as Site 3 (Aliwal North), may benefit from smaller catchment populations, lower pharmaceutical usage, and/or more effective WWTP performance or natural attenuation capacity (Aus der Beek et al., 2016). Conversely, high discharge-point concentrations indicate WWTPs as major point sources, underscoring the limitations of conventional treatment in removing persistent pharmaceuticals.

The detection of both antibiotics and glucocorticoids year-round has ecological implications. Continuous low-level antibiotic exposure can promote antimicrobial resistance development (Boxall et al., 2012), while glucocorticoids can disrupt endocrine function and impact aquatic organism health (Carvalho and Santos, 2016). The persistence of these compounds underscores the importance of targeted monitoring at high-risk sites and investment in advanced treatment technologies, such as ozonation or activated carbon, to improve pharmaceutical removal.

Site	AZI				PRDL		DEX	PRD		
	S50	W50	S84	W84	S	W	S	S	W	
	RQ									HI
P1	0.36	0.27	0.21	0.16						1.00
P2	0.13	0.06	0.08	0.04						0.32
U4	0.33	0.28	0.20	0.17			1.30			2.27
P4		0.27		0.16						0.44
D4	0.14	0.27	0.08	0.16		0.09				0.74
U5	0.04		0.02					0.11		0.17
P5	0.25		0.15				3.04	0.06		3.50
D5		0.07		0.04		0.14		0.07		0.32
U6	0.33	0.08	0.20	0.05	0.45					1.10
P6		0.08		0.05					0.13	0.27
D6	0.34	0.10	0.20	0.06	0.20					0.91
U7								0.09		0.09
P7	0.16	0.39	0.10	0.23						0.87
D7	0.02	0.17	0.01	0.10						0.31
U8	0.04		0.02				0.74			0.80
P8	0.30	0.39	0.18	0.23		0.13				1.22
D8	0.22	0.23	0.13	0.13			1.40			2.11

Figure 7.2 Heat map
Showing the risk quotient (RQ) and hazard index (HI) for glucocorticoids (prednisolone (PRDL), dexamethasone (DEX), and prednisone (PRD)) and the antibiotic azithromycin (AZI). U: upstream, P: point of discharge, D: downstream. Red – very high-risk, orange - high risk, blue - moderate risk, green - low risk and light green – no risk. Sites: 1 Gariep Dam WWTP*, 2 - Gariep Dam, 3 - Aliwal North, 4 -

Zastron WWTP, 5 - Ladybrand WWTP, 6 – Maseru Thetsane River, 7 - Ficksburg Sewage Work Final Effluent and 8 - Clarens WWTP. *WWTP – wastewater treatment plant.

For ecological risk, a deterministic approach was used to calculate the risk quotient (RQ) and hazard index (HI) using single-point values for each parameter, based on the measured environmental concentrations (MECs in µg/ℓ) upstream (U), at the point of discharge (P) and downstream (D) of the WWTPs at the eight sites (Figure 7.2). The PNEC value selected for azithromycin in fish from the literature was 84 ug/ℓ (Pereira et al 2020), which was calculated from available acute or chronic ecotoxicity data for several aquatic organisms, according to the European Technical Guidance Document (European Commission 2003). The PNEC is typically derived by dividing the lowest observed ecotoxicological threshold by an assessment factor to account for uncertainties. The PNEC of 50 µg/ℓ for azithromycin, determined by Stanton et al. (2020) by taking the no-observed-selection concentration and applying an assessment factor of 10, was also applied in this study.

The heat map (Figure 7.2) visually represents the RQ and HI values for pharmaceutical compounds across different sampling sites. It uses a colour gradient to indicate the RQ values, where red regions signify very high-risk zones (RQ ≥ 2), orange represents high risk (1 ≤ RQ < 2), blue indicates moderate risk (0.1 < RQ < 1), and green shows low risk (0.01 ≤ RQ < 0.1). The light green zones indicate sites where pharmaceutical residues were not detected.

Azithromycin shows consistent detection across most sites, with moderate RQs observed at several locations, reinforcing its widespread use and persistence in the environment. Using 84 µg L^{-1} as the PNEC for azithromycin, RQs spanned 0–0.21 in summer and 0–0.23 in winter. By the commonly used categories, six sites in summer and winter were classified as low risk, and seven as moderate risk. Using the alternative 50 µg L^{-1} PNEC for azithromycin, RQs rose accordingly (0–0.36 in summer and 0 - 0.39 in winter) but still did not exceed 1 at any site. The winter maximum occurred at P7 (0.39) and P8 (0.39). In the summer, 10 sites were classified as moderate risk and 3 as low risk. In winter, eight sites were classified as moderate and five as low risk. In all cases, there were no high-risk sites. Under both PNEC choices, the RQs remain < 1, indicating that all sites are at low to moderate ecological risk (Chen et al., 2022). Winter generally elevated RQs at several sites (P7, P8), consistent with reduced dilution/flow rates, or seasonal usage patterns, often observed for antibiotics in catchments (Zhang et al., 2022).

Spatial trends show clustering of moderate-risk zones, particularly at specific sampling sites, which may correspond to discharge points or areas downstream. This aligns with the pattern of pharmaceutical pollution linked to urban or agricultural wastewater inputs. The general trend from the environmental risk assessment reveals significant variability in the risk profiles across the monitored sites. The RQ values for individual pharmaceutical compounds reveal substantial variability across the sites and between summer and winter. Dexamethasone presents localised high risks, particularly at P5 (summer: 3.0) and U4 (summer: 1.3). Prednisolone tends to contribute less to overall risk, with moderate RQs (U6 summer: 0.4), reflecting its relatively lower environmental impact. On the other hand, prednisone shows the lowest RQs across most

sites, with a maximum value of 0.11 at U5 in summer, possibly due to its rapid conversion to prednisolone or other factors influencing its persistence in the environment.

The HI, which aggregates the risks of pharmaceutical inputs, provides insight into the cumulative ecological risks across sites. The highest-risk sites, characterised by elevated HI values, include P5 (HI: 3.5), U4 (HI: 2.27), and D8 (HI: 2.11). These sites are dominated by high dexamethasone contributions and highlight localised contamination, likely driven by concentrated pharmaceutical discharges or upstream inputs, thereby significantly elevating environmental risks.

For health risk, a deterministic approach was used to calculate the hazard quotient (HQ) using single-point values for each parameter based on measured environmental concentrations (MECs in μg L^{-1}) at the eight sites (Table 7.2). The predicted concentration in fish tissue (C_{fish} μg g^{-1}, ww) was estimated using bioaccumulation data from the literature due to a lack of locally derived fish data, introducing some uncertainty as these predictions rely on assumptions. To address this, a sensitivity analysis using different bioaccumulation factors (BAFs) and bioconcentration factors (BCFs) was conducted to assess a range of bioaccumulation scenarios.

According to the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation, azithromycin, with a reported logBAF of 4.8 (BAF = 6.3×10^4 L kg^{-1}), is classified as a very bioaccumulative compound, warranting close attention to its environmental occurrence (Zu et al., 2022). This logBAF value was determined from laboratory studies using *Cyprinus carpio*, *Danio rerio*, *Carassius auratus*, *Carassius carassius*, *Acipenser schrenckii*, and *Oryzias melastigma* (Zu et al., 2022). In contrast, the more conservative BCF value of 4.4 L kg^{-1} (range 2.3-7.3 L kg^{-1}) applied here was derived from field studies by Terzić et al. (2024), which included fish species such as Common barbel (*Barbus barbus*), Prussian carp (*Carassius gibelio*), Common chub (*Squalius cephalus*), Black bullhead (*Ameiurus melas*), Common carp (*Cyprinus carpio*), Common nase (*Chondrostoma nasus*), and Common rudd (*Scardinius erythrophthalmus*).

The EDI approach used followed standard practice using an adult ingestion rate of 0.02 kg of fish per day and an average body weight of 70 kg for adults (Moodley et al., 2021). For azithromycin, the microbiological ADI of 1.7 μg kg^{-1} per day was used in the absence of robust human toxicological NOAEL/NOAEC and is widely adopted in environmental and food-risk derivations (Leung et al., 2013). This is a calculated microbiological ADI for azithromycin, based on effects on human gut bacteria, used for human health derivations considered protective and appropriate for screening (Leung et al., 2013).

The sensitivity analysis demonstrates a stark divergence between bioaccumulation assumptions. In the case of the BAF being 6.3×10^4 L kg^{-1}, the predicted fish concentrations (C_{fish}) are hundreds to thousands of μg g^{-1}, yielding relatively high HQs at all non-zero sites with HQs in summer ranging from 11.6 to 191 and 34.2 to 204.5 in winter. In the BCF scenario (4.4 L kg^{-1}), the predicted fish concentrations are in the 10^{-2} μg g^{-1} range, yielding relatively low HQs at all non-zero sites (summer HQ range: 0.0008 - 0.0133; winter: 0.00239 - 0.0143).

The BAF reflects uptake from both water and diet and is usually field-derived, while the BCF considers only waterborne uptake under steady-state conditions (European Commission, 2003). For ionisable, polar pharmaceuticals such as azithromycin, typical low BCFs are due to reduced passive diffusion at environmental pH, rapid biotransformation, and sorption to particulate matter (Zhang et al., 2022). In contrast, high BAFs can reflect trophic exposure, gut content, or tissue binding, which may overestimate edible muscle residues relevant for dietary risk (European Commission, 2003).

Given azithromycin's ionisable nature and data indicating generally low laboratory BCFs for antibiotics, the BCF-based estimate is the more realistic measure for edible fish parts in EDI calculations. However, the BAF can be viewed as a conservative upper limit, representing a screening-level high bioaccumulation scenario (European Commission, 2003; Zhang et al., 2022; Zu et al., 2022; Senka Terzić et al., 2024).

Overall, the ecological risk assessment showed moderate to low impact at most sites under either PNEC choice, while the human health risk assessment via fish consumption depended entirely on the chosen bioaccumulation metric, with the impact converting from negligible using the BCF to unacceptably high using the BAF. in this study, ecological risk levels that were mostly moderate to low are consistent with many riverine antibiotic assessments that report sub-PNEC RQs at μg L^{-1} MECs, especially where dilution is moderate and PNECs are derived from fish and invertebrate endpoints (Zhang et al., 2022; European Commission, 2003; Pereira et al., 2020; Stanton et al., 2020). The human health risk via fish for macrolides is typically negligible when BCF-based edible tissue concentrations are used with realistic IR/BW, but can appear high under field-based BAF extrapolations. This divergence has been highlighted in discussions of antibiotic bioaccumulation for ionisable pharmaceuticals (European Commission, 2003; Zhang et al., 2022; Zu et al., 2022; Senka Terzić et al., 2024).

Table 7.2 Concentration in fish tissue, estimated daily intake (EDI) and hazard quotient (HQ) based on two bioaccumulation scenarios for the antibiotic azithromycin (AZI) U: upstream, P: point of discharge, D: downstream

	BAF = 6.3×10^4 L kg^{-1},						BCF = 4.4 L kg^{-1}					
	Concentration Fish (ug g^{-1})		EDI (ug kg^{-1})		HQ		Concentration Fish (ug g^{-1})		EDI (ug kg^{-1})		HQ	
Site	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
P1	1136.5	841.1	324.7	240.3	191.0	141.4	0.079	0.059	0.023	0.017	0.013	0.010
P2	422.7	203.5	120.8	58.1	71.0	34.2	0.030	0.014	0.008	0.004	0.005	0.002
U4	1050.2	878.2	300.1	250.9	176.5	147.6	0.073	0.061	0.021	0.018	0.012	0.010
D4	441.0	849.2	126.0	242.6	74.1	142.7	0.031	0.059	0.009	0.017	0.005	0.010
P4	0.0	861.2	0.0	246.1	0.0	144.7	0.000	0.060	0.000	0.017	0.000	0.010
D5	0.0	215.5	0.0	61.6	0.0	36.2	0.000	0.015	0.000	0.004	0.000	0.003
P5	786.2	0.0	224.6	0.0	132.1	0.0	0.055	0.000	0.016	0.000	0.009	0.000
U5	117.8	0.0	33.7	0.0	19.8	0.0	0.008	0.000	0.002	0.000	0.001	0.000
U6	1037.6	257.7	296.5	73.6	174.4	43.3	0.072	0.018	0.021	0.005	0.012	0.003
D6	1083.6	326.3	309.6	93.2	182.1	54.8	0.076	0.023	0.022	0.007	0.013	0.004
P6	0.0	266.5	0.0	76.1	0.0	44.8	0.000	0.019	0.000	0.005	0.000	0.003
P7	506.5	1215.3	144.7	347.2	85.1	204.2	0.035	0.085	0.010	0.024	0.006	0.014
D7	69.3	534.9	19.8	152.8	11.6	89.9	0.005	0.037	0.001	0.011	0.001	0.006
P8	929.9	1217.2	265.7	347.8	156.3	204.6	0.065	0.085	0.019	0.024	0.011	0.014
U8	117.2	0.0	33.5	0.0	19.7	0.0	0.008	0.000	0.002	0.000	0.001	0.000
D8	693.0	709.4	198.0	202.7	116.5	119.2	0.048	0.050	0.014	0.014	0.008	0.008

Sites: 1 Gariep Dam WWTP*, 2 - Gariep Dam, 3 - Aliwal North, 4 - Zastron WWTP, 5 - Ladybrand WWTP, 6 – Maseru Thetsane River, 7 - Ficksburg Sewage Work Final Effluent and 8 - Clarens WWTP. *WWTP – wastewater treatment plant.

CHAPTER 8
PREDICTIVE DEPOSITION AND BUILD-UP OF SELECTED PHARMACEUTICAL COMPOUNDS IN THE COLUMNS OF THE ORANGE-SENQU RIVER BASIN AQUATIC SYSTEM CHANNELS

8.1 Introduction

The presence and occurrence of pollutants in the environment are primarily dependent on the sources of generation and release, the state and speciation of the pollutants, and the quantity of pollutants released (Shetty et al., 2023; Zhou et al., 2024). Moreover, the presence of these compounds is also influenced by the frequency of release, the nature of the receiving environmental medium, and prevailing climatic factors (Atuyambe et al., 2024; Bolan et al., 2024; Sundas et al., 2024). The variables at play in establishing occurrences are numerous and interdependent (Li et al., 2025a). These variables include, but are not limited to, the chemical, physical, and biological properties of the pollutants (Cachada et al., 2018). Consequently, determining the levels of contaminants/pollutants in receiving environmental media, such as water and sediment/soil, and their quality can be complicated.

The deposition of pharmaceutical and organic compounds into the environment has been established (Belle et al., 2025; Eapen et al., 2024; Zampelli and Verna, 2025). However, the environmental processes, mechanisms, and fate of these compounds in the environment depend on the receiving matrices and the physical and chemical characteristics of these matrices (Habimana and Sauvé, 2025; Waleng and Nomngongo, 2022). Weather and climatic factors have been reported to affect the levels of pharmaceutical and other organic compounds in the atmosphere and in water/sediment in aquatic or marine environments (Sousa et al., 2023; Wollenweber et al., 2025). Nevertheless, the amount present in a given water column per unit time is a function of the compound's solubility, aqueous density, the retention capacity of the aqueous system (also density-dependent), and the fluvial processes and hydrodynamic characteristics of the channel (Nguyen, 2020; Walch et al., 2022). Notably, among the processes that determine sediment concentration are flow dynamics, fluvial transport, erosion and deposition rates, sediment particle characteristics (fine or coarse), particle behaviour, and channel geometry (Li et al., 2025b). Thus, although complex, predicting the sediment retention capacity of PCs may be described using models such as the sediment transport model, which accounts for particle deposition in fluvial processes involving the movement of sediment by flowing water (Molina et al., 2024; Mu et al., 2021). Furthermore, this model utilises sediment transport capacity equations that incorporate the influences of flow velocity, channel geometry, particle size, and density (Jiang et al., 2023).

The model for particle deposition in fluvial systems (air currents and flow channels) involves understanding the interplay between fluid dynamics and particle characteristics and the geometry of the flow path, categorised as Eulerian and Lagrangian approaches, which treat particles as pseudo-fluids tracked by the Eulerian−Lagrangian individual trajectories (Akter and Saha, 2024; Sakib et al., 2023). It is essential to recognise that false presumptions can impact the accuracy of a model in predicting the processes that a pharmaceutical may undergo and its spatiotemporal distribution throughout its journey (Shaheen et al., 2022). Additionally, no single model can provide an absolute predictive concentration for pharmaceuticals in terrestrial soils or aqueous systems. While the pollutant deposition rate is a crucial factor in determining the presence of pollutants in the environment, their occurrence levels depend on biotransformation or environmental

transformation rates (Ashfield et al., 2025; Waleng and Nomngongo, 2022). It is therefore pertinent to project into the deposition and enrichment (deposition) rate of pharmaceuticals upon release from different sources into the environment, as well as their biotransformation and or environmental transformation.

In this study, the data generated during the monitoring campaigns (during the summer and winter of 2024) for occurrence levels of selected PCs, azithromycin (AZI), dexamethasone (DEX), prednisone (PRD) and prednisolone (m-PRD), were interpolated in the normalised Stokes equation for fluid flow processes (models), taking into consideration the ambient weather conditions per expedition season.

8.2 Methodology

Fluvial deposition process model for laminar current flow in a strait of an aquatic column and or river channel using the Stokes theorem (Yang, 2024), which described the transient distribution of the flow parameter, was employed in this study. This included fluid flow and particle movement (in this case, seeded pharmaceutical particles) to predict particle deposition. This assumed the viscosity of fluidic systems (air/water) as a function of the density or concentration of pollutants and other solution parameters, as well as the load or content of the media (Baranovskii et al., 2021). The Stokes theorem employed in this study is presented in Equation 1.

$$F=6\pi\eta rv \qquad \text{Equation 1}$$

Given that

F = the viscous drag force (acting opposite to the direction of motion)

6π = a dimensionless constant derived from the mathematical analysis of fluid flow around a sphere

η(eta) = the coefficient of viscosity of the fluid (a measure of its resistance to flow)

r = the radius of the spherical object

v = the velocity of the object relative to the fluid

Since flow is assumed to be continuous, the differential form of the Navier-Stokes equation (Equation 2), founded on the continuity equation that relies on the principle of mass conservation, is applied to predict fluid flow.

$$f\rightarrow -\mu\Delta u\rightarrow+\rho(u\rightarrow\cdot\nabla)u\rightarrow+\nabla p \qquad \text{Equation 2}$$

Given that $\nabla\cdot u\rightarrow = 0\nabla\cdot u\rightarrow = 0$

Where

$\rho\rho$ = the density, kg/m^3;

u = the velocity of the flow, m/s;

μ = the dynamic viscosity, Pa·s;

p = the pressure, Pa; and

f represents the external force applied to the fluid, Pa.

Particle deposition is examined based on the assumption that pollutants/content particles in the flow column do not interact with one another, are independent, and of sufficiently low particle concentrations, such that the

deposited pollutant particles do not significantly impact the fluid flow velocity in the column of a flow channel. This may account for the residual concentration of pollutants in an entrapment (sediment system), given its physical, chemical, and dynamic interactions. Thus, particle movement in a flow column can be estimated using Equations 3:

For particle deposition

$$mpdup\rightarrow dt = 3\pi\mu dpCc\ (ua\rightarrow - up\rightarrow + 2kBT\gamma \sqrt{\text{-----}} dW\rightarrow(t)dt) \quad \text{Equation 3}$$

$$dxpdt = up$$

Where:

mp = the particle mass, kg;

ua = the fluid velocity, m/s,

up = the particle velocity, m/s;

dp = the particle diameter, m;

kB = the Boltzmann constant, J/K;

T = the temperature (K),

$\gamma\gamma$ = the friction factor;

dW = the Wiener measure, which is used to describe Brownian motion; and

xp = the displacement of the particle, m.

8.3 Results and discussion

The understanding of the potential hazards caused by pollutants is a function of the pollutant concentration (dose), exposure (resident time), and pollutant dissipation (Martins et al., 2025; Manisalidis et al., 2020). Thus, there is a need to investigate the mechanisms of PC retention, mass transfer, and fate in the water/sediment system, which is the ultimate aquatic sink. The speciation and behaviour of pollutants vary across different matrices. Their transport and migration patterns in saturated and unsaturated systems depend on their physicochemical properties and the nature of the interaction between pollutants and different components of the systems (i.e., various water systems) (Christensen et al., 2022).

The deposition and entrapment of pharmaceuticals in an aqueous environment are crucial for predicting environmental health and safety and for informing decisions on sustainable environmental management by promoting the development of effective strategies to reduce or eliminate the emission of pharmaceuticals into the environment. Generally, the biotransformation of pharmaceuticals is pharmacokinetic-dependent at the molecular level (Kramlinger et al., 2021; Shanu-Wilson et al., 2020). Environmental transformation, on the other hand, is dependent on many factors, such as the characteristics of the withholding environmental media, site action interaction, and meteorological parameters. Therefore, the quantitative structure for determining the activity relationships of PCs depends on detailed knowledge of contaminant flow, solution viscosity, and depositional patterns.

The physicochemical characterisation of the water columns across the sampling sites, as presented in Supplementary Information 9a and 9b, revealed that the water temperature ranged from 19.8 ºC to 37.4 ºC at Gariep Dam and the Ladybrand Wastewater Treatment Plant (WWTP), respectively. Consistently, the electrical conductivity of the water samples ranged from 585 µS/cm to 1 414 µS/cm. The sum concentration of

the investigated compounds detected across the study sites, as shown in Supplementary Information 1a and 1b, was $\sum PCs$, 225.22 µg/ℓ during winter and $\sum PCs$, 313.41 µg/ℓ during summer, with some of the PCs occurring at not-detectable concentration in the sampled waters. The $\sum PCs$, observed for each of the tested PCs at the different sampling sites during summer and winter were fitted into the fluvial flow deposition model and the particle flow deposition model, except for DEX, which was not detected across all sampling sites during winter.

The models simulating fluvial processes in river channels incorporated sediment-transport equations to predict the formation of bedforms, erosion patterns, and sediment deposition. Since m-PRD, DEX, and PRD were not detected at concentrations above the limit of detection (LoD) during the winter season, except at the following sites: S4 (7.70 µg/ℓ), S5 (12.14 µg/ℓ), and S6 (13.49 µg/ℓ), they were not tested using the particle flow and deposition model. To explain and simulate the fluvial and depositional likelihood of the tested pharmaceuticals, the dependent variables in all five equations (an interdependent coupled system) are simultaneously solved. Consequently, the normalised Navier-Stokes equations presented in Supplementary Information 5 are used as the governing equations for pharmaceutical flow and deposition. The $\sum PCs$ values of AZI, m-PRD, PRD, and DEX were interpolated into the particle movement flow equation and the normalised Navier-Stokes equation to determine the potential for the deposition and subsequent sequestration of the investigated PCs along the tidal and mainstream sediments. The results of the tested model parameters for pharmaceutical flow and deposition along the column, under steady-state and turbulent conditions, are presented in Table 8.1.

Table 8.1 Tested model parameters for the pharmaceutical flow/deposition along the column of the tested parameters under steady state and turbulent conditions

	AZI	m-PRD	PRD	DEX
Continuity	> 1	> 1	> 1	> 1
x−momentum	+ve	+ve	+ve	+ve
y−momentum	–ve	–ve	–ve	–ve
z−momentum	–ve	–ve	–ve	–ve
Energy				
Flow tendency (t_{95})	0.5	0.5	0.5	0.5
Deposition (D_{lam})	0.5	0.5	0.5	0.5
Deposition (D_{turb})	0	0	0	0

Where:

fluid flow continuity (C_f)

C_f can be between 0 and 1

If C_f is 0 = seeding (deposition) and sequestration of contaminants occur

1 = No deposition

Examining the possible flow characteristics, which depend on column velocity, laminar/turbulent flow, shear stress, and channel characteristics, it is uncertain whether sediment sequestration by PCs can result in significant PC build-up at each location, given unpredictable depositional patterns. However, there is potential for sediment accumulation in channels with bowlike entrapments and discrete blebs, where re-suspension or

re-dissolution of sediment is minimal. Water column temperature factors are a co-determinant of PC concentrations in a water-sediment system (Giebułtowicz et al., 2025). Naturally, temperature strongly influences the solubility of solutes in a solvent. It is therefore not unexpected that temperature's impact on solubility across different PCs could affect their occurrence levels, and this could vary due to differences in solubility. Hence, the resuspension of particles from tidal sediment, the sediment bed (epilimnion), and the sub-sediment bed (hypolimnion), as well as the transportation and deposition downstream, is energy- and obstacle-dependent. This is also the determinant of aqueous concentrations in water systems not exposed to continuous input. The distribution of PCs between water and sediment is influenced by particle size, shape, density, and settling velocity, as well as their behaviour in the water column during channel flow (Li et al., 2024). It is essential to note that this may not be true in a water system with a channel morphology/geometry dominated by tidal flats, where flow dynamics differ, thereby affecting the mass transfer and sediment transport rates of PCs and many other soluble and particulate pollutants.

The momentum terms derived from the fluvial and deposition testing suggest that both the flow and the shifting (mass transfer) of the tested pharmaceuticals are convection- and diffusion-controlled. Hence, the random nature of the carriage of pollutants significantly affects the equilibrium in the flow momentum of the pharmaceutical along the x, y, and z directions and is influenced by viscosity, boundary layers, and other flow conditions. It is important to note that the convection nature of the pharmaceutical in the flow column is limited by the physicochemical characteristics (nature) of both the tidal and mainstream boundary layers.

Dexamethasone may have the highest mobility and translocation rate (possibly the reason it was the least frequently detected), while AZI is the most immobile, hence its relatively high detection levels in the sediment system. Barring certain factors, such as chemical and physical processes related to transport, the relationship between the concentrations observed in the sediment at the epilimnion and hypolimnion layers in fluid flow/deposition hydrodynamics provides insight into the mass transfer of pharmaceutical contaminants in unsaturated and saturated aqueous-sediment systems. Geochemical sorption kinetics and thermodynamics, as well as biological transformations, were accounted for in the models describing pollutant transport, dispersion, and immiscible-phase flows.

CHAPTER 9
CONCLUSIONS AND RECOMMENDATIONS

This study sought to assess the levels of four pharmaceutical compounds, namely azithromycin, dexamethasone, prednisone, and prednisolone, in the Orange-Senqu River Basin in summer and winter seasons in 2023 and 2024. The analysis revealed that rapid population growth, combined with increased pharmaceutical use after the COVID-19 pandemic, has led to higher rates of pharmaceutical compound release into aquatic ecosystems. The study argued that although many pharmaceutical compounds, such as acetaminophen and ciprofloxacin, have sufficient environmental data, little is known about the occurrence, fate, and toxicological effects of the chosen corticosteroids and macrolide antibiotics in Southern African water systems. Therefore, the study has bridged this gap and provided important information for water management and monitoring in the region. A rapid liquid chromatography-mass spectrometry (LC-MS) method was optimised, developed, and validated in accordance with the International Conference on Harmonisation guidelines to analyse the four pharmaceuticals, with recoveries above 80% for all. This indicates that the method is reliable and can be used over the long term without issues with accuracy and precision.

Based on the seasonal samples, all four pharmaceuticals were detected in the point of discharge, upstream, and downstream areas. The study found that the macrolide AZI had the highest detection frequency in water samples (79%), although its concentration was low in sediments (38%). On the other hand, m-PRD was found to have the highest concentration in the water samples, while DEX accumulated the most in the sediments. In addition, a significant portion of PRD was intermittently present and was mostly detected at discharge points in summer, suggesting recent inputs. These results demonstrate the importance of pharmaceutical contamination in the Orange-Senqu system, which is attributed to a complex interplay of point and non-point sources, including poor wastewater management, urban runoff, and informal settlements.

Bioassay-based studies further clarified the ecological risks associated with pharmaceutical pollution in the Orange-Senqu system. Yeast-based bioassays, such as the YES, YAAS, and AhR, indicated significant estrogenic, anti-androgenic, and dioxin-like activities in the system, especially in the discharge zones. Furthermore, there was an indication of temporal variability in the system's toxicity profile, with embryotoxicity assays showing 100% mortality in February, followed by a reduction in May. The results of the ecological risk assessment indicated low to moderate risks in the system, with none of the sites exceeding a risk quotient (RQ) of 1. Hotspots were identified in P5, U4, and D8, which were attributed to DEX. The results of the human health risk assessment via fish consumption showed variability depending on assumptions about bioaccumulation. While conservative estimates based on BAFs overestimated risks, but estimates based on BCFs indicated negligible risks. The study further revealed that the behaviour of PCs, as predicted by a normalised Stokes-based approach and the Navier-Stokes equations, is dominated by hydrodynamic convection-diffusion processes, channel morphology, and temperature-dependent effects. Moreover, AZI was the least mobile, as evidenced by its retention of PCs in sediment. In contrast, the results showed that DEX was the most mobile, as observed in the distribution of PCs. This study showed the complex interplay of hydrodynamics, sorption kinetics, and environmental transformation processes in the sequestration of pharmaceuticals in aquatic sediments.

The consistent detection of bioactive compounds such as AZI and DEX in the aquatic environment indicates the potential to disrupt microbial activity, bioaccumulate in benthic organisms, and promote the development of antimicrobial resistance. Therefore, there is an urgent need to implement routine monitoring of pharmaceutical residues, especially in aquatic sediments and discharge points, and this should be complemented by monitoring pharmaceutical use patterns at the source, by evaluating existing wastewater treatment facilities. It is also recommended that studies on the transformation products and the combined toxicological effects of pharmaceutical mixtures in sediments should be conducted, as well as predictive modelling of the fate and transport of pharmaceuticals in aquatic systems. Additionally, quantitative environmental kinetic studies should be conducted to determine the rates of biotransformation and the trigger concentrations of pharmaceuticals that might induce toxic effects in non-target species. Furthermore, stakeholders should focus on remediation and source reduction strategies. At the municipal level, source reduction strategies for pharmaceutical contaminants should focus on preventing their entry into wastewater streams through proper waste disposal and management, as well as public awareness campaigns. These preventive measures, in addition to advanced waste treatment and monitoring, will provide a holistic approach to reducing pharmaceutical contaminants in a transboundary river basin, protecting the ecosystem, and promoting public health.

REFERENCES

ADEDIPE DT, CHEN C, LAI RWS, XU S, LUO Q, ZHOU G-J, BOXALL A, BROOKS BW, DOBLIN MA, WANG X, and co-authors (2024) Occurrence and Potential Risks of Pharmaceutical Contamination in Global Estuaries: A Critical Review and Analysis. Environment International **192** 109031.

ADEEL M, SONG X, WANG Y, FRANCIS D and YANG Y (2017) Environmental Impact of Estrogens On Human, Animal and Plant Life: A Critical Review. Environment International **99** 107–119.

AGUNBIADE FO and MOODLEY B (2014) Pharmaceuticals As Emerging Organic Contaminants in Umgeni River Water System, Kwazulu-Natal, South Africa. Environmental Monitoring and Assessment **186** 7273–7291.

AHMED MB, ZHOU JL, NGO HH and GUO W (2015) Adsorptive Removal of Antibiotics from Water and Wastewater: Progress and Challenges. Science of the Total Environment **532** 112–126.

ALESSI J, OLIVEIRA GB, SCHAAN BD and TELO GH (2020) Dexamethasone in the Era of Covid-19: Friend or Foe? An Essay On The Effects of Dexamethasone Potential Risks of Its Inadvertent Use in Patients with Diabetes. Diabetology & Metabolic Syndrome **12 (80).**

ALI M, KHAN I, KHAN A, RAFIQUE Z and WASEEM H (2022) Chapter 7 - Advances in Biodegradation and Bioremediation of Emerging Contaminants in the Environment. In: *Biological Approaches to Controlling Pollutants* KUMAR S and HASHMI MZ. Woodhead Publishing.

ALMEIDA A, MELLO-SAMPAYO DE, C LOPES, A CARVALHODASILVA, R VIANAP and MEISEL L (2023) Predicted environmental risk assessment of antimicrobials with increased consumption in Portugal during the COVID-19 pandemic; The groundwork for the forthcoming water quality survey. Antibiotics **12 (4)** 652.

ALMEIDA AC, GOMES T, LOMBA JAB and LILLICRAP A (2021) Specific toxicity of azithromycin to the freshwater microalga Raphidocelis subcapitata. Ecotoxicology and Environmental Safety **222** 112553.

AL-SHAMI K, AWADI S, KHAMEES AA, ALSHEIKH AM, AL-SHARIF S, ALA' BERESHY R, AL-EITAN SF, BANIKHALED SH, AL-QUDIMAT AR, AL-ZOUBI RM, and AL-ZOUBI MS (2023) Estrogens and the Risk of Breast Cancer: a Narrative Review of Literature. Heliyon **9(9)**.

AMÉZQUETA S, SUBIRATS X, FUGUET E, ROSÉS M and RÀFOLS C (2020) Octanol-Water Partition Constant. In: *Liquid-Phase Extraction* 183-208.

AMOS SIBEKO P, NAICKER D, MDLULI PS and MADIKIZELA LM (2019) Naproxen, Ibuprofen, and Diclofenac Residues in River Water, Sediments and Eichhornia Crassipes of Mbokodweni River in South Africa: An Initial Screening. Environmental Forensics **20** 129–138.

ANDREU-SÁNCHEZ Ó, GARCÍA-LORENZO ML, ESBRÍ JM, SÁNCHEZ-DONOSO R, IGLESIAS-MARTÍNEZ M, ARROYO X, CRESPO-FEO E, RUIZ-COSTA N, ROCA-PÉREZ L and CASTIÑEIRAS P (2022) Soil and freshwater bioassays to assess ecotoxicological impact on soils affected by mining activities in the Iberian Pyrite Belt. Toxics **10 (7)** 353.

ANTONIEWICZ MR (2013) Tandem Mass Spectrometry for Measuring Stable-Isotope Labeling. Current Opinion in Biotechnology **24 (1)** 48–53.

ANTUNES SC, FREITAS R, FIGUEIRA E, GONÇALVES F and NUNES B (2013) Biochemical Effects of Acetaminophen in Aquatic Species: Edible Clams Venerupis Decussata and Venerupis Philippinarum. Environmental Science and Pollution Research **20** 6658–6666.

ARCHER E, WOLFAARDT GM, WYK VAN and J.H. (2017) Pharmaceutical and personal care products (PPCPs) as endocrine-disrupting contaminants (EDCs) in South African surface waters. Water SA **43 (4)** 684–706.

ARCHER E and WYK JH (2015) The potential Anti-Androgenic effect of agricultural pesticides used in the western cape: in vitro investigation of mixture effects. Water SA **41** 129–137.

ARUMUGAM A, LEE KE, NG PY, SHAMSUDDIN AS, ZULKIFLI A and GOH TL (2025) Pharmaceuticals As Emerging Pollutants: Implications for Water Resource Management in Malaysia. Emerging Contaminants **11 (2)** 100470.

ARUN S, XIN L, GAONKAR O, NEPPOLIAN B, ZHANG G and CHAKRABORTY P (2022) Antibiotics in Sewage Treatment Plants, Receiving Water Bodies and Groundwater of Chennai City and the Suburb, South India: Occurrence, Removal Efficiencies, and Risk Assessment. Science of the Total Environment **851** 158195.

BACKHAUS T and FAUST M (2012) Predictive Environmental Risk Assessment of Chemical Mixtures: a Conceptual Framework. Environmental Science & Technology **46** 2564–2573.

BARRIOS-ESTRADA C, JESÚS ROSTRO-ALANIS M, MUÑOZ-GUTIÉRREZ BD, IQBAL HMN, KANNAN S and PARRA-SALDÍVAR R (2018) Emergent Contaminants: Endocrine Disruptors and their Laccase-Assisted Degradation–A Review. Science of the Total Environment **612** 1516–1531.

BASHAR T, APU MNH, MOSTAID MS, ISLAM MS and HASNAT A (2018) Pharmacokinetics and Bioavailability Study of A Prednisolone Tablet as A Single Oral Dose in Bangladeshi Healthy Volunteers. Volume 16. A Publication of International Hormesis Society, Dose-Response **16(3)** 1559325818783932.

BEEK T, WEBER F-A, BERGMANN A, HICKMANN S, EBERT I, HEIN A and KÜSTER A (2016) Pharmaceuticals in the environment—Global occurrences and perspectives. Environ. Toxicol. Chem **35** 823–835.

BERCU JP, PARKE NJ, FIORI JM and MEYERHOFF RD (2008) Human Health Risk Assessments For Three Neuropharmaceutical Compounds in Surface Waters. Regulatory Toxicology and Pharmacology **50** 420–427.

BHARDWAJ LK, RATH P, BAJPAI S, UPADHYAY D, JAIN H, KUMAR N and SINHA S (2024) Covid-19 and the Interplay with Antibacterial Drug Resistance. In: *Frontiers in Combating Antibacterial Resistance: Current Perspectives and Future Horizons* 246-273. IGI Global Scientific Publishing.

BONNIÈRE A, KHASKA S, SALLE C, LOUVAT P and VERDOUX P (2024) Long-Term Impact of Wastewater Effluent Discharge On Groundwater: Identification of Contaminant Plume By Geochemical. Isotopic, and Organic Tracers' Approach. Water Research **257** 121637.

BOVEE TFH, HELSDINGEN RJR, KOKS PD, KUIPER HA, HOOGENBOOM RLAP and KEIJER J (2004) Development of a rapid yeast estrogen bioassay, based on the expression of green fluorescent protein. Gene 325 187–200.

BOWMAN JC, ZHOU JL and READMAN JW (2002) Sediment–Water Interactions of Natural Oestrogens Under Estuarine Conditions. Marine Chemistry **77** 263–276.

BOXALL ABA, RUDD MA, BROOKS BW, CALDWELL DJ, CHOI K, HICKMANN S, INNES E, OSTAPYK K, STAVELEY JP, VERSLYCKE T, ANKLEY (2012). Pharmaceuticals in the environment: What are the big questions? Environmental. Health Perspectives **120(9)**, 1221–1229.

BOY-ROURA M, MAS-PLA J, PETROVIC M, GROS M, SOLER D, BRUSI D and MENCIÓ A (2018) Towards The Understanding of Antibiotic Occurrence and Transport in Groundwater: Findings from The Baix Fluvià Alluvial Aquifer (Ne Catalonia, Spain). Science of the Total Environment. **612** 1387–1406.

BRACK W, AISSA SA, BACKHAUS T, DULIO V, ESCHER BI, FAUST M, HILSCHEROVA K, HOLLENDER J, HOLLERT H, MÜLLER C, and co-authors (2019) Effect-based methods are key. The European Collaborative Project SOLUTIONS recommends integrating effect-based methods for diagnosis and monitoring of water quality. Environmental Sciences Europe **31(1)** 1–6.
BRAUNS B, CHANDRA S, CIVIL W, LAPWORTH DJ, MACDONALD AM, MCKENZIE AA, READ DS, SEKHAR M, SINGER AC, THANKACHAN A, and co-authors (2024) Presence of Emerging Organic Contaminants and Microbial Indicators in Surface Water and Groundwater in Urban India. Environmental Pollution **362** 124983.

BUTLER CC, DORWARD J, YU L-M, GBINIGIE O, HAYWARD G, SAVILLE BR, HECKE O, BERRY N, DETRY M, SAUNDERS C, and co-authors (2021) Azithromycin for Community Treatment of Suspected Covid-19 in People At Increased Risk of An Adverse Clinical Course in the Uk (Principle): A Randomised, Controlled, Open-Label. Adaptive Platform Trial. The Lancet **397 (10279)** 1063–1074.

BYERS EN, MESSER TL, UNRINE J, BARTON C, AGOURIDIS C and MILLER DN (2025) The Occurrence and Persistence of Surface Water Contaminants Across Different Landscapes. Science of the Total Environment **958** 177837.

CABAN M, KUMIRSKA J, BIALK-BIELINSKA A and STEPNOWSKI P (2016) Analytical Techniques For Determining Pharmaceutical Residues in Drinking Water–State of Art and Future Prospects. Current Analytical Chemistry **12** 237–248.

CAPELA R, GARRIC J, LFC C and SANTOS MM (2020) Embryo bioassays with aquatic animals for toxicity testing and hazard assessment of emerging pollutants: A review. Science of the Total Environment **705** 135740.

CAPPELLI F, LONGONI O, RIGATO J, RUSCONI M, SALA A, FOCHI I, PALUMBO MT, POLESELLO S, ROSCIOLI C and SALERNO F (2022) Suspect Screening of Wastewaters to Trace Anti-Covid-19 Drugs: Potential Adverse Effects On Aquatic Environment. Science of the Total Environment **824** 153756.

CÂRSTEA AP, MITĂ A, FORTOFOIU MC, DOICA IP, CÂRSTEA D, BEZNĂ CM, NEGROIU CE, DIACONU ID, GEORGESCU AR, KAMAL AM, and Mahler B (2023) How Dexamethasone Used in Anti-Covid-19 Therapy Influenced Antihypertensive Treatment in Patients with Sars-Cov-2. Healthcare **11(10)** 1399.

CARTER LJ, ARMITAGE JM, BROOKS BW, NICHOLS JW and TRAPP S (2024) Predicting The Accumulation of Ionizable Pharmaceuticals and Personal Care Products in Aquatic and Terrestrial Organisms. Environmental Toxicology and Chemistry **43** 502–512.

CARVALHO RN and SANTOS MM (2016) Environmental impacts of human pharmaceuticals. In: *Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks*. Springer, 237–261.

ČELIĆ M, GROS M, FARRÉ M, BARCELÓ D and PETROVIĆ M (2019) Pharmaceuticals As Chemical Markers of Wastewater Contamination in the Vulnerable Area of the Ebro Delta (Spain). Science of the Total Environment **652** 952–963.

CENTRE FOR DRUG EVALUATION and RESEARCH (1994) Reviewer Guidance: Validation of Chromatographic Methods. 1994. Https://Edisciplinas.Usp.Br/Pluginfile.Php/2716487/Mod_Folder/Content/0/Cder%2c%201994%20-%20validation%20of%20chromatographic%20methods.Pdf?Forcedownload=1 (Accessed 18th December 2023).

MODARRESI CHAHARDEHI A, ARSAD H and LIM V (2020) Zebrafish as a successful animal model for screening toxicity of medicinal plants. Plants **9 (10)** 1345.

CHATANGA P, NTULI V, MUGOMERI E, KEKETSI T and CHIKOWORE NV (2019) Situational analysis of physico-chemical, biochemical and microbiological quality of water along Mohokare River, Lesotho. The Egyptian Journal of Aquatic Research **45 (1)** 45–51.

CHAUHAN B, DODAMANI S, MALIK S, ALMALKI WH, HAQUE S and SAYYED RZ (2023) Microbial Approaches for Pharmaceutical Wastewater Recycling and Management for Sustainable Development: A Multicomponent Approach. Environmental Research **237** 116983.

CHEN C, SONG J, PU Q, LIU X, YAN J, WANG X, WANG H and QIAN Q (2023) Azithromycin induces neurotoxicity in zebrafish by interfering with the VEGF/Notch signaling pathway. Science of The Total Environment **903** 166505.

CHEN J, AHN KC, GEE NA, GEE SJ, HAMMOCK BD and LASLEY BL (2007) Antiandrogenic properties of parabens and other phenolic containing small molecules in personal care products. Toxicol Appl Pharmacol **221** 278–284.

CHEN X, LEI L, LIU S, HAN J, LI R, MEN J, LI L, WEI L, SHENG Y, YANG L, and co-authors (2021) Occurrence and Risk Assessment of Pharmaceuticals and Personal Care Products (Ppcps) Against Covid-19 in Lakes and Wwtp-River-Estuary System in Wuhan, China. The Science of the Total Environment **792** 148352.

CHEN Y, JIANG C, WANG Y, SONG R, TAN Y, YANG Y and ZHANG Z (2022) Sources, environmental fate, and ecological risks of antibiotics in sediments of Asia's longest river: a whole-basin investigation. Environ. Sci. Technol **56 (20)** 14439–14451.

CHOUDHURY M, ADHIKARI MD, AGARWAL S, SAMANTA P, SHARMA A, KUNDU D and KUMAR S (2025) Pharmaceuticals and Personal Care Products in Soil: Sources, Impacts and Myco-Remediation Strategies. Emerging Contaminants **11 (2)** 100488.

CHUN R, GARRETT LD and VAIL DM (2007) Chapter 11 - Cancer Chemotherapy. In: *Withrow & Macewen's Small Animal Clinical Oncology*. 4th edition. W.B. Saunders, Saint Louis.

CUNNINGHAM C, CAMPION S, LUNNON K, MURRAY CL, WOODS JF, DEACON RM, RAWLINS JN and PERRY VH (2009) Systemic Inflammation Induces Acute Behavioral and Cognitive Changes and Accelerates Neurodegenerative Disease. Biological Psychiatry **65** 304–312.

DANKWA BE, GYESI JN, NYAABA BA, AMPAH MA, BOADU CK, LARYEA MK, DARKO G and BORQUAYE LS (2024) Environmental Impact of Pharmaceutical Contaminants in Dumpsites: A Study from Ejisu-Juaben Municipality, Ghana. Discover Soil **1 (1)** 6

DELLAGRECA M, FIORENTINO A, IESCE M, ISIDORI M, NARDELLI A, PREVITERA L and TEMUSSI F (2003) Identification of Phototransformation Products of Prednisone By Sunlight: Toxicity of the Drug and Its Derivatives On Aquatic Organisms. Environmental Toxicology and Chemistry/Setac **22** 534–539.

DESGENS-MARTIN V and AA KELLER (2021) COVID-19 Treatment agents: Do they pose an environmental risk? American Chemical Society Environmental Science and Technology Water **1 (7)** 1555–1565.

DINIZ V, RATH G, RATH S, RODRIGUES-SILVA C, GUIMARÃES JR and CUNHA DGF (2021) Long-term ecotoxicological effects of ciprofloxacin in combination with caffeine on the microalga Raphidocelis subcapitata. Toxicology Reports **8**, 429-435.

DO NASCIMENTO MTL, OLIVEIRA SANTOS AD, CUNHA D, FELIX LC, GOMES G, RANGEL CMA, HAUSER-DAVIS RA, FONSECA EM, BILA DM and NETO JAB (2022) Endocrine disruptors, estrogenic activity by the YES bioassay and acute toxicity in southeastern Brazil metropolitan surface waters. Geochimica Brasiliensis **36**.

DUARTE DJ, OLDENKAMP R and RAGAS AMJ (2022) Human Health Risk Assessment of Pharmaceuticals in the European Vecht River. Integrated Environmental Assessment and Management **18** 1639–1654.

DU B, HADDAD SP, LUEK A, SCOTT WC, SAARI GN, KRISTOFCO LA, CONNORS KA, RASH C, RASMUSSEN JB, CHAMBLISS CK, and co-authors (2014) Bioaccumulation and Trophic Dilution of Human Pharmaceuticals Across Trophic Positions of An Effluent-Dependent Wadeable Stream. Philosophical Transactions of the Royal Society B: Biological Sciences **369** (1656).

DULSAT-MASVIDAL M, CIUDAD C, COLOMER-VIDAL P, INFANTE O, MATEO R and LACORTE S (2025) Assessing Sediment Contamination Status in Important Bird and Biodiversity Areas in Spain. Environmental Research **278** 121716.

EAPEN JV, THOMAS S, ANTONY S, GEORGE P and ANTONY J (2024) A Review of the Effects of Pharmaceutical Pollutants On Humans and Aquatic Ecosystem. Exploration of Drug Science **2 (5)** 484–507.

EARL K, SLEIGHT H, ASHFIELD N and BOXALL ABA (2024) Are Pharmaceutical Residues in Crops a Threat to Human Health? Journal of Toxicology and Environmental Health, Part A **87** 773–791.

EBELE AJ, ABDALLAH MA-E and HARRAD S (2017) Pharmaceuticals and Personal Care Products (Ppcps) in the Freshwater Aquatic Environment. Emerging Contaminants **3** 1–16.

EBRAHIMZADEH G, NODEHI RN, ALIMOHAMMADI M, REZAEI KAHKAH MR and MAHVI AH (2021) Monitoring of Caffeine Concentration in Infused Tea, Human Urine, Domestic Wastewater and Different Water Resources in Southeast of Iran- Caffeine An Alternative Indicator For Contamination of Human Origin. Journal of Environmental Management **283** 111971.

Fokunang ET, NGUIDJOE EM, KATHLEENNGU K, FOKUNANG ET and FOKUNANG CN (2018) Detection and Analysis of Pharmaceutical Products from The Waste Water System at the University Teaching Hospital of Yaoundé, Cameroon. Moj Toxicol **4(1).**

EFRAIN MERMA CHACCA D, MALDONADO I and VILCA FZ (2022) Environmental and Ecotoxicological Effects of Drugs Used for the Treatment of Covid 19. Frontiers in Environmental Science **10** 940975**.**

EGLI M, RAPP-WRIGHT H, OLOYEDE O, FRANCIS W, PRESTON-ALLEN R, FRIEDMAN S, WOODWARD G, PIEL FB and BARRON LP (2023) A One-Health Environmental Risk Assessment of Contaminants of Emerging Concern in London's Waterways Throughout The Sars-Cov-2 Pandemic. Environment International **180** 108210.

EL-KALLINY AS, ABDEL-WAHED MS, EL-ZAHHAR AA, HAMZA IA and GAD-ALLAH TA (2023) Nanomaterials: A Review of Emerging Contaminants with Potential Health or Environmental Impact. Discover Nano **18** 68.

EO OMOTOLA and OS OLATUNJI (2020) Quantification of selected pharmaceutical compounds in water using liquid chromatography-electrospray ionisation mass spectrometry (LC-ESI-MS). Heliyon **6 (12)**.

EUROPEAN COMMISSION (2003) Technical Guidance Document in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC. In: No 1488/94 on Risk Assessment for Existing Substances, Part II. Brussels, Belgium.

FAIRBAIRN DJ, KARPUZCU ME, ARNOLD WA, BARBER BL, KAUFENBERG EF, KOSKINEN WC, NOVAK PJ, RICE PJ and SWACKHAMER DL (2015) Sediment–Water Distribution of Contaminants of Emerging Concern in a Mixed Use Watershed. Science of the Total Environment **505** 896–904.

FANG TY, PRAVEENA SM, DEBURBURE C, ARIS AZ, ISMAIL SNS and RASDI I (2016) Analytical Techniques For Steroid Estrogens in Water Samples-A Review. Chemosphere **165** 358–368.

FEKADU S, ALEMAYEHU E, DEWIL R and BRUGGEN B (2019) Pharmaceuticals in Freshwater Aquatic Environments: A Comparison of the African and European Challenge. Science of the Total Environment **654** 324–337.

FILIST M, BUŚ-KWAŚNIK K, KSYCIŃSKA H and RUDZKI PJ (2014) Simplified Lc–Ms/Ms Method Enabling The Determination of Azithromycin in Human Plasma After a Low 100mg Dose Administration. Journal of Pharmaceutical and Biomedical Analysis **100** 184–189.

FLOWER RJ (2009) Prednisone. In: Xpharm: The Comprehensive Pharmacology Reference ENNA SJ, BYLUND DB.1-6 Elsevier, New York.

GARG V and JUSKO WJ (1991) Simultaneous Analysis of Prednisone, Prednisolone and their Major Hydroxylated Metabolites in Urine By High-Performance Liquid Chromatography. Journal of Chromatography **567** 39–47.

GHANNAM HE (2021) Risk assessment of pollution with heavy metals in water and fish from River Nile, Egypt. Applied Water Science **11** 125.

GOLDSCHEIDER N (2010) Chapter 8 - Delineation of Spring Protection Zones. In: *Groundwater hydrology of springs.* Butterworth-Heinemann, Boston. 305–338.

GONG J, LIN C, XIONG X, CHEN D, CHEN Y, ZHOU Y, WU C and DU Y (2019) Occurrence, distribution, and potential risks of environmental corticosteroids in surface waters from Pearl River Delta, South China. Environmental Pollution **251** 102–109.

GORITO AM, RIBEIRO ARL, RAMOS S, SILVA AMT and ALMEIDA CMR (2024) Occurrence of Micropollutants in Surface Waters: Monitoring of Portuguese Lima and Douro River Estuaries and Interconnecting Northwest Coast. Marine Pollution Bulletin **209** 117140.

GORITO AM, RIBEIRO ARL, RODRIGUES P, PEREIRA MFR, GUIMARÃES L, ALMEIDA CMR and SILVA AMT (2022) Antibiotics Removal from Aquaculture Effluents By Ozonation: Chemical and Toxicity Descriptors. Water Research **218** 118497.

GRANADOS JC, FALAH K, KOO I, MORGAN EW, PERDEW GH, PATTERSON AD, JAMSHIDI N and NIGAM SK (2022) AHR is a master regulator of diverse pathways in endogenous metabolism. Scientific Reports **12(1)** 16625.

GUETTAI N, KADMI Y, PURI M, KERKICH K and BOUARGANE B (2024) Occurrence, Analysis and Removal Processes of Emerging Pharmaceuticals from Waters for the Protection and Preservation of A Sustainable Environment: A Review. Journal of Cleaner Production **466** 142654.

GUILOSKI IC, RIBAS JLC, PEREIRA LDS, NEVES APP and ASSIS HC (2015) Effects of Trophic Exposure to Dexamethasone and Diclofenac in Freshwater Fish. Ecotoxicology and Environmental Safety **114** 204–211.

GUIMARÃES RE, ALBORNOZ LL, BELTRAME TF, GABALDÓN MG, BORBA FH and DA SILVA SW (2025) on-site electro-generation of H2O2 combined with uv process: multiple pharmaceutically active compounds degradation for drinking water systems. separation and purification technology **361** 131608.

GUMBI BP, MOODLEY B, BIRUNGI G and NDUNGU PG (2017) Assessment of Nonsteroidal Anti-Inflammatory Drugs By Ultrasonic-Assisted Extraction and Gc-Ms in Mgeni and Msunduzi River Sediments, Kwazulu-Natal, South Africa. Environmental Science and Pollution Research **24** 20015–20028.

GUO X, LIU Y, DONG W, HU Q, LI Y, SHUANG S, DONG C, CAI L and GONG X (2021) Azithromycin Detection in Cells and Tablets By N,S Co-Doped Carbon Quantum Dots. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy **252** 119506.

GWENZI W, SELVASEMBIAN R, OFFIONG N-AO, MAHMOUD AED, SANGANYADO E and MAL J (2022) Covid-19 Drugs in Aquatic Systems: A Review. Environmental Chemistry Letters **20** 1275–1294.

GWOREK B, KIJEŃSKA M, WRZOSEK J and GRANIEWSKA M (2021) Pharmaceuticals in the Soil and Plant Environment: A Review. Water, Air, & Soil Pollution 232 (4) 145.
G YG, B W and R K (2002) Environmental fate of alkylphenols and alkylphenol ethoxylates: a review. Environment International **28 (3)** 215–226.

HALMI MIE (2016) Rapid ecotoxicological tests using bioassay systems—a review. Journal of Biochemistry, Microbiology and Biotechnology **4 (1)** 29–37.

HANAMOTO S and OGAWA F (2019) Predicting The Sorption of Azithromycin and Levofloxacin to Sediments from Mineral and Organic Components. Environmental Pollution **255** 113180.

HANEEF J, SHAHARYAR M, HUSAIN A, RASHID M, MISHRA R, PARVEEN S, AHMED N, PAL M and KUMAR D (2013) Application of Lc–Ms/Ms For Quantitative Analysis of Glucocorticoids and Stimulants in Biological Fluids. Journal of Pharmaceutical Analysis **3(5)** 341–348.

HARROWER J (2024) Urban river environments: Monitoring antibiotics using passive samplers (POCIS) and fugacity modelling techniques. PhD thesis. Glasgow Caledonian University.

HASSAN SH, GINKEL SW, HUSSEIN MA, ABSKHARON R and OH SE (2016) Toxicity Assessment Using Different Bioassays and Microbial Biosensors. Environment International **92** 106–118.

HAYDEN KR, JONES M, ELKIN KR, SHREVE MJ, CLEES WI, CLARK S, MASHTARE ML, VEITH TL, ELLIOTTE HA, WATSON JE, and co-authors (2022) Impacts of the COVID-19 pandemic on pharmaceuticals in wastewater treated for beneficial reuse: Two case studies in central Pennsylvania. Journal of Environmental Quality **51** 1066–1082.

HE C, YIN Z, HE J, LV J and WANG C (2022) Occurrence and photodegradation of typical steroid hormones in surface water of urban lakes Wuhan. China. Journal of Environmental Chemical Engineering **10 (6)** 108602.

HENDRICKS CL, HERD C, NEL M, G TINTINGER and PEPPER MS (2021) The COVID-19 treatment landscape: A South African perspective on a race against time. Frontiers in Medicine **8** 604087.
HERRERO P, BORRULL F, POCURULL E and MARCÉ RM (2012) Determination of Glucocorticoids in Sewage and River Waters By Ultra-High Performance Liquid Chromatography–Tandem Mass Spectrometry. Journal of Chromatography A **1224** 19–26.

HOF D, BING T and Heß S (2024) Temporal and spatial variations in the effect-based ecotoxicological assessment of streams. Environ Sci Eur **36(1)** 167.

HORBY P, S LW, R EJ, MAFHAM M, L BJ, LINSELL L, STAPLIN L, BRIGHTLING C, USTIANOWSKI A and ELMAHI E (2021) Dexamethasone in hospitalized patients with Covid-19. New England Journal of Medicine **384 (8)** 693–704.

HOREL A and SCHIEWER S (2020) microbial degradation of different hydrocarbon fuels with mycoremediation of volatiles. Microorganisms **8 (2)** 163.

HOSSEIN M and RIPANDA AS (2025) Pollution By Antimicrobials and Antibiotic Resistance Genes in East Africa: Occurrence, Sources, and Potential Environmental Implications. Toxicology Reports **14** 101969.

INARMAL N and MOODLEY B (2023) selected pharmaceutical analysis in a wastewater treatment plant during Covid-19 infection waves in South Africa. environmental science: water research & technology **9 (6)** 1566–1576.

INDRANIL D, AMBATI SR, BHOS PN, and PILLI S (2024) Influence of seasons on the effluent quality in sbr-based wastewater treatment plants. Journal of Water Chemistry and Technology **46 (5)** 491–505.

INTERNATIONAL CONFERENCE ON HARMONISATION (2005) International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. ICH harmonised tripartite guideline. Validation of analytical procedures: Text and methodology. Q2(R1). https://pacificbiolabs.com/wp-content/uploads/2017/12/Q2_R1__Guideline-4.pdf. (23 July 2023).

ISLAM P, HOSSAIN MI, KHATUN P, MASUD RI, TASNIM S, ANJUM M, ISLAM MZ, NIBIR SS, RAFIQ K, ISLAM MA (2024). Steroid Hormones in Fish, Caution For Present and Future: A Review. Toxicology Reports **13**, 101733.

ISMAIL NAH, WEE SY, HARON DEM, KAMARULZAMAN NH and ARIS AZ (2020) Occurrence of Endocrine Disrupting Compounds in Mariculture Sediment of Pulau Kukup, Johor, Malaysia. Marine Pollution Bulletin **150** 110735.

ISMAIL NAH, WEE SY, KAMARULZAMAN NH and ARIS AZ (2019) Quantification of Multi-Classes of Endocrine-Disrupting Compounds in Estuarine Water. Environmental Pollution **249** 1019–1028.

JOBLING S, BURN RW, THORPE K, WILLIAMS R and TYLER C (2009) Statistical modeling suggests that antiandrogens in effluents from wastewater treatment works contribute to widespread sexual disruption in fish living in English rivers. Environ Health Perspect **117** 797–802.

JONKERS TJH, HOUTMAN CJ, OORSCHOT Y, LAMOREE MH and HAMERS T (2023) Identification of Antimicrobial and Glucocorticoid Compounds in Wastewater Effluents with Effect-Directed Analysis. Environmental Research **231** 116117.

KAYODE-AFOLAYAN SD, AHUEKWE EF and NWINYI OC (2022) Impacts of pharmaceutical effluents on aquatic ecosystems. Scientific AFRICAN **17** 01288.

KHAN AH, KHAN NA, AHMED S, DHINGRA A, SINGH CP, KHAN SU, MOHAMMADI AA, CHANGANI F, YOUSEFI M, ALAM S, and co-authors (2020) Application of Advanced Oxidation Processes Followed By Different Treatment Technologies For Hospital Wastewater Treatment. Journal of Cleaner Production **269**.

KHAN K, KAR S and ROY K (2023) Are We Ready to Combat The Ecotoxicity of Covid-19 Pharmaceuticals? An in Silico Aquatic Risk Assessment. Aquatic Toxicology **256** 106416.

KHAN S, NAUSHAD M, GOVARTHANAN M, IQBAL J and ALFADUL SM (2022) Emerging Contaminants of High Concern for the Environment: Current Trends and Future Research. Environmental Research **207** 112609.

KHAN WA, ARAIN MB, YAMINI Y, SHAH N, KAZI TG, PEDERSEN-BJERGAARD S and TAJIK M (2020) Hollow Fiber-Based Liquid Phase Microextraction Followed By Analytical Instrumental Techniques For Quantitative Analysis of Heavy Metal Ions and Pharmaceuticals. Journal of Pharmaceutical Analysis **10** 109–122.

KIDD KA, BACKHAUS T, BRODIN T, INOSTROZA PA and MCCALLUM ES (2024) Environmental Risks of Pharmaceutical Mixtures in Aquatic Ecosystems: Reflections On A Decade of Research. Environmental Toxicology and Chemistry **43** 549–558.

KLEIN EY, BOECKEL TP, MARTINEZ EM, PANT S, GANDRA S, LEVIN SA, GOOSSENS H and LAXMINARAYAN R (2018) Global Increase and Geographic Convergence in Antibiotic Consumption Between 2000 and 2015. In: Proceedings of the National Academy of Sciences **115**.

KOCK A, GLANVILLE HC, LAW AC, STANTON T, CARTER LJ and TAYLOR JC (2023) Emerging Challenges of the Impacts of Pharmaceuticals On Aquatic Ecosystems: A Diatom Perspective. Science of the Total Environment **878** 162939.

KONDOR AC, MOLNÁR É, JAKAB G, VANCSIK A, FILEP T, SZEBERÉNYI J, SZABÓ L, MAÁSZ G, PIRGER Z, WEIPERTH A, and co-authors (2022) Pharmaceuticals in Water and Sediment of Small Streams Under The Pressure of Urbanization: Concentrations, Interactions, and Risks. Science of the Total Environment **808** 152160.

KORKMAZ NE, CAGLAR NB and AKSU A (2022) Presence and Distribution of Selected Pharmaceutical Compounds in Water and Surface Sediment of the Golden Horn Estuary, Sea of Marmara, Turkey. Regional Studies in Marine Science **51** 102221.

KOT-WASIK A, JAKIMSKA A and ŚLIWKA-KASZYŃSKA M (2016) Occurrence and Seasonal Variations of 25 Pharmaceutical Residues in Wastewater and Drinking Water Treatment Plants. Environ Monit Assess **188 (12)** 661.

KRANZ N, INTERWIES E and VIDAURRE R (2005) Transboundary Regimes in the Orange Senqu Basin, Contribution to Deliverable 1.3.1 of the NeWater Project. 2005. Berlin.

KRUĆ-FIJAŁKOWSKA R, DROŻDŻYŃSKI D and MATUSIAK M (2025) Occurrence and temporal changes of pharmaceuticals in the Warta River in Poland during and after the Covid-19 pandemic. Sci Rep **15** 29451.

KUMAR A and XAGORARAKI I (2010) Pharmaceuticals, Personal Care Products and Endocrine-Disrupting Chemicals in U.S. Surface and Finished Drinking Waters: A Proposed Ranking System. Science of the Total Environment **408** 5972–5989.

KUMARI M and KUMAR A (2021) Can pharmaceutical drugs used to treat COVID-19 infection leads to human health risk? A hypothetical study to identify potential risk. Sci Total Environ **778** (146303).

KUMARI M and KUMAR A (2022) Environmental and human health risk assessment of mixture of Covid-19 treating pharmaceutical drugs in environmental waters. Science of the Total Environment **812** 152485.

KUMAR V, LAKKABOYANA SK, SHARMA N, CHAKRABORTY P, UMESH M, PASRIJA R, THOMAS J, KALEBAR VU, JAYARAJ I, AWASTHI MK, and co-authors (2023) A Critical Assessment of Technical Advances in Pharmaceutical Removal from Wastewater – A Critical Review. Case Studies in Chemical and Environmental Engineering **8** 100363.

KUNKEL U and RADKE M (2008) Biodegradation of Acidic Pharmaceuticals in Bed Sediments: Insight from A Laboratory Experiment. Environmental Science & Technology **42 (19)** 7273–7279.

KURODA K, LI C, K D and KUMAR M (2021) Predicted occurrence, ecotoxicological risk and environmentally acquired resistance of antiviral drugs associated with COVID-19 in environmental waters. Science of the Total Environment **776** 145740.

KUSCHIK-MACZOLLEK N, GLOCK M and SCHMITZ M (2024) in vitro effect-based monitoring of water, sediment and soil from a floodplain restoration site in Central Europe. Environmental Sciences Europe **36(1)** 119.

KUSI J, OJEWOLE CO, OJEWOLE AE and NWI-MOZU I (2022) Antimicrobial Resistance Development Pathways in Surface Waters and Public Health Implications. Antibiotics **11(6)** 821 .

KUSKOPF L, SHEEHAN M and WHELAN A (2020) Contaminants of Emerging Concern: Developing A Sampling and Analysis Quality Plan for the Cleveland Bay Sewage Treatment Plant. Water E-Journal **5**. 2020. Australian Water.

LACOUTURE A, LAFRONT C, PEILLEX C, PELLETIER M and AUDET-WALSH É (2022) Impacts of Endocrine-Disrupting Chemicals On Prostate Function and Cancer. Environmental Research **204** 112085.

LA TROBE UNIVERSITY T (2025) T-Tests. Independent and paired-samples t-tests. 2025. https://latrobe.libguides.com/Ibmspss/Ttest (Accessed July 9, 2025).

LENTZ MP, GRAHAM DJ and VLIET MTH (2024) Drought Impact On Pharmaceuticals in Surface Waters in Europe: Case Study for the Rhine and Elbe Basins. Science of the Total Environment **922** 171186.

LETSOALO MR, SITHOLE T, MUFAMADI S, MAZHANDU Z, SILLANPAA M, KAUSHIK A and MASHIFANA T (2023) Efficient Detection and Treatment of Pharmaceutical Contaminants to Produce Clean Water for Better Health and Environmental. Journal of Cleaner Production **387** 135798.

LI JJ, ZHANG XJ, YANG Y, HUANG T, LI C, SU L, ZHAO YH and CRONIN MTD (2018) Development of Thresholds of Excess Toxicity for Environmental Species and their Application to Identification of Modes of Acute Toxic Action. Science of the Total Environment **616–617** 491–499.

LIN H, ZHOU L, LU S, YANG H, LI Y and YANG X (2024) Occurrence and Spatiotemporal Distribution of Natural and Synthetic Steroid Hormones in Soil, Water, and Sediment Systems in Suburban Agricultural Area of Guangzhou City, China. Journal of Hazardous Materials **470** 134288.

LIN H, ZHOU L, LU S, YANG H, LI Y and YANG X (2024) Occurrence and Spatiotemporal Distribution of Natural and Synthetic Steroid Hormones in Soil, Water, and Sediment Systems in Suburban Agricultural Area of Guangzhou City, China. Journal of Hazardous Materials **470** 134288.

LIN L, DAI Y and XIA Y (2022) An overview of aryl hydrocarbon receptor ligands in the Last two decades (2002–2022): A medicinal chemistry perspective. European Journal of Medicinal Chemistry **244** 114845.

LIU S, YING G-G, ZHOU L-J, ZHANG R-Q, CHEN Z-F and LAI H-J (2012) Steroids in a Typical Swine Farm and their Release into the Environment. Water Research **46 (12)** 3754–3768.

LIU Z, BAHADORAN A, ALIZADEH AA, EMAMI N, ALAWADI AL-MUSAWTJ, AHR ALJEBOREE, AM SHAMSBORHAN, M NAJAFIPOUR, I MOUSAVI, SE MOSALLANEZHADM, and co-authors (2023) Sonocrystallization of a novel ZIF/zeolite composite adsorbent with high chemical stability for removal of the pharmaceutical pollutant azithromycin from contaminated water. Ultrasonics Sonochemistry **97** 106463.

LI Y, MA Y, YANG L, DUAN S, ZHOU F, CHEN J, LIU Y and ZHANG B (2020) Effects of azithromycin on feeding behavior and nutrition accumulation of Daphnia magna under the different exposure pathways. Ecotoxicology and Environmental Safety **197 (5)** 110573.

LI Y, NIU X, YAO C, YANG W and LU G (2019) Distribution, Removal, and Risk Assessment of Pharmaceuticals and their Metabolites in Five Sewage Plants. International Journal of Environmental Research and Public Health **16(23)** 4729.

LI Y, SALLACH JB, ZHANG W, BOYD SA and LI H (2019) Insight into the distribution of pharmaceuticals in soil-water-plant systems. Water Research **152** 38–46.

LI Y, TAGGART MA, MCKENZIE C, ZHANG Z, LU Y, PAP S and GIBB SW (2021) A SPE−HPLC−MS/MS Method for the Simultaneous Determination of Prioritised Pharmaceuticals and EDCs with High Environmental Risk Potential in Freshwater. Journal of Environmental Sciences **100** 18–27.
LOMARTIRE S, MARQUES JC and GONÇALVES AMM (2021) Biomarkers Based Tools to Assess Environmental and Chemical Stressors in Aquatic Systems. Ecological Indicators **122** 107207.

LOOS R, MARINOV D, SANSEVERINO I, NAPIERSKA D and LETTIERI T (2018) *Review of the 1st Watch List Under The Water Framework Directive and Recommendations for the 2nd Watch List*. Publications Office of the European Union, Luxembourg.

LOSKOTOVÁ B, STRAKA M and PAŘIL P (2019) Sediment Characteristics Influence Benthic Macroinvertebrate Vertical Migrations and Survival Under Experimental Water Loss Conditions; Sediment Characteristics Influence Benthic Macroinvertebrate Vertical Migrations and Survival Under Experimental Water Loss Conditions. Fundamental and Applied Limnology **193 (1)** 39–49.

LU M-C, CHEN YY, CHIOU M-R, CHEN MY and FAN H-J (2016) Occurrence and Treatment Efficiency of Pharmaceuticals in Landfill Leachates. Waste Management **55** 257–264.

LV Y-Z, LUO X-J, ZHAO J-L, WANG S-Q and MAI B-X (2021) Occurrence and Distribution of Antibiotics in Sediments from Black-Odor Ditches in Urban Areas from China. Science of the Total Environment **787** 147554.

MAHLAKENG MK (2020) The Orange–Senqu River Basin: AN ANALYSIS OF REGIME CAPACITY AND NASCENT ENVIRONMENTAL CONFLICT. World Affairs: The Journal of International Issues **24 (1)** 142–66.

MALMQVIST E, FUMAGALLI D, MUNTHE C and LARSSON DGJ (2023) Pharmaceutical Pollution from Human Use and the Polluter Pays Principle. Public Health Ethics **16** 152–164.

MANBOHI A, RAHNAMA R, TAHERI M, HAMZEH MA and HAMZEHPOUR A (2024) Antibiotics in Surface Waters of the South Caspian Sea: Occurrence, Spatial Distribution and Ecological Risks. Environmental Research **261** 119709.

MAREMANE S, BELLE G, OBERHOLSTER P and OMOTOLA E (2024) Occurrence of selected Covid-19 drugs in surface water resources: a review of their sources, pathways, receptors, fate, ecotoxicity, and

possible interactions with heavy metals in aquatic ecosystems. Environmental Geochemistry and Health **47**. 31 10 1007 10653-024-02293–9.

MAREMANE S, BELLE G, OBERHOLSTER P and OMOTOLA E (2024) Occurrence of selected Covid-19 drugs in surface water resources: a review of their sources, pathways, receptors, fate, ecotoxicity, and possible interactions with heavy metals in aquatic ecosystems. Environmental Geochemistry and Health **47 (31)**.

MARTÍNEZ-POLANCO MP, VALDERRAMA-RINCÓN JA, MARTÍNEZ-ROJAS AJ, LUNA-WANDURRAGA HJ, DÍAZ-BÁEZ MC, BUSTOS-LÓPEZ MC and VALDERRAMA-RINCON JD (2022) Degradation of High Concentrations of Azithromycin When Present in a High Organic Content Wastewater By Using A Continuously Fed Laboratory-Scale Uasb Bioreactor. Chemosphere **287** 132191.

MATHEW BB, SINGH H, BIJU VG and KRISHNAMURTHY NB (2017) Classification, Source, and Effect of Environmental Pollutants and their Biodegradation. Journal of Environmental Pathology, Toxicology and Oncology: Official Organ of the International Society for Environmental Toxicology and Cancer **36(1)** 55–71.

MATONGO S, BIRUNGI G, MOODLEY B and NDUNGU P (2015) Pharmaceutical Residues in Water and Sediment of Msunduzi River, Kwazulu-Natal, South Africa. Chemosphere **134** 133–140.

MEZZELANI M, GORBI S, FATTORINI D, D'ERRICO G, CONSOLANDI G, MILAN M, BARGELLONI L and REGOLI F (2018) Long-Term Exposure of Mytilus Galloprovincialis to Diclofenac, Ibuprofen and Ketoprofen. Insights Into Bioavailability, Biomarkers and Transcriptomic Changes. Chemosphere **198** 238–248.

MEZZELANI M, GORBI S, ROS Z, FATTORINI D, D'ERRICO G, MILAN M, BARGELLONI L and REGOLI F (2016) Ecotoxicological Potential of Non-Steroidal Anti-Inflammatory Drugs (Nsaids) in Marine Organisms: Bioavailability, Biomarkers and Natural Occurrence in Mytilus Galloprovincialis. Marine Environmental Research **121** 31–39.

MEZZELANI M, GORBI S and REGOLI F (2018) Pharmaceuticals in the Aquatic Environments: Evidence of Emerged Threat and Future Challenges for Marine Organisms. Marine Environmental Research **140** 41–60.

MHEIDLI N, MALLI A, MANSOUR F and AL-HINDI M (2022) Occurrence and Risk Assessment of Pharmaceuticals in Surface Waters of the Middle East and North Africa: A Review. The Science of the Total Environment **851** 158302.

MHUKA V, DUBE S and NINDI MM (2020) Occurrence of Pharmaceutical and Personal Care Products (Ppcps) in Wastewater and Receiving Waters in South Africa Using Lc-OrbitrapTM Ms. Emerging Contaminants **6** 250–258.

MICHAEL I, RIZZO L, MCARDELL CS, MANAIA CM, MERLIN C, SCHWARTZ T, DAGOT C and FATTA-KASSINOS D (2013) Urban wastewater treatment plants as hotspots for antibiotic-resistant bacteria and genes spread into the environment: A review. Science of the Total Environment **447** 345–360.

MIRZAIE F, TEYMORI F, SHAHCHERAGH S, DOBARADARAN S, ARFAEINIA H, KAFAEI R, SAHEBI S, FARJADFARD S and RAMAVANDI B (2022) Occurrence and Distribution of Azithromycin in Wastewater Treatment Plants, Seawater, and Sediments of the Northern Part of the Persian Gulf Around Bushehr Port: A Comparison with Pre-Covid 19 Pandemic. Chemosphere **307 (4)** 135996.

MOLINARI R, LAVORATO C and ARGURIO P (2024) Chapter 32 - Hybrid Membrane Processes in Advanced Wastewater Treatment. In: *Current Trends and Future Developments On (Bio-) Membranes*. Elsevier, 811-844.

MOODLEY R, MAHLANGENI NT and REDDY P (2021) Determination of heavy metals in selected fish species and seawater from the South Durban Industrial Basin, KwaZulu-Natal, South Africa. Environ Monit Assess **193** 206.

MORALES-PAREDES CA, RODRÍGUEZ-DÍAZ JM and BOLUDA-BOTELLA N (2022) Pharmaceutical Compounds Used in the Covid-19 Pandemic: A Review of their Presence in Water and Treatment Techniques for their Elimination. Science of the Total Environment **814** 152691.

MORENO-GONZÁLEZ R, RODRÍGUEZ-MOZAZ S, GROS M, PÉREZ-CÁNOVAS E, BARCELÓ D and LEÓN VM (2014) Input of Pharmaceuticals Through Coastal Surface Watercourses Into a Mediterranean Lagoon (Mar Menor, Se Spain): Sources and Seasonal Variations. Science of the Total Environment **490** 59–72.

MORYS C, POWILLEIT M and FORSTER S (2017) Bioturbation in Relation to the Depth Distribution of Macrozoobenthos in the Southwestern Baltic Sea. Marine Ecology Progress Series **579** 19–36.

MOSHARAF MK, GOMES RL, COOK S, ALAM MS and RASMUSSSEN A (2024) Wastewater Reuse and Pharmaceutical Pollution in Agriculture: Uptake, Transport, Accumulation and Metabolism of Pharmaceutical Pollutants Within Plants. Chemosphere **364** 143055.

MOYIB OK, AYEDUN MA, OJ A and OMOTOLA OE (2017) Waste printing paper as analogous adsorbents for heavy metals in aqueous solution. Nigerian Journal of Chemical Research **22 (1)** 29–38.

MUAMBO KE, KIM M-G, KIM D-H, PARK S and OH J-E (2024) Pharmaceuticals in Raw and Treated Water from Drinking Water Treatment Plants Nationwide: Insights Into Sources and Exposure Risk Assessment. Water Research X **(24)** 100256.

MÜLLER A, ÖSTERLUND H, MARSALEK J and VIKLANDER M (2020) The Pollution Conveyed By Urban Runoff: A Review of Sources. Science of the Total Environment **709** 136125.

MUNZHELELE EP, AYINDE WB, GITARI WM, PINDIHAMA GK and MUDZIELWANA R (2025) Occurrence of Efavirenz, Levonorgestrel, Ibuprofen, and Diclofenac in Wastewaters of Limpopo Province, South Africa. Heliyon **11 (2)** 41524.

MUNZHELELE EP, MUDZIELWANA R, AYINDE WB and GITARI WM (2024) Pharmaceutical Contaminants in Wastewater and Receiving Water Bodies of South Africa: A Review of Sources, Pathways, Occurrence, Effects, and Geographical Distribution. Water **16(6)** 796.

MUSEE N, KEBAABETSWE LP, TICHAPONDWA S, TUBATSI G, MAHAYE N, LEARENG SK and NOMNGONGO PN (2021) Occurrence, fate, effects, and risks of dexamethasone: Ecological implications post-Covid-19. International Journal of Environmental Research and Public Health **18 (21)** 11291.

NAND S, SINGH PP, VERMA S, MISHRA S, PATEL A, SHUKLA S and SRIVASTAVA PK (2025) Biochar for Mitigating Pharmaceutical Pollution in Wastewater: A Sustainable Solution. Science of the Total Environment **966** 178743.

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION (2023a) Pubchem Compound Summary for Cid 5865, Prednisone. https://pubchem.ncbi.nlm.nih.gov/compound/prednisone. (Accessed 23 July 2023)

NEALE PA, ESCHER BI, BAAT ML, ENAULT J and LEUSCH FDL (2023) Effect-Based Trigger Values Are Essential for the Uptake of Effect-Based Methods in Water Safety Planning. Environmental Toxicology and Chemistry **42** 714–726.

NETH M, MATTSSON A, WILÉN B-M and MODIN O (2023) Weighted Score Ratios (Wrs) Give Transparent Weighting in Multicriteria Sustainability Assessments - A Case Study On Removal of Pharmaceutical Residues from Wastewater. Science of the Total Environment **893** 164792.

NGQWALA NP and MUCHESA P (2020) Occurrence of Pharmaceuticals in Aquatic Environments: A Review and Potential Impacts in South Africa. South African Journal of Science **116 (7-8)** 1-7.

NIETO-JUÁREZ JI, TORRES-PALMA RA, BOTERO-COY AM and HERNÁNDEZ F (2021) Pharmaceuticals and Environmental Risk Assessment in Municipal Wastewater Treatment Plants and Rivers from Peru. Environment International **155** 106674.

NIPPES RP, MACRUZ PD, SILVA GN and SCALIANTE MHNO (2021) A Critical Review On Environmental Presence of Pharmaceutical Drugs Tested for the Covid-19 Treatment. Process Safety and Environmental Protection **152** 568–582.

NKOOM M, LU G, LIU J and DONG H (2020) Biological Uptake, Depuration and Biochemical Effects of Diclofenac and Carbamazepine in Carassius Carassius. Ecotoxicology and Environmental Safety **205** 111106.

OECD (2023) Endocrine Disrupting Chemicals in Freshwater: Monitoring and Regulating Water Quality, Oecd Studies On Water. Oecd Publishing, Paris. https://doi.org/10.1787/5696d960-en

OGUNBANWO OM, KAY P, BOXALL AB, WILKINSON J, SINCLAIR CJ, SHABI RA, FASASI AE, LEWIS GA, AMODA OA and BROWN LE (2022) High Concentrations of Pharmaceuticals in a Nigerian River Catchment. Environmental Toxicology and Chemistry **41 (3)** 551–558.

OGUNBIYI OD, AJIBOYE TO, OMOTOLA EO, OLADOYE PO, CA O and QUINETE N (2023) Analytical approaches for screening of per- and poly fluoroalkyl substances in food items: A review of recent advances and improvements. Environmental Pollution **329** 121705.

OHORE OE, IFON BE, WANG Y, KAZMI SSUH, ZHANG J, SANGANYADO E, JIAO X, LIU W and WANG Z (2023) Vertical Changes in Water Depth and Environmental Variables Drove The Antibiotics and Antibiotic Resistomes Distribution, and Microbial Food Web Structures in the Estuary and Marine Ecosystems. Environment International **178** 108118.

OHORO CR, AMAKU JF, CONRADIE J, OLISAH C, AKPOMIE KG, MALLOUM A, AKPOTU SO, ADEGOKE KA, OKEKE ES and OMOTOLA EO (2024) Effect of Physicochemical Parameters On The Occurrence of Per- and Polyfluoroalkyl Substances (Pfas) in Aquatic Environment. Marine Pollution Bulletin **208** 117040.

OKEKE ES, CHUKWUDOZIE KI, NYARUABA R, ITA RE, OLADIPO A, EJEROMEDOGHENE O, ATAKPA EO, AGU CV and OKOYE CO (2022) Antibiotic Resistance in Aquaculture and Aquatic Organisms: A Review of Current Nanotechnology Applications for Sustainable Management. Environmental Science and Pollution Research International **29 (46)** 69241–69274.

OLADOYE PO, AJIBOYE TO, WANYONYI WC, EO OMOTOLA and OLADIPO ME (2023) Insights into remediation technology for malachite green wastewater treatment. Water Science and Engineering **16(3)** 261-270.

OLIVEIRA LC, MELO BISNETO AV, PUGA SC, FERNANDES AS, VÉRAS JH, CARDOSO CG, RIBEIRO ESC, CARNEIRO CC and CHEN-CHEN L (2021) Prednisone Is Genotoxic in Mice and Drosophila Melanogaster. Mutation Research. Genetic Toxicology and Environmental Mutagenesis **865** 503334.

OLIVEIRA LLD, ANTUNES SC, GONÇALVES F, ROCHA O and NUNES B (2015) Evaluation of Ecotoxicological Effects of Drugs On Daphnia Magna Using Different Enzymatic Biomarkers. Ecotoxicology and Environmental Safety **119** 123–131.

OLIVEIRA LLD, ANTUNES SC, GONÇALVES F, ROCHA O and NUNES B (2016) Acute and Chronic Ecotoxicological Effects of Four Pharmaceuticals Drugs On Cladoceran Daphnia Magna. Drug and Chemical Toxicology **39** 13–21.

OLUWOLE AO, OMOTOLA EO and OLATUNJI OS (2020) Pharmaceuticals and personal care products in water and wastewater: A review of treatment processes and use of photocatalyst immobilized on functionalized carbon in AOP degradation. 2020. BioMed Central (BMC) Chemistry **14 (1)** 62.

OMOTOLA EO, GENTHE B, L NDLELA and OLATUNJI SO (2023a) Insights into the ecotoxic impact of diclofenac using Daphnia magna as a model organism. TASUED Journal of Pure and Applied Sciences **2 (1)** 19–26.

OMOTOLA EO, GENTHE B, NDLELA L and OLATUNJI SO (2023) Phytotoxicity and apoptotic impact assessment of an over-the-counter drug (paracetamol) residue using Allium cepa as a bioindicator. TASUED Journal of Pure and Applied Sciences **2 (1)** 39–49.

OMOTOLA EO, GENTHE B, L NDLELA and OLATUNJI SO (2023a) Insights into the ecotoxic impact of diclofenac using Daphnia magna as a model organism. TASUED Journal of Pure and Applied Sciences **2 (1)** 19–26.

OMOTOLA EO, GENTHE B, NDLELA L and OLATUNJI OS (2021) Environmental Risk Characterization of An Antiretroviral (Arv) Lamivudine in Ecosystems. International Journal of Environmental Research and Public Health **18(16)** 8358.

OMOTOLA EO, GENTHE B, NDLELA L and OLATUNJI SO (2023) Phytotoxicity and Apoptotic Impact Assessment of An Over-The-Counter Drug (Paracetamol) Residue Using Allium Cepa As A Bioindicator. TASFUED Journal of Pure and Applied Sciences **2 (1)** 39–49.

OMOTOLA EO, OLUWOLE AO, OLADOYE PO and OLATUNJI OS (2022) Occurrence, Detection and Ecotoxicity Studies of Selected Pharmaceuticals in Aqueous Ecosystems- A Systematic Appraisal. Environmental Toxicology and Pharmacology **91** 103831.

OMOTOLA EO and OLATUNJI OS (2020) Quantification of Selected Pharmaceutical Compounds in Water Using Liquid Chromatography-Electrospray Ionisation Mass Spectrometry (Lc-Esi-Ms) Heliyon **6 (12)**.

ORTON F, ROSIVATZ E, SCHOLZE M and KORTENKAMP A (2011) Widely used pesticides with previously unknown endocrine activity revealed as in Vitro antiandrogens. Environmental Health Perspectives **119(6)** 794.

ORTÚZAR M, ESTERHUIZEN M, OLICÓN-HERNÁNDEZ DR, GONZÁLEZ-LÓPEZ J and ARANDA E (2022) Pharmaceutical Pollution in Aquatic Environments: A Concise Review of Environmental Impacts and Bioremediation Systems. Frontiers in Microbiology **13** 869332.

OTAROLA G, CASTILLO H and MARCELLINI S (2018) Aryl hydrocarbon receptor-based bioassays for dioxin detection: Thinking outside the box. Journal of Applied Toxicology **38 (4)** 437–449.

OUTRIDGE PM and WANG F (2015) The Stability of Metal Profiles in Freshwater and Marine Sediments. In:*Environmental Contaminants: Using Natural Archives to Track Sources and Long-Term Trends of Pollution* 35-60. Springer Netherlands, Dordrecht.

O'FLYNN D, LAWLER J, A YUSUF, PARLE-MCDERMOTT A, HAROLD D, MCCLOUGHLIN T, HOLLAND L, F REGAN and WHITE B (2021) A review of pharmaceutical occurrence and pathways in the aquatic environment in the context of a changing climate and the COVID-19 pandemic. Analytical methods **5 (13)** 575–594.

PAMPLONA-SILVA MT, MAZZEO DEC, BIANCHI J and MARIN-MORALES MA (2018) Estrogenic Compounds: Chemical Characteristics, Detection Methods, Biological and Environmental Effects. Water Air Soil Pollution **229**.

PAN C, YANG H, GAO W, WEI Z, SONG C and MI J (2024) Optimization of Organic Solid Waste Composting Process Through Iron-Related Additives: A Systematic Review. Journal of Environmental Management **351** 119952.

PAOLA D, ABBATE JM, IARIA C, CORDARO M, CRUPI R, SIRACUSA R, D'AMICO R, FUSCO R, IMPELLIZZERI D, CUZZOCREA S, and SPANO N (2022) Retracted: Environmental Risk Assessment of Dexamethasone Sodium Phosphate and Tocilizumab Mixture in Zebrafish Early Life Stage (Danio Rerio). Toxics **10(6)** 279.

PAPICH MG (2016) Prednisone. In: Saunders Handbook of Veterinary Drugs PAPICH MG. Fourth edition. W.B. Saunders, St. Louis 670-672.

PAPPAS JJ, DESROCHERS N, TUTEJA B, HUGHES D, MCLAUGHLIN A, SABOURIN L, RENAUD JB, LITTLEJOHN C, PARROTT J, LAPEN DR, and co-authors (2025) Ecotoxicological Implications of Increased Antidepressant Concentrations in the Laurentian Great Lakes Basin, 2018–2023. Science of the Total Environment **981** 179331.

PATEL M, KUMAR R, KISHOR K, MLSNA T, PITTMAN JR CU, MOHAN D (2019) Pharmaceuticals of Emerging Concern in Aquatic Systems: Chemistry, Occurrence, Effects, and Removal Methods. Chemical Reviews **119** 3510–3673.

FANALI S, HADDAD PR, POOLE CF, SCHOENMAKERS P and LLOYD A .Elsevier, Amsterdam.

PEMBERTHY D, PADILLA Y, A E and PEÑUELA GA (2020) Monitoring pharmaceuticals and personal care products in water and fish from the Gulf of Urabá, Colombia. Heliyon **6 (6)**.

PEREIRA A, SILVA L, LARANJEIRO C, LINO C and PENA A (2020). Selected pharmaceuticals in different aquatic compartments: Part I—Source, fate and occurrence. Molecule **25 (5)** 1026.

PHAM HTM, GIERSBERG M, UHLIG S, HANKE G, SIMON K, KUNATH K, BARONIAN K and KUNZE G (2012) EstraMonitor - A monitor for amperometric detection of estrogenic activity with Arxula adeninivorans yeast cells as the biocomponent. Sens Actuators B: Chemical **161** 137–145.

PIAZZA A, MERLO F, ABUALSHAAR A, PISCOPIELLO F, MARASCHI F, BERNINI A, SPALLA M, STURINI M, MIGLIAVACCA R, PILLA G, and co-authors (2025) Occurrence of Micropollutants and Enterobacteria in Ticino Valley: An Insight of Water Contamination in an Agricultural Area with Highly Anthropogenic Impact. Emerging Contaminants **11 (3)** 100509.

PIEDADE F, S BIO and NUNES B (2020) Effects of common pharmaceutical drugs (paracetamol and acetylsalicylic acid) short term exposure on biomarkers of the mussel Mytilus spp. Environmental Toxicology and Pharmacology **73** 103276.

PINTO I, SIMÕES M and GOMES IB (2022) An Overview of the Impact of Pharmaceuticals On Aquatic Microbial Communities. Antibiotics **11(12)** 1700.

PIZZINI S, GIUBILATO E, MORABITO E, BARBARO E, BONETTO A, CALGARO L, FELTRACCO M, SEMENZIN E, VECCHIATO M, ZANGRANDO R, and Gambaro A (2024) Contaminants of Emerging Concern in Water and Sediment of the Venice Lagoon, Italy. Environmental Research **249** 118401.

POPEK E (2018) Chapter 2 - Environmental Chemical Pollutants. In: *Sampling and Analysis of Environmental Chemical Pollutants* 13-69.

PRAVEENA SM, MOHD RASHID MZ, MOHD NASIR FA, SZE YEE W and ARIS AZ (2019) Occurrence and Potential Human Health Risk of Pharmaceutical Residues in Drinking Water from Putrajaya (Malaysia) Ecotoxicology and Environmental Safety **180** 549–556.

PRAVEENA SM, SHAIFUDDIN SNM, SUKIMAN S, NASIR FAM, HANAFI Z, KAMARUDIN N, ISMAIL THT and ARIS AZ (2018) Pharmaceuticals Residues in Selected Tropical Surface Water Bodies from Selangor (Malaysia): Occurrence and Potential Risk Assessments. Science of the Total Environment **642** 230–240.

QUESADA HB, BAPTISTA ATA, CUSIOLI LF, SEIBERT D, OLIVEIRA BEZERRA C and BERGAMASCO R (2019) Surface Water Pollution By Pharmaceuticals and An Alternative of Removal By Low-Cost Adsorbents: A Review. Chemosphere **222** 766–780.

RAHMAN A, JAHANARA I and JOLLY YN (2021) Assessment of Physicochemical Properties of Water and their Seasonal Variation in An Urban River in Bangladesh. Water Science and Engineering **14** 139–148.

RAIMONDO S, VIVIAN DN and BARRON MG (2010) *Web-based interspecies correlation estimation (Web-ICE) for acute toxicity: User manual.* Office of Research and Development. US Environmental Protection Agency, Gulf Breeze, FL, USA.

RAJPOOT K, DESAI N, KOPPISETTI H, TEKADE M, SHARMA MC, BEHERA SK and TEKADE RK (2022). In *Silico Methods for the Prediction of Drug Toxicity.* In:Pharmacokinetics and Toxicokinetic Considerations TEKADE RK. Academic Press. 357-383.

RANJAN N, SINGH PK and MAURYA NS (2022) Pharmaceuticals in Water As Emerging Pollutants for River Health: A Critical Review Under Indian Conditions. Ecotoxicology and Environmental Safety **247** 114220.

REIS EO, Foureaux AFS, Rodrigues JS, Moreira VR, Lebron YAR, Santos LVS, Amaral MCS, Lange LC (2019). **250** 773–781.Occurrence, removal and seasonal variation of pharmaceuticals in Brasilian drinking water treatment plants. Environmental Pollution **250** 773-781.

REN C, TAN X, HUANG C, ZHAO H and LAN W (2022) Sources, Pollution Characteristics, and Ecological Risk Assessment of Steroids in Beihai Bay, Guangxi. Water **14 (9)**.

REYES NJD, GERONIMO FKF, YANO KAV, GUERRA HB and KIM LH (2021) Pharmaceutical and Personal care products in different matrices: Occurrence, pathways, and treatment processes. Water **13 (9)** 1159.

RIMAYI C, ODUSANYA D, WEISS JM, BOER J and CHIMUKA L (2018) Contaminants of Emerging Concern in the Hartbeespoort Dam Catchment and the Umngeni River Estuary 2016 Pollution Incident, South Africa. Science of the Total Environment **627** 1008–1017.

ROUTLEDGE EJ and SUMPTER JP (1996) Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environmental Toxicology and Chemistry: An International Journal **15 (3)** 241–248.

ROYANO S, NAVARRO I, TORRE ADL and MARTÍNEZ MÁ (2024) Investigating the Presence, Distribution and Risk of Pharmaceutically Active Compounds (Phacs) in Wastewater Treatment Plants. River Sediments and Fish. Chemosphere **368** 143759.

SACKEY LNA, OKOBENG A, OBIDIEH PY, NGALA FM, OTOO EB, QUARTEY J, BENTIL JA and AZANU D (2024) Risk Assessment of Pharmaceutical Contaminants in Pharmaceutical Wastewater. The Scientific World Journal **2024(1)** 5538398.

SAITMAN A (2019) Overview of Analytical Methods in Drugs of Abuse Analysis: Gas Chromatography/Mass Spectrometry, Liquid Chromatography Combined with Tandem Mass Spectrometry and Related Methods. In*: Critical Issues in Alcohol and Drugs of Abuse Testing,* 157-171 Academic Press.

SAMAL K, MAHAPATRA S and HIBZUR ALI M (2022) Pharmaceutical Wastewater As Emerging Contaminants (Ec): Treatment Technologies, Impact On Environment and Human Health. Energy Nexus **6** 100076.

SANGANYADO E (2025) Chiral Pharmaceuticals in Environmental Samples. In: *Comprehensive Analytical Chemistry*. Elsevier.

SANTOS AV, COUTO CF, LEBRON YAR, MOREIRA VR, FOUREAUX AFS, REIS EO, SANTOS LVDS, DE ANDRADE LH, AMARAL MCS, LANGE LCS (2020) Occurrence and Risk Assessment of Pharmaceutically Active Compounds in Water Supply Systems in Brazil. Science of the Total Environment **746** 141011.

SARDAR MF, YOUNAS F, LI H, ALI J, ZHU P, YU X, CUI Z and GUO W (2025) Current Scenario of Emerging Pollutants in Farmlands and Water Reservoirs: Prospects and Challenges. Ecotoxicology and Environmental Safety **291** 117829.

SCACCIA N, SILVA FONSECA JV, MEGUEYA AL, ARAGÃO GL, RASOLOFOARISON T, PAULA AV, VINCI KANDA KUPA L, TCHATCHUENG J, MAKUETCHE K, RASOLOJAONA TZ, and co-authors (2024) Analysis of Chlorhexidine, Antibiotics and Bacterial Community Composition in Water Environments from Brazil, Cameroon and Madagascar During The Covid-19 Pandemic. Science of the Total Environment **932** 173016.

SCHUIJT LM, PENG FJ, BERG SJP, DINGEMANS MML and BRINK PJ (2021) Ecotoxicological tests for assessing impacts of chemical stress to aquatic ecosystems: Facts, challenges, and future. The Science of the Total Environment **795** 148776.

SELWE KP, THORN JPR, DESROUSSEAUX AOS, DESSENT CEH and SALLACH JB (2022) Emerging Contaminant Exposure to Aquatic Systems in the Southern African Development Community. Environmental Toxicology and Chemistry **41** 382–395.

SERLETTI L, DUTCHER L, DEGNAN KO, SZYMCZAK JE, CLUZET V, DAVID MZ, CRESSMAN L, GLASSMAN LW and HAMILTON KW (2023) Analysis of Seasonal Variation of Antibiotic Prescribing for Respiratory Tract Diagnoses in Primary Care Practices. Antimicrobial Stewardship & Healthcare Epidemiology, **3 (1)** p.e147.

SHARMA J, JOSHI M, BHATNAGAR A, CHAURASIA AK and NIGAM S (2022) Pharmaceutical Residues: One of the Significant Problems in Achieving ‘Clean Water for All’ and Its Solution. Environmental Research **215** 114219.

SHEN X, CHANG H, SUN Y and WAN Y (2020) Determination and Occurrence of Natural and Synthetic Glucocorticoids in Surface Waters. Environment International **134** 105278.

SHRIVASTAVA A and GUPTA V (2011) Methods for the Determination of Limit of Detection and Limit of Quantitation of the Analytical Methods. Chronicles of Young Scientists **2** 21.

SIGONYA S, MOKHOTHU TH, MOKHENA TC and MAKHANYA TR (2023) Mitigation of Non-Steroidal Anti-Inflammatory and Antiretroviral Drugs As Environmental Pollutants By Adsorption Using Nanomaterials As Viable Solution—A. Critical Review. Applied Sciences **13(2)** 772.

SILVESTER R, PERRY WB, WEBSTER G, RUSHTON L, BALDWIN A, PASS DA, BYRNES NA, FARKAS K, HEGINBOTHOM M, CRAINE N, and co-authors (2025) Metagenomic Profiling of Hospital Wastewater: A Comprehensive National Scale Analysis of Antimicrobial Resistance Genes and Opportunistic Pathogens. Journal of Infection 90 **(6)** 106503.

SOARES DMM, PROCÓPIO DP, ZAMUNER CK, NÓBREGA BB, BETTIM MR, REZENDE G, LOPES PM, PEREIRA ABD, BECHARA EJH, OLIVEIRA AG, and co-authors (2022) Fungal bioassays for environmental monitoring. Frontiers in Bioengineering and Biotechnology **10** 954579.

SOHONI P and SUMPTER JP (1998) Several environmental oestrogens are also anti-androgens. Journal of Endocrinology **158** 327–339.

SOLAUN O, RODRÍGUEZ JG, BORJA Á, LÓPEZ-GARCÍA E, ZONJA B, POSTIGO C, BARCELÓ D, ALDA ML and LARRETA J (2022) Antibiotics in the Basque Coast (N Spain): Occurrence in Waste and Receiving Waters, and Risk Assessment (2017–2020). Science of the Total Environment **847** 157563.

SOLÉ M, RALDUA D, BARCELÓ D and PORTE C (2003) Long-Term Exposure Effects in Vitellogenin, Sex Hormones, and Biotransformation Enzymes in Female Carp in Relation to A Sewage Treatment Works. Ecotoxicology and Environmental Safety **56** 373–380

SOUSA JCG, RIBEIRO AR, BARBOSA MO, PEREIRA MFR and SILVA AMT (2018) A Review On Environmental Monitoring of Water Organic Pollutants Identified By EU Guidelines. Journal of Hazardous Materials **344** 146–162.

SOUTH AFRICAN NATIONAL DEPARTMENT OF HEALTH (NDOH) MODULE 5HEALTH SOUTH AFRICA (2021) NDoH COVID-19 Guidelines Module 5- Drug Therapy_v7_13December2 (Accessed 20 July 2023). Version 6. https://knowledgehub.health.gov.za/system/files/elibdownloads/2021-11/drug%20therapy,%20version%206%20(28sep2021)_0.pdf. (Accessed 20 July 2023).

SOUZA A, MORENO BB, ALMEIDA JE, ROGERO SO, PEREIRA CDS and ROGERO JR (2016) Cytotoxicity Evaluation of Amoxicillin and Potassium Clavulanate in Perna Perna Mussels. Ecotoxicology and Environmental Contamination **11** 21–26.

STANTON IC, MURRAY AK and ZHANG L (2020) Evolution of antibiotic resistance at low antibiotic concentrations including selection below the minimal selective concentration. Communications Biology **3(1)** 467.

STRESSMARQ BIOSCIENCES S (2023) Dexamethasone. https://www.stressmarq.com/products/small-molecules/inducer/dexamethasone-sih-259/?v=3e8d115eb4b3#product-citations. (Accessed 08 July 2023)

SULEMAN S, VERHEUST Y, DUMOULIN A, WYNENDAELE E, D'HONDT M, VANDERCRUYSSEN K, VERYSER L, DUCHATEAU L and SPIEGELEER B (2015) Gas Chromatographic Method for the Determination of Lumefantrine in Antimalarial Finished Pharmaceutical Products. Journal of Food and Drug Analysis **23** 552–559.

SUN X, LIU M, LIU H, LI L and DING Y (2024) A Molecularly Imprinted Electrochemical Aptasensor-Based Dual Recognition Elements for Selective Detection of Dexamethasone. Talanta **277** 126404.

SUZUKI G, SATO K, ISOBE T, TAKIGAMI H, BROUWER A and NAKAYAMA K (2015) Detection of Glucocorticoid Receptor Agonists in Effluents from Sewage Treatment Plants in Japan. Science of the Total Environment **527–528** 328–334.

SWARTZ CD, GENTHE B, CHAMIER J, PETRIK LF, TIJANI JO, ADELEYE A, COOMANS CJ, OHLIN A, FALK D and MENGE JG (2018) *Emerging Contaminants in Wastewater Treated for Direct Potable Re-Use: The Human Health Risk Priorities in South Africa*. *Vol Ii: A Prioritization Framework for Monitoring Contaminants of Emerging Concern in Reclaimed Water for Potable Use*. Wrc Report No. Tt 742/2/17.

SZARKA A, VNUKOVÁ L, KERŠŇÁKOVÁ Z, VIKTORYOVÁ N and HROUZKOVÁ S (2024) Contamination with Pharmaceuticals in Aquatic Environment: Focus On Analytical Methodologies. Applied Sciences.

TANG Y, YIN M, YANG W, LI H, ZHONG Y, MO L, LIANG Y, MA X and SUN X (2019) Emerging Pollutants in Water Environment: Occurrence, Monitoring, Fate, and Risk Assessment. Water Environment Research: A Research Publication of the Water Environment Federation **91** 984–991.

TANG Z, LIU ZH, WANG H, DANG Z and LIU Y (2021) A review of 17α-ethynylestradiol (EE2) in surface water across 32 countries: Sources, concentrations, and potential estrogenic effects. Journal of Environmental Management **292** 112804.

TAN L, XU K, ZHANG S, TANG F, ZHANG M, GE F and ZHANG K (2024) Pollution characteristics and ecological risk assessment of glucocorticoids in the Jiangsu section of the Yangtze River Basin. Frontiers of Environmental Science & Engineering, **18 (11)** 143.

TANUI IC, KANDIE F, KRAUSS M, PIOTROWSKA A, FINCKH S, KIPROP A, HOLLERT H, SHAHID N, LIESS M and BRACK W (2025) Occurrence and Potential Risk of Steroid Hormones in Selected Surface Water and Wastewater Treatment Plants in Western Kenya. Environmental Pollution **367** 125623.

TARAZONA JV, MARTÍNEZ M, MARTÍNEZ M-A and ANADÓN A (2021) Environmental Impact Assessment of Covid-19 Therapeutic Solutions. A Prospective Analysis. Science of the Total Environment **778** 146257.

TEGEGNE AA, MEKASHA YT, AYU AA, HASEN G and SULEMAN S (2024) A Review On Emerging Pharmaceutical Residues in Ethiopia: Occurrence, Ecotoxicological Aspects, and Regulatory Concerns. Frontiers in Microbiology **15** 1499487.

TENG R-L and BENET LZ (1989) Simultaneous Measurement of Prednisone, Prednisolone and 6β-Hydroxyprednisolone in Urine By High-Performance Liquid Chromatography Using Dexamethasone As The Internal Standard. Journal of Chromatography B: Biomedical Sciences and Applications **493** 421–423.

TERZIC S, IVANKOVIC K, JAMBROSIC K, KURTOVIC B and AHEL M (2024) Bioaccumulation and tissue distribution of pharmaceuticals and their transformation products in fish along the pollution gradients of a wastewater-impacted river. Science of The Total Environment **956**.

TEWARI S, JINDAL R, KHO YL, EO S and CHOI K (2013) Major Pharmaceutical Residues in Wastewater Treatment Plants and Receiving Waters in Bangkok, Thailand, and Associated Ecological Risks. Chemosphere **91 (5)** 697–704.

THAVARAYAN L and MOODLEY B (2025) Development of Solid Phase Extraction Method for the Lc-Pda Detection of Selected Pharmaceuticals and their Metabolites in Surface Water and Sediment from The Isipingo River, Kzn, South Africa. Scientific African **28** 02655.

TRUTER JC, WYK JH and OBERHOLSTER PJ (2016) An in Vitro and in Vivo Assessment of Endocrine Disruptive Activity in a Major South African River. Water, Air and Soil Pollution, **227(2)** 54.

U.S. ENVIRONMENTAL PROTECTION AGENCY (2008). White Paper: Aquatic Life Criteria for Contaminants of Emerging Concern. Part 1: General Challenges and Recommendations. https://www.epa.gov/sites/default/files/2015-08/documents/white_paper_aquatic_life_criteria_for_contaminants_of_emerging_concern_part_i_general_challenges_and_recommendations_1.pdf (Accessed 14 August 2024).

VAIDELIENE A and MICHAILOV N (2008) Dam Influence on The River Self-Purification. In: 7th International Conference Environmental Engineering, Vilnius, Lithuania, 22-23 May 2008. Vilnius:Vilnius Gediminas Technical University. Available at: https://www.researchgate.net/profile/Adele-Vaideliene/publication/228599400_Dam_influence_on_the_river_self_-_Purification/links/5fd75a13299bf140880a9a67/Dam-influence-on-the-river-self-Purification.pdf. (Accessed 15 April 2024).

VERMILLION MAIER ML and TJEERDEMA RS (2018) Azithromycin Sorption and Biodegradation in a Simulated California River System. Chemosphere 190 471–480.
VIEGAS CA (2021) Microbial bioassays in environmental toxicity testing. Advances in Applied Microbiology **115** 115–158.

VILLA S, NICA V, CASTIGLIONI S and FINIZIO A (2020) Environmental Risk Classification of Emerging Contaminants in An Alpine Stream Influenced By Seasonal Tourism. Ecological Indicators **115** 106428.

VUMAZONKE S, KHAMANGA SM and NGQWALA NP (2020) Detection of Pharmaceutical Residues in Surface Waters of the Eastern Cape Province. International Journal of Environmental Research and Public Health **17 (11)** 4067.

WADA OZ and OLAWADE DB (2025) Recent Occurrence of Pharmaceuticals in Freshwater, Emerging Treatment Technologies, and Future Considerations: A Review. Chemosphere **374** 144153.

WALENG NJ and NOMNGONGO PN (2022) Occurrence of Pharmaceuticals in the Environmental Waters: African and Asian Perspectives. Environmental Chemistry and Ecotoxicology **4** 50–66.

WANG F, XIANG L, SZE-YIN LEUNG K, ELSNER M, ZHANG Y, GUO Y, PAN B, SUN H, AN T, YING G, and co-authors (2024) Emerging Contaminants: A One Health Perspective. The Innovation **5** 100612.

WANG J, SHEN J, YE D, YAN X, ZHANG Y, YANG W, LI X, WANG J, ZHANG L and PAN L (2020) Disinfection Technology of Hospital Wastes and Wastewater: Suggestions for Disinfection Strategy During Coronavirus Disease 2019 (Covid-19) Pandemic in China. Environmental Pollution **262** 114665.

WANG R, LU Y, SONG S, YANG S, WU Y and CUI H (2022) Industrial Source Discharge Estimation for Pharmaceutical and Personal Care Products in China. Journal of Cleaner Production **381** 135129.

WANG R, LU Y, SONG S, YANG S, WU Y and CUI H (2022) Industrial Source Discharge Estimation for Pharmaceutical and Personal Care Products in China. Journal of Cleaner Production **381** 135129.

WILKINSON JL, BOXALL ABA, KOLPIN DW, LEUNG KMY, LAI RWS, GALBÁN-MALAGÓN C, ADELL AD, MONDON J, METIAN M and MARCHANT RA (2022) Pharmaceutical Pollution of the World's Rivers. Proceedings of the National Academy of Sciences **119 (8)** p.e2113947119.

WORLD HEALTH ORGANIZATION (2012) Pharmaceuticals in drinking-water. World Health Organization. 2012. : https://apps.who.int/iris/handle/10665/44630. (Accessed 24 July 2023).

WU F, ZHAO X, NI S and YANG B (2020) A LC−MS/Ms Validated Method for Determination of Azithromycin in Human Tears and Its Application to An Ocular Pharmacokinetic Study. Die Pharmazie - An International Journal of Pharmaceutical Sciences **75** 478–482.

WU X, REN J, XU Q, XIAO Y, LI X and PENG Y (2023) Priority Screening of Contaminant of Emerging Concern (CECs) in Surface Water from Drinking Water Sources in the Lower Reaches of the Yangtze River Based On Exposure-Activity Ratios (Ears). Science of the Total Environment **856** 159016.

WU Y, GUO H, ZHANG J, CHEN X, WU M and QIN W (2019) Multiple Applications of Enzymes Induced By Algal Biomasses from A New Bacillus Isolate to Saccharify Algae and Degrade Chemical Dyes. Waste and Biomass Valorization **10** 2517–2526.

XIANG Q, SHEN X, LI K, WANG Z, ZHAO X and CHEN Q (2024) Occurrence, distribution, and environmental risk of 61 glucocorticoids in surface water of the Yellow River Delta, China. Science of the Total Environment **906** 167504.

XIANG Q, SHEN X, LI K, WANG Z, ZHAO X and CHEN Q (2024) Occurrence, Distribution, and Environmental Risk of 61 Glucocorticoids in Surface Water of the Yellow River Delta, China. Science of the Total Environment **906** 167504.

XING L, HADDAO KM, EMAMI N, NALCHIFARD F, HUSSAIN W, JASEM H, DAWOOD AH, TOGHRAIE D and HEKMATIFAR M (2022) Fabrication of Hkust-1/Zno/Sa Nanocomposite for Doxycycline and Naproxen Adsorption from Contaminated Water. Sustainable Chemistry and Pharmacy **29** 100757.

XU M, LU S, CHEN W, HU L, ZHOU L and YANG X (2025) Ten-Month Comprehensive Assessment of Steroid Hormones in the Tributaries of Baiyun District, Guangzhou City, China: Spatiotemporal Dynamics, Source Attribution, and Environmental Implications. Science of the Total Environment **958** 177908.

YASIR M, GOYAL A and SONTHALIA S (2023) Corticosteroid Adverse Effects. Treasure Island (Fl), Statpearls Publishing. https://www.ncbi.nlm.nih.gov/books/nbk531462/. (Accessed 18 August 2024).

YATES EA and RUDD TR (2016) Recent innovations in the structural analysis of heparin. International Journal of Cardiology **212** 5–9.

YAZDAN MMD, AHAD TMD, MALLICK Z, MALLICK SP, JAHAN I and MAZUMDER M (2021) An overview of the glucocorticoids pathways in the environment and their removal using conventional. Wastewater Treatment Systems Pollutants **1 (3)** 141–155.

YING G, WILLIAMS B AND KOOKANA (2002) Environmental fate of alkylphenols and alkylphenol ethoxylates: a review. Environment International **28 (3)** 215–226.

YUSUF A, O'FLYNN D, WHITE B, HOLLAND L, PARLE-MCDERMOTT A, LAWLER J, MCCLOUGHLIN T, HAROLD D, HUERTA B and REGAN F (2021) Monitoring of Emerging Contaminants of Concern in the Aquatic Environment: A Review of Studies Showing The Application of Effect-Based Measures. Analytical Methods **13** 5120–5143.

YU X, LYU S, ZHAO W, GUO C, XU J and SUI Q (2024) A Picture of Pharmaceutical Pollution in Landfill Leachates: Occurrence, Regional Differences and Influencing Factors. Waste Management **184** 20–27.

ZENKER A, CICERO MR, PRESTINACI F, BOTTONI P and CARERE M (2014) Bioaccumulation and Biomagnification Potential of Pharmaceuticals with A Focus to the Aquatic Environment. Journal of Environmental Management **133** 378–387.

ZHANG D, LIU W, YIN C, SHE L, REN J, XU Q, WANG S and PENG Y (2024) Occurrence of Contaminants of Emerging Concern in Surface and Waste Water from The Yangtze River Chemical Contiguous Zone, China: Distribution. Sources and Ecological Risk Assessment. Science of the Total Environment **949** 175151.

ZHANG H, WANG XC, ZHENG Y and DZAKPASU M (2023) Removal of Pharmaceutical Active Compounds in Wastewater By Constructed Wetlands: Performance and Mechanisms. Journal of Environmental Management **325** 116478.

ZHOU C and WANG X (2017) Rapid Determination of Isomeric Benzoylpaeoniflorin and Benzoylalbiflorin in Rat Plasma By LC−MS/Ms Method. International Journal of Analytical Chemistry 1693464.

ZHOU J and BROODBANK N (2014) Sediment-Water Interactions of Pharmaceutical Residues in the River Environment. Water Research **48** 61–70.

ZHU M, CHEN J, PEIJNENBURG WJGM, XIE H, WANG Z and ZHANG S (2022) Controlling factors and toxicokinetic modeling of antibiotics bioaccumulation in aquatic organisms: A review. Critical Reviews in Environmental Science and Technology **53 (15)** 1431–1451.

SUPPLEMENTARY INFORMATION

Supplementary Information 1a: Levels (µg/ℓ) of azithromycin in water during summer

S/N	Sampling Site	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1	30733	32435	17.55	18.53	18.04	0.69	3.84
2	P2	12446	11379	7.01	6.40	6.71	0.43	6.48
3	Site 3	<LoD	<LoD	-	-	-	-	-
4	U4	29611	28778	16.91	16.43	16.67	0.34	2.04
5	D4	12125	12723	6.83	7.17	7.00	0.24	3.48
6	P5	21222	22640	12.07	12.89	12.48	0.58	4.63
7	U5	3057	4001	1.60	2.15	1.87	0.38	20.52
8	D5	<LoD	<LoD	-	-	-	-	-
9	U6	28842	28875	16.46	16.48	16.47	0.01	0.08
10	D6	29927	30322	17.09	17.31	17.20	0.16	0.94
11	P7	13755	14714	7.77	8.32	8.04	0.39	4.86
12	U7	<LoD	<LoD	-	-	-	-	-
13	D7	2167	2216	1.09	1.12	1.10	0.02	1.81
14	P8	25694	26088	14.65	14.87	14.76	0.16	1.09
15	U8	3486	3508	1.85	1.86	1.86	0.01	0.48
16	D8	20239	18502	11.50	10.50	11.00	0.71	6.43

P=PoD; U=U.Strm; D=D.Strm; Site 3=S3

Supplementary Information 1b: Levels (µg/ℓ) of azithromycin in water during winter

S/N	Sampling Site	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1	34112	34047	13.37	13.34	13.35	0.02	0.12
2	P2	4851	5079	3.19	3.26	3.23	0.06	1.74
3	P3	<LoD	<LoD	_	_	_	_	_
4	P4	35694	34284	13.92	13.43	13.67	0.35	2.54
5	U4	35564	35978	13.87	14.01	13.94	0.10	0.73
6	D4	34643	34222	13.55	13.40	13.48	0.10	0.77
7	U5	<LoD	<LoD	_	_	_	_	_
8	D5	5460	5572	3.40	3.44	3.42	0.03	0.81
9	P6	7895	7794	4.24	4.21	4.23	0.02	0.59
10	U6	7569	7321	4.13	4.04	4.09	0.06	1.49
11	D6	10464	10708	5.14	5.22	5.18	0.06	1.16
12	P7	50583	51689	19.10	19.48	19.29	0.27	1.41
13	U7	<LoD	<LoD	_	_	_	_	_
14	D7	20889	19280	8.77	8.21	8.49	0.40	4.66
15	P8	50706	51751	19.14	19.50	19.32	0.26	1.33
16	U8	<LoD	<LoD	_	_	_	_	_
17	D8	28038	28102	11.25	11.27	11.26	0.02	0.14

Supplementary Information 2a: Levels (µg/ℓ) of prednisolone in water during summer

S/N	Sampling Site	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1	<LoD	<LoD	-	-	-	-	-
2	P2	<LoD	<LoD	-	-	-	-	-
3	Site 3	<LoD	<LoD	-	-	-	-	-
4	U4	<LoD	<LoD	-	-	-	-	-
5	D4	<LoD	<LoD	-	-	-	-	-
6	P5	<LoD	<LoD	-	-	-	-	-
7	U5	<LoD	<LoD	-	-	-	-	-
8	D5	<LoD	<LoD	-	-	-	-	-
9	U6	47232	45663	40.61	39.28	39.94	0.94	2.35
10	D6	20684	19352	18.11	16.98	17.54	0.80	4.55
11	P7	<LoD	<LoD	-	-	-	-	-
12	U7	<LoD	<LoD	-	-	-	-	-
13	D7	<LoD	<LoD	-	-	-	-	-
14	P8	<LoD	<LoD	-	-	-	-	-
15	U8	<LoD	<LoD	-	-	-	-	-
16	D8	<LoD	<LoD	-	-	-	-	-

Supplementary Information 2b: Levels (µg/ℓ) of prednisolone in water during winter

S/N	Sampling Site	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1	<LoD	<LoD	-	-	-	-	-
2	P2	<LoD	<LoD	-	-	-	-	-
3	P3	<LoD	<LoD	-	-	-	-	-
4	P4	<LoD	<LoD	-	-	-	-	-
5	U4	<LoD	<LoD	-	-	-	-	-
6	D4	6442	6644	7.60	7.79	7.70	0.13	1.71
7	U5	<LoD	<LoD	-	-	-	-	-
8	D5	10964	11766	11.77	12.51	12.14	0.52	4.30
9	P6	<LoD	<LoD	-	-	-	-	-
10	U6	<LoD	<LoD	-	-	-	-	-
11	D6	<LoD	<LoD	-	-	-	-	-
12	P7	<LoD	<LoD	-	-	-	-	-
13	U7	<LoD	<LoD	-	-	-	-	-
14	D7	<LoD	<LoD	-	-	-	-	-
15	P8	10892	11230	11.70	12.02	11.86	0.22	1.86
16	U8	<LoD	<LoD	-	-	-	-	-
17	D8	<LoD	<LoD	-	-	-	-	-

Supplementary Information 3a: Levels (µg/ℓ) of dexamethasone in water during summer

S/N	Sampling Site	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1	<LoD	<LoD	-	-	-	-	-
2	P2	<LoD	<LoD	-	-	-	-	-
3	Site 3	<LoD	<LoD	-	-	-	-	-
4	U4	20992	20542	17.98	17.62	17.80	0.25	1.43
5	D4	<LoD	<LoD	-	-	-	-	-
6	P5	49046	52431	40.44	43.14	41.79	1.92	4.58
7	U5	<LoD	<LoD	-	-	-	-	-
8	D5	<LoD	<LoD	-	-	-	-	-
9	U6	<LoD	<LoD	-	-	-	-	-
10	D6	<LoD	<LoD	-	-	-	-	-
11	P7	<LoD	<LoD	-	-	-	-	-
12	U7	<LoD	<LoD	-	-	-	-	-
13	D7	<LoD	<LoD	-	-	-	-	-
14	P8	<LoD	<LoD	-	-	-	-	-
15	U8	10372	11951	9.48	10.75	10.12	0.89	8.83
16	D8	22119	22837	18.89	19.46	19.17	0.41	2.12

Supplementary Information 3b: Levels (µg/ℓ) of dexamethasone in water during winter

S/N	Sampling Site	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1	<LoD	<LoD	-	-	-	-	-
2	P2	<LoD	<LoD	-	-	-	-	-
3	P3	<LoD	<LoD	-	-	-	-	-
4	P4	<LoD	<LoD	-	-	-	-	-
5	U4	<LoD	<LoD	-	-	-	-	-
6	D4	<LoD	<LoD	-	-	-	-	-
7	U5	<LoD	<LoD	-	-	-	-	-
8	D5	<LoD	<LoD	-	-	-	-	-

9	P6	<LoD	<LoD	-	-	-	-	-
10	U6	<LoD	<LoD	-	-	-	-	-
11	D6	<LoD	<LoD	-	-	-	-	-
12	P7	<LoD	<LoD	-	-	-	-	-
13	U7	<LoD	<LoD	-	-	-	-	-
14	D7	<LoD	<LoD	-	-	-	-	-
15	P8	<LoD	<LoD	-	-	-	-	-
16	U8	<LoD	<LoD	-	-	-	-	-
17	D8	<LoD	<LoD	-	-	-	-	-

Supplementary Information 4a: Levels (µg/ℓ) of prednisone in water during summer

S/N	Sampling Site	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1	<LoD	<LoD	-	-	-	-	-
2	P2	<LoD	<LoD	-	-	-	-	-
3	Site 3	<LoD	<LoD	-	-	-	-	-
4	U4	<LoD	<LoD	-	-	-	-	-
5	D4	<LoD	<LoD	-	-	-	-	-
6	P5	7018	8125	6.01	6.87	6.44	0.61	9.44
7	U5	12474	14911	10.25	12.14	11.19	1.34	11.95
8	D5	7794	9143	6.61	7.66	7.14	0.74	10.38
9	U6	<LoD	<LoD	-	-	-	-	-
10	D6	<LoD	<LoD	-	-	-	-	-
11	P7	<LoD	<LoD	-	-	-	-	-
12	U7	11563	10345	9.54	8.59	9.07	0.67	7.37
13	D7	<LoD	<LoD	-	-	-	-	-
14	P8	<LoD	<LoD	-	-	-	-	-
15	U8	<LoD	<LoD	-	-	-	-	-
16	D8	<LoD	<LoD	-	-	-	-	-

Supplementary Information 4b: Levels (µg/ℓ) of prednisone in water during winter

S/N	Sampling Site	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1	<LoD	<LoD	-	-	-	-	-
2	P2	<LoD	<LoD	-	-	-	-	-
3	P3	<LoD	<LoD	-	-	-	-	-
4	P4	<LoD	<LoD	-	-	-	-	-
5	U4	<LoD	<LoD	-	-	-	-	-
6	D4	<LoD	<LoD	-	-	-	-	-
7	U5	<LoD	<LoD	-	-	-	-	-
8	D5	<LoD	<LoD	-	-	-	-	-

9	P6	13321	14691	12.81	14.17	13.49	0.96	7.14
10	U6	<LoD	<LoD	-	-	-	-	-
11	D6	<LoD	<LoD	-	-	-	-	-
12	P7	<LoD	<LoD	-	-	-	-	-
13	U7	<LoD	<LoD	-	-	-	-	-
14	D7	<LoD	<LoD	-	-	-	-	-
15	P8	<LoD	<LoD	-	-	-	-	-
16	U8	<LoD	<LoD	-	-	-	-	-
17	D8	<LoD	<LoD	-	-	-	-	-

Supplementary Information 5a: Levels (ng/g) of azithromycin in sediments during summer

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1 5 cm	<LoD	<LoD	-	-	-	-	-
2	P1 10 cm	4415	4683	0.48	0.51	0.49	0.02	4.43
3	P1 15 cm	3652	3912	0.39	0.42	0.40	0.02	5.24
4	P2 5 cm	10633	9319	1.19	1.04	1.12	0.11	9.58
5	P2 10 cm	<LoD	<LoD	-	-	-	-	-
6	P2 15 cm	<LoD	<LoD	-	-	-	-	-
7	Site 3 5 cm	<LoD	<LoD	-	-	-	-	-
8	Site 3 10 cm	<LoD	<LoD	-	-	-	-	-
9	Site 3 15 cm	<LoD	<LoD	-	-	-	-	-
10	P4 5 cm	<LoD	<LoD	-	-	-	-	-
11	U4 5 cm	<LoD	<LoD	-	-	-	-	-
12	D4 5 cm	<LoD	<LoD	-	-	-	-	-
13	P5 5 cm	<LoD	<LoD	-	-	-	-	-
14	P5 10 cm	<LoD	<LoD	-	-	-	-	-
15	P5 15 cm	<LoD	<LoD	-	-	-	-	-
16	U5 5 cm	7746	7847	0.86	0.87	0.87	0.01	0.95
17	U5 10 cm	<LoD	<LoD	-	-	-	-	-
18	U5 15 cm	<LoD	<LoD	-	-	-	-	-
19	D5 5 cm	<LoD	<LoD	-	-	-	-	-
20	D5 10 cm	<LoD	<LoD	-	-	-	-	-
21	D5 15 cm	<LoD	<LoD	-	-	-	-	-
22	U6 5 cm	7949	8308	0.88	0.93	0.91	0.03	3.23
23	U6 10 cm	6789	5636	0.75	0.62	0.68	0.09	13.73
24	U6 15 cm	<LoD	<LoD	-	-	-	-	-
25	D6 5 cm	<LoD	<LoD	-	-	-	-	-
26	D6 10 cm	<LoD	<LoD	-	-	-	-	-
27	D6 15 cm	<LoD	<LoD	-	-	-	-	-
28	P7 5 cm	<LoD	<LoD	-	-	-	-	-
29	P7 10 cm	<LoD	<LoD	-	-	-	-	-
30	P7 15 cm	10173	9191	1.14	1.03	1.08	0.08	7.38
31	U7 5 cm	2256	2371	0.23	0.24	0.23	0.01	3.99
32	U7 10 cm	5734	5725	0.63	0.63	0.63	0.00	0.12

33	U7 15 cm	12459	13898	1.40	1.57	1.49	0.12	7.89
34	D7 5 cm	<LoD	<LoD	-	-	-	-	-
35	D7 10 cm	<LoD	<LoD	-	-	-	-	-
36	D7 15 cm	<LoD	<LoD	-	-	-	-	-
37	P8 5 cm	<LoD	<LoD	-	-	-	-	-
38	P8 10 cm	<LoD	<LoD	-	-	-	-	-
39	U8 5 cm	<LoD	<LoD	-	-	-	-	-
40	U8 10 cm	<LoD	<LoD	-	-	-	-	-
41	U8 15 cm	<LoD	<LoD	-	-	-	-	-
42	D8 5 cm	29227	30077	3.34	3.43	3.39	0.07	2.05
43	D8 10 cm	<LoD	<LoD	-	-	-	-	-
44	D8 15 cm	<LoD	<LoD	-	-	-	-	-

Supplementary Information 5b: Levels (ng/g) of azithromycin in sediments during winter

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1 5 cm	20388	21034	1.72	1.76	1.74	0.03	1.83
2	P1 10 cm	<LoD	<LoD	_	_	_	_	_
3	P1 15 cm	<LoD	<LoD	_	_	_	_	_
4	P2 5 cm	<LoD	<LoD	_	_	_	_	_
5	P2 10 cm	<LoD	<LoD	_	_	_	_	_
6	P2 15 cm	<LoD	<LoD	_	_	_	_	_
7	P3 5 cm	<LoD	<LoD	_	_	_	_	_
8	P3 10 cm	<LoD	<LoD	_	_	_	_	_
9	P3 15 cm	6012	6010	0.72	0.72	0.72	0.00	0.01
10	P4 5 cm	25313	25003	2.06	2.04	2.05	0.02	0.74
11	P4 10 cm	33962	34278	2.66	2.68	2.67	0.02	0.58
12	U4 5 cm	11655	12006	1.11	1.13	1.12	0.02	1.54
13	U4 10 cm	11558	11412	1.10	1.09	1.10	0.01	0.65
14	D4 5 cm	6208	6197	0.73	0.73	0.73	0.00	0.07
15	P5 5 cm	7116	7367	0.79	0.81	0.80	0.01	1.54
16	P5 10 cm	<LoD	<LoD	_	_	_	_	_
17	P5 15 cm	<LoD	<LoD	_	_	_	_	_
18	U5 5 cm	8336	8264	0.88	0.87	0.88	0.00	0.40
19	U5 10 cm	<LoD	<LoD	_	_	_	_	_
20	U5 15 cm	<LoD	<LoD	_	_	_	_	_
21	P6 5 cm	5601	5608	0.69	0.69	0.69	0.00	0.05
22	P6 10 cm	9102	9292	0.93	0.95	0.94	0.01	0.99
23	P6 15 cm	5114	5147	0.66	0.66	0.66	0.00	0.25
24	U6 5 cm	11468	10574	1.10	1.04	1.07	0.04	4.12

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
25	U6 10 cm	7753	7614	0.84	0.83	0.83	0.01	0.82
26	U6 15 cm	3578	3495	0.55	0.54	0.55	0.00	0.75
27	D6 5 cm	<LoD	<LoD	_	_	_	_	_
28	D6 10 cm	10700	10906	1.04	1.06	1.05	0.01	0.96
29	D6 15 cm	10675	10228	1.04	1.01	1.03	0.02	2.14
30	P7 5 cm	<LoD	<LoD	_	_	_	_	_
31	P7 10 cm	<LoD	<LoD	_	_	_	_	_
32	P7 15 cm	<LoD	<LoD	_	_	_	_	_
33	U7 5 cm	<LoD	<LoD	_	_	_	_	_
34	U7 10 cm	5685	5527	0.70	0.68	0.69	0.01	1.13
35	U7 15 cm	10635	10268	1.04	1.01	1.03	0.02	1.76
36	D7 5 cm	5990	5964	0.72	0.71	0.72	0.00	0.18
37	D7 10 cm	<LoD	<LoD	_	_	_	_	_
38	D7 15 cm	<LoD	<LoD	_	_	_	_	_
39	P8 5 cm	<LoD	<LoD	_	_	_	_	_
40	U8 5 cm	5629	5793	0.69	0.70	0.70	0.01	1.16
41	U8 10 cm	<LoD	<LoD	_	_	_	_	_
42	U8 15 cm	<LoD	<LoD	_	_	_	_	_
43	D8 5 cm	31264	30948	2.47	2.45	2.46	0.02	0.63
44	D8 10 cm	5788	5691	0.70	0.70	0.70	0.00	0.68
45	D8 15 cm	<LoD	<LoD	_	_	_	_	_

Supplementary Information 6a: Levels (ng/g) of prednisolone in sediments during summer

S/N	Sampling sites	Peak Area 1	Peak Area 2	conc.1 (ng/g)	conc.2 (ng/g)	Mean	S.D	%RSD
1	P1 5 cm	<LoD	<LoD	-	-	-	-	-
2	P1 10 cm	<LoD	<LoD	-	-	-	-	-
3	P1 15 cm	<LoD	<LoD	-	-	-	-	-
4	P2 5 cm	<LoD	<LoD	-	-	-	-	-
5	P2 10 cm	<LoD	<LoD	-	-	-	-	-
6	P2 15 cm	<LoD	<LoD	-	-	-	-	-
7	Site 3 5 cm	6762	6942	1.26	1.29	1.28	0.02	1.69
8	Site 3 10 cm	<LoD	<LoD	-	-	-	-	-
9	Site 3 15 cm	<LoD	<LoD	-	-	-	-	-
10	P4 5 cm	<LoD	<LoD	-	-	-	-	-
11	U4 5 cm	11677	10338	2.09	1.87	1.98	0.16	8.10
12	D4 5 cm	<LoD	<LoD	-	-	-	-	-
13	P5 5 cm	13166	14837	2.35	2.63	2.49	0.20	8.05
14	P5 10 cm	5505	5033	1.05	0.97	1.01	0.06	5.61
15	P5 15 cm	8342	8927	1.53	1.63	1.58	0.07	4.44
16	U5 5 cm	<LoD	<LoD	-	-	-	-	-
17	U5 10 cm	4049	3705	0.80	0.74	0.77	0.04	5.34
18	U5 15 cm	3254	3518	0.67	0.71	0.69	0.03	4.60
19	D5 5 cm	<LoD	<LoD	-	-	-	-	-
20	D5 10 cm	<LoD	<LoD	-	-	-	-	-
21	D5 15 cm	3609	3813	0.73	0.76	0.74	0.02	3.29
22	U6 5 cm	<LoD	<LoD	-	-	-	-	-
23	U6 10 cm	1821	1747	0.42	0.41	0.42	0.01	2.13
24	U6 15 cm	<LoD	<LoD	-	-	-	-	-
25	D6 5 cm	<LoD	<LoD	-	-	-	-	-
26	D6 10 cm	3278	3271	0.67	0.67	0.67	0.00	0.13
27	D6 15 cm	<LoD	<LoD	-	-	-	-	-
28	P7 5 cm	3100	2853	0.64	0.60	0.62	0.03	4.78
29	P7 10 cm	2553	2377	0.55	0.52	0.53	0.02	3.96
30	P7 15 cm	<LoD	<LoD	-	-	-	-	-
31	U7 5 cm	<LoD	<LoD	-	-	-	-	-
32	U7 10 cm	<LoD	<LoD	-	-	-	-	-

33	U7 15 cm	<LoD	<LoD	-	-	-	-	-
34	D7 5 cm	6277	7138	1.18	1.32	1.25	0.10	8.25
35	D7 10 cm	2263	2248	0.50	0.50	0.50	0.00	0.36
36	D7 15 cm	2019	2188	0.46	0.49	0.47	0.02	4.30
37	P8 5 cm	26443	24184	4.60	4.21	4.41	0.27	6.15
38	P8 10 cm	<LoD	<LoD	-	-	-	-	-
39	U8 5 cm	13043	13918	2.33	2.47	2.40	0.10	4.37
40	U8 10 cm	6668	6085	1.24	1.15	1.20	0.07	5.85
41	U8 15 cm	<LoD	<LoD	-	-	-	-	-
42	D8 5 cm	<LoD	<LoD	-	-	-	-	-
43	D8 10 cm	<LoD	<LoD	-	-	-	-	-
44	D8 15 cm	<LoD	<LoD	-	-	-	-	-

Supplementary Information 6b: Levels (ng/g) of prednisolone in sediments during winter

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1 5 cm	<LoD	<LoD	_	_	_	_	_
2	P1 10 cm	<LoD	<LoD	_	_	_	_	_
3	P1 15 cm	<LoD	<LoD	_	_	_	_	_
4	P2 5 cm	<LoD	<LoD	_	_	_	_	_
5	P2 10 cm	<LoD	<LoD	_	_	_	_	_
6	P2 15 cm	<LoD	<LoD	_	_	_	_	_
7	P3 5 cm	<LoD	<LoD	_	_	_	_	_
8	P3 10 cm	<LoD	<LoD	_	_	_	_	_
9	P3 15 cm	<LoD	<LoD	_	_	_	_	_
10	P4 5 cm	<LoD	<LoD	_	_	_	_	_
11	P4 10 cm	<LoD	<LoD	_	_	_	_	_
12	U4 5 cm	<LoD	<LoD	_	_	_	_	_
13	U4 10 cm	<LoD	<LoD	_	_	_	_	_

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
14	D4 5 cm	<LoD	<LoD	_	_	_	_	_
15	P5 5 cm	<LoD	<LoD	_	_	_	_	_
16	P5 10 cm	<LoD	<LoD	_	_	_	_	_
17	P5 15 cm	<LoD	<LoD	_	_	_	_	_
18	U5 5 cm	3437	3387	0.97	0.96	0.96	0.01	0.68
19	U5 10 cm	<LoD	<LoD	_	_	_	_	_
20	U5 15 cm	<LoD	<LoD	_	_	_	_	_
21	P6 5 cm	<LoD	<LoD	_	_	_	_	_
22	P6 10 cm	10646	10537	2.30	2.28	2.29	0.01	0.62
23	P6 15 cm	<LoD	<LoD	_	_	_	_	_
24	U6 5 cm	<LoD	<LoD	_	_	_	_	_
25	U6 10 cm	<LoD	<LoD	_	_	_	_	_
26	U6 15 cm	<LoD	<LoD	_	_	_	_	_
27	D6 5 cm	<LoD	<LoD	_	_	_	_	_
28	D6 10 cm	<LoD	<LoD	_	_	_	_	_
29	D6 15 cm	<LoD	<LoD	_	_	_	_	_
30	P7 5 cm	<LoD	<LoD	_	_	_	_	_
31	P7 10 cm	<LoD	<LoD	_	_	_	_	_
32	P7 15 cm	<LoD	<LoD	_	_	_	_	_
33	U7 5 cm	<LoD	<LoD	_	_	_	_	_
34	U7 10 cm	<LoD	<LoD	_	_	_	_	_
35	U7 15 cm	<LoD	<LoD	_	_	_	_	_
36	D7 5 cm	<LoD	<LoD	_	_	_	_	_
37	D7 10 cm	<LoD	<LoD	_	_	_	_	_
38	D7 15 cm	<LoD	<LoD	_	_	_	_	_

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
39	P8 5 cm	<LoD	<LoD	_	_	_	_	_
40	U8 5 cm	<LoD	<LoD	_	_	_	_	_
41	U8 10 cm	<LoD	<LoD	_	_	_	_	_
42	U8 15 cm	<LoD	<LoD	_	_	_	_	_
43	D8 5 cm	<LoD	<LoD	_	_	_	_	_
44	D8 10 cm	1296	1114	0.57	0.54	0.56	0.02	4.27
45	D8 15 cm	<LoD	<LoD	_	_	_	_	_

Supplementary Information 7a: Levels (ng/g) of dexamethasone in sediments during summer

S/N	Sampling Sites	Peak Area 1	Peak Area 2	conc.1 (ng/g)	conc.2 (ng/g)	Mean	S.D	%RSD
1	P1 5 cm	<LoD	<LoD	-	-	-	-	-
2	P1 10 cm	3478	3452	0.79	0.79	0.79	0.00	0.37
3	P1 15 cm	<LoD	<LoD	-	-	-	-	-
4	P2 5 cm	<LoD	<LoD	-	-	-	-	-
5	P2 10 cm	<LoD	<LoD	-	-	-	-	-
6	P2 15 cm	<LoD	<LoD	-	-	-	-	-
7	Site 3 5 cm	<LoD	<LoD	-	-	-	-	-
8	Site 3 10 cm	<LoD	<LoD	-	-	-	-	-
9	Site 3 15 cm	<LoD	<LoD	-	-	-	-	-
10	P4 5 cm	<LoD	<LoD	-	-	-	-	-
11	U4 5 cm	<LoD	<LoD	-	-	-	-	-
12	D4 5 cm	<LoD	<LoD	-	-	-	-	-
13	P5 5 cm	<LoD	<LoD	-	-	-	-	-
14	P5 10 cm	<LoD	<LoD	-	-	-	-	-
15	P5 15 cm	<LoD	<LoD	-	-	-	-	-
16	U5 5 cm	5862	5291	1.17	1.08	1.13	0.06	5.72
17	U5 10 cm	8025	9238	1.52	1.72	1.62	0.14	8.48
18	U5 15 cm	<LoD	<LoD	-	-	-	-	-
19	D5 5 cm	75858	73352	12.38	11.98	12.18	0.28	2.33
20	D5 10 cm	6996	7083	1.36	1.37	1.36	0.01	0.72
21	D5 15 cm	25625	25053	4.34	4.25	4.29	0.06	1.51
22	U6 5 cm	<LoD	<LoD	-	-	-	-	-
23	U6 10 cm	<LoD	<LoD	-	-	-	-	-
24	U6 15 cm	<LoD	<LoD	-	-	-	-	-
25	D6 5 cm	<LoD	<LoD	-	-	-	-	-
26	D6 10 cm	<LoD	<LoD	-	-	-	-	-
27	D6 15 cm	<LoD	<LoD	-	-	-	-	-
28	P7 5 cm	<LoD	<LoD	-	-	-	-	-
29	P7 10 cm	<LoD	<LoD	-	-	-	-	-
30	P7 15 cm	21014	23153	3.60	3.94	3.77	0.24	6.42
31	U7 5 cm	<LoD	<LoD	-	-	-	-	-
32	U7 10 cm	<LoD	<LoD	-	-	-	-	-
33	U7 15 cm	5789	7393	1.16	1.42	1.29	0.18	14.06

34	D7 5 cm	5392	6846	1.10	1.33	1.22	0.16	13.53
35	D7 10 cm	<LoD	<LoD	-	-	-	-	-
36	D7 15 cm	<LoD	<LoD	-	-	-	-	-
37	P8 5 cm	28351	27258	4.77	4.60	4.69	0.12	2.64
38	P8 10 cm	7762	7655	1.48	1.46	1.47	0.01	0.82
39	U8 5 cm	37287	38834	6.20	6.45	6.33	0.18	2.77
40	U8 10 cm	<LoD	<LoD	-	-	-	-	-
41	U8 15 cm	<LoD	<LoD	-	-	-	-	-
42	D8 5 cm	<LoD	<LoD	-	-	-	-	-
43	D8 10 cm	<LoD	<LoD	-	-	-	-	-
44	D8 15 cm	<LoD	<LoD	-	-	-	-	-

Supplementary Information 7b: Levels (ng/g) of dexamethasone in sediments during winter

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1 5 cm	<LoD	<LoD	_	_	_	_	_
2	P1 10 cm	<LoD	<LoD	_	_	_	_	_
3	P1 15 cm	<LoD	<LoD	_	_	_	_	_
4	P2 5 cm	<LoD	<LoD	_	_	_	_	_
5	P2 10 cm	<LoD	<LoD	_	_	_	_	_
6	P2 15 cm	<LoD	<LoD	_	_	_	_	_
7	P3 5 cm	<LoD	<LoD	_	_	_	_	_
8	P3 10 cm	<LoD	<LoD	_	_	_	_	_
9	P3 15 cm	<LoD	<LoD	_	_	_	_	_
10	P4 5 cm	<LoD	<LoD	_	_	_	_	_
11	P4 10 cm	<LoD	<LoD	_	_	_	_	_
12	U4 5 cm	<LoD	<LoD	_	_	_	_	_
13	U4 10 cm	<LoD	<LoD	_	_	_	_	_
14	D4 5 cm	<LoD	<LoD	_	_	_	_	_

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
15	P5 5 cm	<LoD	<LoD	_	_	_	_	_
16	P5 10 cm	<LoD	<LoD	_	_	_	_	_
17	P5 15 cm	<LoD	<LoD	_	_	_	_	_
18	U5 5 cm	<LoD	<LoD	_	_	_	_	_
19	U5 10 cm	<LoD	<LoD	_	_	_	_	_
20	U5 15 cm	<LoD	<LoD	_	_	_	_	_
21	P6 5 cm	<LoD	<LoD	_	_	_	_	_
22	P6 10 cm	<LoD	<LoD	_	_	_	_	_
23	P6 15 cm	<LoD	<LoD	_	_	_	_	_
24	U6 5 cm	<LoD	<LoD	_	_	_	_	_
25	U6 10 cm	<LoD	<LoD	_	_	_	_	_
26	U6 15 cm	<LoD	<LoD	_	_	_	_	_
27	D6 5 cm	<LoD	<LoD	_	_	_	_	_
28	D6 10 cm	<LoD	<LoD	_	_	_	_	_
29	D6 15 cm	<LoD	<LoD	_	_	_	_	_
30	P7 5 cm	<LoD	<LoD	_	_	_	_	_
31	P7 10 cm	<LoD	<LoD	_	_	_	_	_
32	P7 15 cm	<LoD	<LoD	_	_	_	_	_
33	U7 5 cm	<LoD	<LoD	_	_	_	_	_
34	U7 10 cm	<LoD	<LoD	_	_	_	_	_
35	U7 15 cm	<LoD	<LoD	_	_	_	_	_
36	D7 5 cm	<LoD	<LoD	_	_	_	_	_
37	D7 10 cm	<LoD	<LoD	_	_	_	_	_
38	D7 15 cm	<LoD	<LoD	_	_	_	_	_
39	P8 5 cm	<LoD	<LoD	_	_	_	_	_

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
40	U8 5 cm	25185	25542	4.88	4.95	4.91	0.05	1.01
41	U8 10 cm	<LoD	<LoD	_	_	_	_	_
42	U8 15 cm	<LoD	<LoD	_	_	_	_	_
43	D8 5 cm	<LoD	<LoD	_	_	_	_	_
44	D8 10 cm	<LoD	<LoD	_	_	_	_	_
45	D8 15 cm	<LoD	<LoD	_	_	_	_	_

Supplementary Information 8a: Levels (ng/g) of prednisone in sediments during summer

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1 5 cm	5624	6080	0.99	1.06	1.02	0.05	4.90
2	P1 10 cm	<LoD	<LoD	-	-	-	-	-
3	P1 15 cm	<LoD	<LoD	-	-	-	-	-
4	P2 5 cm	<LoD	<LoD	-	-	-	-	-
5	P2 10 cm	<LoD	<LoD	-	-	-	-	-
6	P2 15 cm	7725	6572	1.31	1.13	1.22	0.13	10.36
7	Site 3 5 cm	3193	2913	0.61	0.56	0.59	0.03	5.24
8	Site 3 10 cm	<LoD	<LoD	-	-	-	-	-
9	Site 3 15 cm	<LoD	<LoD	-	-	-	-	-
10	P4 5 cm	<LoD	<LoD	-	-	-	-	-
11	U4 5 cm	<LoD	<LoD	-	-	-	-	-
12	D4 5 cm	<LoD	<LoD	-	-	-	-	-
13	P5 5 cm	<LoD	<LoD	-	-	-	-	-
14	P5 10 cm	<LoD	<LoD	-	-	-	-	-
15	P5 15 cm	<LoD	<LoD	-	-	-	-	-
16	U5 5 cm	4638	4892	0.83	0.87	0.85	0.03	3.27
17	U5 10 cm	<LoD	<LoD	-	-	-	-	-
18	U5 15 cm	<LoD	<LoD	-	-	-	-	-
19	D5 5 cm	<LoD	<LoD	-	-	-	-	-
20	D5 10 cm	<LoD	<LoD	-	-	-	-	-
21	D5 15 cm	<LoD	<LoD	-	-	-	-	-

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
22	U6 5 cm	<LoD	<LoD	-	-	-	-	-
23	U6 10 cm	<LoD	<LoD	-	-	-	-	-
24	U6 15 cm	<LoD	<LoD	-	-	-	-	-
25	D6 5 cm	<LoD	<LoD	-	-	-	-	-
26	D6 10 cm	<LoD	<LoD	-	-	-	-	-
27	D6 15 cm	<LoD	<LoD	-	-	-	-	-
28	P7 5 cm	<LoD	<LoD	-	-	-	-	-
29	P7 10 cm	<LoD	<LoD	-	-	-	-	-
30	P7 15 cm	1842	2010	0.40	0.42	0.41	0.02	4.48
31	U7 5 cm	8620	8485	1.45	1.43	1.44	0.01	1.03
32	U7 10 cm	<LoD	<LoD	-	-	-	-	-
33	U7 15 cm	<LoD	<LoD	-	-	-	-	-
34	D7 5 cm	<LoD	<LoD	-	-	-	-	-
35	D7 10 cm	<LoD	<LoD	-	-	-	-	-
36	D7 15 cm	<LoD	<LoD	-	-	-	-	-
37	P8 5 cm	<LoD	<LoD	-	-	-	-	-
38	P8 10 cm	<LoD	<LoD	-	-	-	-	-
39	U8 5 cm	12043	14276	1.98	2.33	2.16	0.25	11.37
40	U8 10 cm	<LoD	<LoD	-	-	-	-	-
41	U8 15 cm	<LoD	<LoD	-	-	-	-	-
42	D8 5 cm	<LoD	<LoD	-	-	-	-	-
43	D8 10 cm	<LoD	<LoD	-	-	-	-	-
44	D8 15 cm	<LoD	<LoD	-	-	-	-	-

Supplementary Information 8b: Levels (ng/g) of prednisone in sediments during winter

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
1	P1 5 cm	<LoD	<LoD	_	_	_	_	_
2	P1 10 cm	<LoD	<LoD	_	_	_	_	_
3	P1 15 cm	<LoD	<LoD	_	_	_	_	_
4	P2 5 cm	<LoD	<LoD	_	_	_	_	_

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
5	P2 10 cm	<LoD	<LoD	_	_	_	_	_
6	P2 15 cm	<LoD	<LoD	_	_	_	_	_
7	P3 5 cm	<LoD	<LoD	_	_	_	_	_
8	P3 10 cm	<LoD	<LoD	_	_	_	_	_
9	P3 15 cm	<LoD	<LoD	_	_	_	_	_
10	P4 5 cm	<LoD	<LoD	_	_	_	_	_
11	P4 10 cm	<LoD	<LoD	_	_	_	_	_
12	U4 5 cm	<LoD	<LoD	_	_	_	_	_
13	U4 10 cm	<LoD	<LoD	_	_	_	_	_
14	D4 5 cm	<LoD	<LoD	_	_	_	_	_
15	P5 5 cm	<LoD	<LoD	_	_	_	_	_
16	P5 10 cm	<LoD	<LoD	_	_	_	_	_
17	P5 15 cm	<LoD	<LoD	_	_	_	_	_
18	U5 5 cm	<LoD	<LoD	_	_	_	_	_
19	U5 10 cm	<LoD	<LoD	_	_	_	_	_
20	U5 15 cm	<LoD	<LoD	_	_	_	_	_
21	P6 5 cm	<LoD	<LoD	_	_	_	_	_
22	P6 10 cm	<LoD	<LoD	_	_	_	_	_
23	P6 15 cm	<LoD	<LoD	_	_	_	_	_
24	U6 5 cm	<LoD	<LoD	_	_	_	_	_
25	U6 10 cm	<LoD	<LoD	_	_	_	_	_
26	U6 15 cm	<LoD	<LoD	_	_	_	_	_
27	D6 5 cm	<LoD	<LoD	_	_	_	_	_
28	D6 10 cm	<LoD	<LoD	_	_	_	_	_
29	D6 15 cm	<LoD	<LoD	_	_	_	_	_

S/N	Sampling Sites	Peak Area 1	Peak Area 2	Conc 1	Conc 2	Mean	S.D	%RSD
30	P7 5 cm	<LoD	<LoD	_	_	_	_	_
31	P7 10 cm	<LoD	<LoD	_	_	_	_	_
32	P7 15 cm	<LoD	<LoD	_	_	_	_	_
33	U7 5 cm	<LoD	<LoD	_	_	_	_	_
34	U7 10 cm	<LoD	<LoD	_	_	_	_	_
35	U7 15 cm	<LoD	<LoD	_	_	_	_	_
36	D7 5 cm	<LoD	<LoD	_	_	_	_	_
37	D7 10 cm	<LoD	<LoD	_	_	_	_	_
38	D7 15 cm	<LoD	<LoD	_	_	_	_	_
39	P8 5 cm	<LoD	<LoD	_	_	_	_	_
40	U8 5 cm	<LoD	<LoD	_	_	_	_	_
41	U8 10 cm	<LoD	<LoD	_	_	_	_	_
42	U8 15 cm	<LoD	<LoD	_	_	_	_	_
43	D8 5 cm	<LoD	<LoD	_	_	_	_	_
44	D8 10 cm	<LoD	<LoD	_	_	_	_	_
45	D8 15 cm	<LoD	<LoD	_	_	_	_	_

Supplementary Information 9a: Water physicochemical properties for summer sampling

Summer (Sampling was done from 12 to 15 February 2024)

Sampling sites	Name of WWTP	Coordinates	Sampling Date/ Time	River system into which effluent is discharged	Point sampled	Ph	Temperature (°C)	EC (µS/cm)	Redox
Site 1	Gariep Dam WWTP	30°35'7.56"S and 25°30'54.62"E	12/02 @ 12:00	Effluent is channelled into a farm Dam which then flows into a wetland and then into the Upper Orange System	PoD	8.06	37.4	1414	107.4

Site 2	Gariep Dam	30°35’11.41"S and 25°29’11.45"E	12/02 @18:30pm	Section of the Upper Orange System	PoD	8.55	26.2	200.1	50.8
Site 3	Aliwal North	30°41’12.71"S and 26°41’39.43"E,	13/02 @7:20am	Upper Orange River System	N/A	8.00	23.5	773	92
Site 4	Zastron WWTP	30°17’57.91"S and 27°6’43.95"E	13/02 @ 11:04am	Effluent falls into the Caledon River System	U.stm	7.97	23.5	773	-0.8
					Do.stm	8.13	22.7	775	-22.5
Site 5	Ladybrand WWTP	29°11’12.81"S and 27°29’7.82"E	14:02 @ 7:00am	Effluent falls into a wetland, which later seeps into the Caledon River System	U.stm	8.15	19.8	585	108.8
					PoD	8.13	22.7	775	-101
					Do.stm	8.07	20.2	613	23.1
Site 6	Maseru Thetsane River)	29°18’53.23"S and 27°28’2.11"E	14/0 @ 2:00pm		U.strm	9.23	30.1	184.7	4
					D.strm	9.13	29.2	198.2	34.2
Site 7	Ficksburg Sewage Work Final Effluent	28°53’26.53"S and 27°53’45.73"E	15/02 @ 10:00 am	Effluent falls into the Caledon River System	U.strm	8.67		203.0	101.4
					PoD	8.48		348	120.7
					D.strm	8.55		228	82.1
Site 8	Clarens WWTP	28°31’17.23"S and 28°25’50.80"E	15/02 @ 2:00pm	Effluent falls into the Little Caledon River System	U.strm	8.17	21.4	636	84.8
					PoD	7.79	25.9	663	-137.7
					D.strm	7.4	25.9	649	-48.3

Supplementary Information 9b: Winter Sampling (Sampling was done from 29 April to 2 May 2024)

Sampling sites	Name of WWTP	Coordinates	Sampling Date/ Time	River system into which effluent is discharged	Point sampled	Ph	Temperature (°C)	EC (µS/cm)	Redox	
Site 1	Gariep Dam WWTP	30°35'7.56"S and 25°30'54.62"E	30/04 @ 7:50 am	Effluent is channelled into a farm Dam which then flows into a wetland and then into the Upper Orange System	PoD	8,13	37.4	702	-77,7	0,93
Site 2	Gariep Dam	30°35'11.41"S and 25°29'11.45"E	30/04 @ 8:13 am	Section of the Upper Orange System	PoD	8.55	26.2	200.1	89,3	7,73
Site 3	Aliwal North	30°41'12.71"S and 26°41'39.43"E,	29/04 @ 2:50 pm	Upper Orange River System	N/A	8.00	23.5	773	129,4	8,3
Site 4	Zastron WWTP	30°17'57.91"S and 27°6'43.95"E	29/04 @ 12;30	Effluent falls into the Caledon River System	U.stm	7.97	23.5	773	101,5	4,85
					Do.stm	8.13	22.7	775	-	5,27
Site 5	Ladybrand WWTP	29°11'12.81"S and 27°29'7.82"E	30/04 @ 12:00	Effluent falls into a wetland, which later seeps into the Caledon River System	U.stm	8.15	19.8	585	36,4	9,17
					PoD	8.13	22.7	775	11,6	5,08
					Do.stm	8.07	20.2	613	136,6	4,96
Site 6	Maseru Thetsane River)	29°18'53.23"S and 27°28'2.11"E	30/04 @ 2: 00pm		U.strm	9.23	30.1	184.7	117	11,07
					D.strm	9.13	29.2	198.2	88,6	11,26
Site 7	Ficksburg Sewage Work Final Effluent	28°53'26.53"S and 27°53'45.73"E	02/05 @10:57am	Effluent falls into the Caledon River System	U.strm	8.67		203.0	172,1	10,02
					PoD	8.48		348	153,1	9,83
					D.strm	8.55		228	182	10,55
Site 8	Clarens WWTP	28°31'17.23"S and 28°25'50.80"E	02/04 @13:25	Effluent falls into the Little Caledon River System	U.strm	8.17	21.4	636	-124,2	8,04
					PoD	7.79	25.9	663	-201,6	0,99
					D.strm	7.4	25.9	649	-120	1

Supplementary Information 10: Navier-Stokes equations

The Navier-Stokes equations consist of a time-dependent continuity equation for conservation of mass, three time-dependent conservation of momentum equations and a time-dependent conservation of energy equation.

There are four independent variables in the problem: the x, y, and z spatial coordinates of some domain, and the time t. There are six dependent variables: the pressure p, density rho (ρ), and temperature T (which is contained in the energy equation through the total energy E_t) and three components of the velocity vector; the u component is in the x direction, the v component is in the y direction, and the w component is in the z direction. All of the dependent variables are functions of all four independent variables. The differential equations are therefore partial differential equations and not the ordinary differential equations that are studied in a beginning calculus class. The partial derivative indicates that we are to hold all the independent variables fixed, except the variable next to the symbol, when computing a derivative. The set of equations is:

Continuity: $\partial\rho/\partial t+\partial(\rho u)/\partial x+\partial(\rho v)/\partial y+\partial(\rho w)/\partial z=0$

X-momentum:

$$\partial(\rho u)/\partial t+\partial(\rho u^2)/\partial x+\partial(\rho uv)/\partial y+\partial(\rho uw)/\partial z = -\partial\rho/\partial x+1/Re_r[\partial\tau_{xx}/\partial x+\partial\tau_{xy}/\partial y+\partial\tau_{xz}/\partial z]$$

Y-momentum:

$$\partial(\rho v)/\partial t+\partial(\rho uv)/\partial x+\partial(\rho v2)/\partial y+\partial(\rho uw)/\partial z = -\partial\rho/\partial y+1/Re_r[\partial\tau_{xy}/\partial x+\partial\tau_{yy}/\partial y+\partial\tau_{yz}/\partial z]$$

Z-momentum:

$$\partial(\rho w)/\partial t+\partial(\rho uw)/\partial x+\partial(\rho uw)/\partial y+\partial(\rho w2)/\partial z = -\partial\rho/\partial z+1/Re_r[\partial\tau_{xz}/\partial x+\partial\tau_{yz}/\partial y+\partial\tau_{zz}/\partial z]$$

Energy:

$$\partial(Et)/\partial t+\partial(uEt)/\partial x+\partial(vEt)/\partial y+\partial(wEt)/\partial z=-\partial(up)/\partial x-\partial(vp)/\partial y-\partial(wp)/\partial z-1/Re_rPr_r[\partial qx/\partial x+\partial qy/\partial y+\partial qz/\partial z]+1/Re_r[\partial/\partial x(u\tau_{xx}+v\tau_{xy}+w\tau_{xz})+\partial/\partial y(u\tau_{xy}+v\tau_{yy}+w\tau_{yz})+\partial/\partial z(u\tau_{xz}+v\tau_{yz}+w\tau_{zz})]$$

where Re is the Reynolds number, which is a similarity parameter that is the ratio of the scaling of the inertia of the flow to the viscous forces in the flow. The q variables are the heat flux components, and P_r is the Prandtl number, which is a similar parameter, which is the ratio of the viscous stresses to the thermal stresses. The tau (τ) variables are components of the stress tensor. A tensor is generated when two vectors are multiplied in a certain way. Our velocity vector had three components; the stress tensor had nine components. Each component of the stress tensor is itself a second derivative of the velocity components.