MICROPLASTICS AS POTENTIAL VECTORS FOR SELECTED ORGANIC CHEMICAL POLLUTANTS IN RIVER ECOSYSTEMS

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by

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DECLARATION

I declare that the thesis entitled ***Microplastics as Potential Vectors for Selected Organic Chemical Pollutants in River Ecosystems*** which I hereby submit for the degree of Doctor of Philosophy at Rhodes University is my own work. I also declare that this thesis has not previously been submitted by me for a degree at this or any other tertiary institution and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

Tumwesigye

Edgar TUMWESIGYE
21st June, 2024.

DEDICATION

This work is dedicated to my dear wife Mrs Sheila T. Tumwesigye and my beloved children Rodney Taremwa Tumwesigye, Randy Tasingurwa Tumwesigye, Nimurungi Ivy Deamah, Murungi Ashley Tessa, and Tashobya Ryley Tumwesigye who missed my full-time attention and company during my study period.

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To God: Thank you for carrying me through this journey! 1 Chronicles 16:34: *"Give thanks to the LORD, for he is good; his love endures forever."*

ABSTRACT

Microplastics (MPs) as pollutants in river ecosystems have received considerable research attention in recent years. However, in Africa, research on MPs is sparse, and more needs to be done. Empirical evidence suggests that MP can act as vectors of organic chemical pollutants due to their diverse functional groups and other physical-chemical properties, such as their small sizes, crystal structure and porosity. MPs acting as vectors of chemical pollutants, adds to the complexity of understanding the risk posed to both the ecosystem and human health. Regarding the so-called vector effect, the role of seasonality, land use type, adsorption kinetics, and MP properties has yet to receive the necessary research in the literature, especially concerning pharmaceutical active compounds and other organic pollutants in river systems. This is particularly true for Africa, including South Africa.

This study, therefore, aimed to fill these existing research gaps. Overall, the study aimed to investigate the potential of selected microplastic polymers of a particular size range as vectors of organic pollutants in urban rivers within the Eastern Cape of South Africa. To achieve this aim, the study explored the influence of spatial-temporal variability, MPs particle sizes, and various physicochemical variables on the adsorption of antibiotics: Sulfamethoxazole, ciprofloxacin, and endocrine disruptors: 17β-Estradiol, 4-(2, 6-dimethyl-2-heptyl) phenol. The adsorption kinetics mechanism was also investigated and established.

Polyethylene Terephthalate (PET) and polypropylene (PP) MPs were seasonally deployed once in the summer and autumn seasons, i.e. 20th January 2022 in Bloukrans River and 21st January 2022 in Swartkops River for the summer season and 7th April 2022 in Bloukrans River and 8th April 2022 in Swartkops River for the autumn season. Deployed MPs were of two size ranges, type 1 (2 mm<-≤5 mm) and type 2 (0.5mm<-≤2 mm). The sites where the MPs were deployed had different land use practices: informal settlements, discharge points of wastewater treatment works (WWTWs), agricultural farms, and control sites, considered as the least impacted sites. This was done to analyse land use types' role in the adsorption of chemical pollutants onto MPs. MPs were retrieved in periodic intervals of 7 days, 14 days and 35days calculated based on the day of deployment for both summer and autumn seasons and analysed for Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol, 4-(2, 6-dimethyl-2-heptyl) phenol using high-resolution liquid chromatography–mass spectrometry LC-MS/MS equipped with a triple quadrupole (QqQ) analyser. Concurrent with MP retrieval water physicochemical variables: pH, dissolved oxygen (DO), temperature, turbidity, electrical conductivity (EC), total suspended solids (TSS), total dissolved solids, total alkalinity and total hardness. The adsorption kinetics mechanism was studied in the laboratory between PET and PP of two size ranges and Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol model chemical.

The results indicate that land use practices significantly impacted the concentration of the adsorbed chemicals on MPs. Sites downstream of the WWTW had higher concentrations of Sulfamethoxazole: 11119.6001±12552.4120ngL^{-1} and ciprofloxacin: 30285.19± 28783.7821ngL^{-1} adsorbed onto MPs in the Bloukrans River compared to the concentration of same compounds from other land use types along the same River catchment. Agriculturally impacted sites had higher concentrations of 17β-Estradiol on MPs; 11624.5611 ± 15382.2923ngL^{-1} and 100.3635± 29.6321ngL^{-1} in Swartkops and Bloukrans Rivers respectively compared to other sites. These results suggest that land use is an essential factor influencing chemical inputs into rivers and their adsorption onto MPs. Adsorption was higher for the MP of smaller sizes compared to MPs with bigger sizes, indicating that size is an essential factor that influences the vector effects of MPs. Adsorption was significantly higher after 35 days than all other days during the two seasons ($P< 0.05$). The adoption kinetics data fitted well with the pseudo-second-order model ($R^2> 0.99$), indicating that chemisorption mechanisms may be the rate-limiting step. Data did not fit the intraparticle diffusion model. Both film diffusion and intraparticle diffusion possibly influenced the rate-limiting adsorption step simultaneously. Regarding the relationship between adsorption and water physico-chemical variables, of special interest a positive correlation between total alkalinity, electrical conductivity, total hardness, and total suspended salts (TDS) and the concentration of the adsorbed chemicals was observed. While the relationship between adsorption and dissolved oxygen was negative. The physicochemical variables with a positive relationship with adsorption are indicative of pollution. Therefore, the result suggests that increasing pollution tends to favour higher adsorption.

The results in this study highlight the insights on i) the influence of land use on adsorption, ii) the role of exposure duration on adsorption, iii) the influence of seasonality and MP sizes on adsorption iv) relationship between water physicochemical parameters and adsorption as well as v) establishing adsorption kinetic mechanism. These findings are critical to better understanding the so-called vector effects of MPs and the management associated with MPs in river systems and form essential data sets needed in developing effective pollution mitigation strategies that are region-specific

LIST OF PUBLICATIONS

1. Tumwesigye, E., Felicitas Nnadozie, C., C Akamagwuna, F., Siwe Noundou, X., William Nyakairu, G., & Odume, O. N. (2023). Microplastics as vectors of chemical contaminants and biological agents in freshwater ecosystems: Current knowledge status and future perspectives. *Environmental Pollution* https://doi.org/10.1016/j.envpol.2023.121829
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ABBREVIATIONS AND ACRONYMS

μ - FTIR	micro (microscope) Fourier transform interferometer
ANOVA	Analysis of variance
$C_{18}H_{24}O$	4-(2, 6-dimethyl-2-heptyl) Phenol
CEC	Contaminants of emerging concern
CIP	Ciprofloxacin
DDE	Dichlorodiphenyldichloroethane
DDT	Dichlorodiphenyltrichloroethane
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
E2	17β-Estradiol
EC	Electrical conductivity
EXAFS	Extended X-ray absorption fine structure
FPMT	Film-pore mass transfer
FTIR-ATR	Fourier transform infrared spectroscopy – attenuated total reflectance.
GC-ECD	Gas chromatography-electron capture detector
GC-MS	Gas chromatography-mass spectrometry
GRC	Grahamstown Recycling Centre
HCAs	Hazardous chemical agents
DO	Dissolved oxygen.
HDPE	High density polyethylene
HOCs	Hydrophobic Organic Chemicals
HOP	Hydrophobic Organic Pollutants.
HPLC	High Performance Liquid Chromatography
ICP-OES	Inductively coupled plasma optical emission spectroscopy.
Kd	Distribution coefficient
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LDPE	low density polyethylene
LLDPE	linear-low-density polyethene
LOD	Limit of Detection
logKow	n-octanol-water partition coefficient
LOQ	Limit of Quantification
MDPE	Medium-density polyethene
MPs	Microplastics

NSAIDS	non-steroidal anti-inflammatorydrugs
OCP	OrganochlorinePesticides
PAHs	PolycyclicAromaticHydrocarbons
PBDEs	PolybrominatedDiphenylEthers
PBTs	Persistent Bioaccumulative and Toxic Compounds
PCA	Principal Component analysis.
PCBs	Polychlorinated Biphenyls
PEC	Predicted Exposure Concentration.
PET	Polyethylene Terephthalate
PFOM	Pseudo-first-order model
PMMA	Poly-methyl methacrylate
POPs	Persistent Organic Pollutants
PP	Polypropylene
PRP	Propranolol
PSOM	Pseudo-second-order model
PVC	Polyvinyl Chloride
RDM	Resources Directed Measures
RPM	Revolutions per minute
SEM-EDX/EDS	Scanning electron microscopy-energy dispersive X-ray analysis
SER	Sertraline
SPFD	Strategic plastic framework directive
SXM	Sulfamethoxazole
TDS	Total Dissolved Solids
TEM	Transmission Electron Microscopy
TSS	Total Suspended Solids
US-EPA	United States Environmental Protection Agency
WWTP	Wastewater Treatment Plant

CHAPTER 1: General Introduction and Literature Review

1.1 Introduction

The world faces increasing complexity of river pollution and its effects. The causes of river ecosystem pollution are complex and often interrelated and include a growing human population, expanding informal settlements, particularly in the developing world, increasing industrialisation, agricultural expansion, and climate change(Liyanage & Yamada, 2017; Döll et al., 2018; Gozlan et al., 2019; Liang et al., 2019). Although the global community has come together to address legacy pollutants, such as persistent organic pollutants (POPs), through the Stockholm Convention and mercury pollution through the Minamata Convention, new water contaminants of emerging concern (CEC) have arisen (Bouwman et al. 2018), one of which are microplastics (MPs) (Wang et al., 2018; Atugoda et al., 2021; Thiele et al., 2021).

Microplastics are understood to be plastic particles ranging from 1µm–5000 µm in size and have been found in aquatic, terrestrial, and air systems (Frias & Nash, 2019; Qiao et al., 2019; Campanale et al., 2020; Wang et al., 2020; Yang et al., 2020). Microplastics are the product of the fragmentation and weathering of plastics, usually categorised as secondary MPs, which accumulate in aquatic ecosystems together with primary MPs (Li et al., 2021). Further successive fragmentation and weathering of these MPs usually produce smaller plastics categorised as secondary nano-plastics (NPs) (Li et al., 2021). The unsustainable use of plastic material and their indiscriminate disposal are the primary reasons for the proliferation of plastics in the environment through atmospheric deposition and direct inputs from surface runoffs, among others (Llorca et al., 2014; Law, 2017; Wang et al., 2018). Regardless of the source, the entry into and durability of MP particles in aquatic environments, especially freshwater systems, coupled with suspected associated human health effects, has recently become a topic of discussion (Yang et al., 2020; Fabra et al., 2021).

The minute sizes of microplastics render them practically and economically unfeasible to recapture them from aquatic ecosystems for recycling or disposal (Fabra et al., 2021), and MP particles have become ubiquitous in water environments. Studies have found MPs in remote habitats, such as Oceanic islands, Antarctic currents, the deep seafloor of the Arctic, and polar sea ice caps, with the highest concentration reportedly observed in heavily urbanised and industrialised waterways (Wang et al., 2018; Kanhai et al., 2018, 2020).

As a result of their physical and chemical properties and behaviour, for example, their small particle sizes and large surface area, MPs can adsorb/absorb other hydrophobic pollutants from the surrounding water systems, including POPs, pharmaceuticals, and heavy metals (Atugoda et al., 2021; Puckowski et al., 2021; Santos et al., 2021), and act as vectors for these chemicals. The interaction between MPs and hydrophobic pollutants can change MPs' chemical composition and biological toxicity. Studies have indicated that chemicals adsorbed onto MP surfaces become concentrated and have been estimated to be in orders of 10^5 to 10^6 magnitude greater than the concentrations in the ambient freshwater environments (Mato et al., 2001; Ziccardi et al., 2016; Wang et al., 2018). The observation that chemicals tend to concentrate on MP surfaces raises severe ecological and human health risks. Thus, there is a need for studies that investigate MPs as potential vectors of chemical pollutants. It is, however, essential to note that the significance of the vector effects of MPs still needs to be more conclusive. For example, research studies by Gouin et al. (2011) and Beckingham and Ghosh (2017) concluded that the transfer of organic pollutants into biological organisms by dietary MPs may be small compared to other natural routes of exposure to environmental chemicals. These uncertainties further demonstrate the need for studies focusing on understanding how MPs act as vectors of chemicals and the potential risk posed to humans and the environment.

Whereas there is an overwhelming number of studies on microplastic pollution in the marine environment, there is limited information on microplastics in freshwater environments, especially river systems, although this is rapidly changing as more studies on freshwater are becoming available (Atugoda et al., 2021; Puckowski et al., 2021; Talbot & Chang, 2022). In response to the recent developments in microplastic research, countries with developing economies, including South Africa, have initiated several studies to improve their understanding of microplastic-associated risks in freshwater systems (Bouwman et al., 2018). The interest in microplastics in the environment is motivated by the unknown human health risks, possibly due to the ubiquity of microplastics in aquatic systems (Wright & Kelly, 2017; Koelmans et al., 2019). Interpreting a growing knowledge base of microplastics in marine systems consistently indicates actual and potential risks at many levels (Kirstein et al., 2016; Y. Liu et al., 2021). As freshwater serves as one of the resource pillars of an economy and is a primary human need, it is necessary to assess the threats to this resource (Owowenu et al., 2023)

Therefore, this study aimed to investigate microplastics as vectors of emerging chemical pollutants–selected antibiotics and endocrine-disrupting compounds in river ecosystems, including examining the adsorption kinetics.

The subsequent part of this chapter consists of the literature review that begins with a review of MPs in river systems and then proceeds to review sources and transport pathways of MPs in river systems. The chapter concludes with a review of MPs as vectors of chemical contaminants, the rationale and significance of the project, the aim and objectives of the project and finally, a structural outline of this thesis.

1.2 Microplastics in Riverine Systems

The accumulation and effects of MPs in riverine systems, like much of all freshwater ecosystems, are much less studied than MPs in marine ecosystems (Eerkes-Medrano et al., 2015; Lambert et al., 2018; Alfonso et al., 2021; Talbot & Chang, 2022). By 2018, only 4% of microplastic research studies were associated with freshwater ecosystems, including riverine systems (Lambert et al., 2018.). There is a need to expand MP research in rivers because rivers are known pathways for transporting plastic fragments (Shruti et al., 2019; Y. Liu et al., 2020) and their associated contaminants into the marine environments (Rodrigues et al., 2018; Hu et al., 2020; Yin et al., 2020). Recent riverine plastic particle flux calculations indicate that MP concentration may exceed previous estimates (Zhao et al., 2019; Huang et al., 2020) due to increased land-based plastic sources. We can only fully comprehend the effects and risks of MP abundance and the associated contaminants in ocean waters if we also understand the transportation pathways, especially in low-order water systems (Talbot & Chang, 2022).

Riverine microplastics, like marine microplastics, enter the river ecosystems from various sources by several pathways (Klein et al., 2018). The sources of both primary and secondary MPs in one region may be different from another, depending on differences in land-use types and, particularly, waste management capabilities; for example, in affluent regions, primary microplastics used in consumer cosmetics are the most significant source of MPs (Lambert et al., 2013a). Other potential environmental sources of primary MPs include i) wastewater treatment works (WWTWs), responsible especially for personal care products (Kruger et al., 2015), and fibres from textiles released during the washing of clothes; ii) biosolids from WWTWs onto agricultural lands; iii) atmospheric deposition of fibres; iv) plastic mulch film used for crop production, which is an essential source of plastic particles in agriculture soils

(Dris et al., 2017). Secondary MPs are generated through the effects of various processes, which can be physical (weather, temperature, mechanical forces), biological (bacteria, photo-degradation (UV-light), fungi, algae), and lastly, through chemical degradation via oxidation of plastic wastes mismanaged and left in the open environment (Kittipongvises et al., 2022). Successive precipitation events wash the MP residues and the disintegrated particles into riverine ecosystems, where they can accumulate (Xu et al., 2006).

Once in riverine systems, the degradation processes of MPs are mainly influenced by MP polymer properties (Table 1.1), such as density, crystallinity and the type of chemical additives (Klein et al., 2018)which alter the physicochemical properties of the polymer, making it brittle. Subsequent mechanical degradation, which is the most important as far as plastic in the aquatic environment, breaks the plastic into smaller particles, creating nanoplastics (Lambert et al., 2013b).

Table 1.1: Crystallinity and density of common microplastic polymers.
(Chubarenko et al., 2016)

Polymer type	**Crystallinity**	**Density(gcm^{-3})**
Polypropylene	50-80%	0.83-0.85
Polyethylene -low density	45-60%	0.89-0.93
Polyethylene - high density	70-95%	0.94-0.97
Polyethylene terephthalate	High	1.29-1.45
Polyoxymethylene	70-80%	1.41
Polyvinyl chloride	High	1.38
Polystyrene	Low	1.04-1.1
Polycarbonate	Low	1.20
Polyamide	35-45%	1.12-1.14

Research studies that have investigated the abundance of MPs in riverine systems around the world include that of Sighicelli et al. (2018), who provided evidence for the Oglio and Mincio Rivers that drain the Iseo and Maggiore lakes in Italy, respectively, as essential sources and sinks of microplastic particles. In these river systems, the highest concentration of microplastics was reported in Iseo freshwater systems, with approximately 40,000 particles.km^{-2} due to sewage pollution. In Japan, 31 of 36 different sites of 29 riverine ecosystems confirmed the presence of microplastics. Kataoka et al. (2019) assessed sources and inflow processes of MPs in river environments in Japan from 2015 to 2018 with Edo River recording a mean concentration of MPs of 0.62 $mg.m^{-3}$ (April to September). This concentration was 2.7 times higher than in the October–March season, which measured 0.23 $mg.m^{-3}$. The difference in microplastic concentration was attributed to flooding and seasonal dynamics (Kataoka et al., 2019).

Wang et al. (2019) reported microplastic concentration in surface water sampled from the freshwater systems of the Yellow River basin of northern China (Ulansuhai Lake basin) ranging from 1760±710 to 10120±4090 n/m^3. This study reported that 80% of the reported MP particles characterised using a laser scanning focal microscope were less than 2 mm in size. Although these studies indicate an increase in research efforts towards MP research in rivers and other freshwater systems, there is still a paucity of data related to the abundance, spatial distribution and polymer identification of MPs in freshwater ecosystems (Koelmans et al., 2019 Rios Mendoza & Balcer, 2019; González-Pleiter et al., 2021). Table 1.2 provides examples of studies on MPs in river systems. The table does not purport to provide an exhaustive list but instead provides an indicative study to shed light on the studies focusing on MPs in rivers.

Table 1.2: Examples of studies on microplastic concentrations in riverine systems

S/N	Area/City/ Country	Sample	Identification technique	Abundant type/size range	MP concentration	Author
1.	Bloukrans River Eastern Cape, South Africa	River sediments and Chironomus *ssp*	Visual microscopy at X100 magnification	2000µm - 63µm	Water sédiments 6.3±4.3particles kg^{-1}summer, 160±139.5particles kg^{-1}winter. Chironomus *ssp* 0.37±0.44particles mg^{-1}, 1.12±1.19 particles mg^{-1}	Nel et al. (2018)
2.	Orange –Vaal River systems South Africa	Bulk water	Stereomicroscope	> 25µm	Bulk water 0.23 ±0.27 items^{-1}	Weideman et al., (2019)
3.	Wen-Rui Tang River watershed in south-east china	Water sediments	µ-FT-IR	< 300µm	28312±14996items.kg^{-1}	Wang et al. (2018)
4.	Thames River basin, UK	Water sediments	Roman spectroscopy	1mm – 4mm	Highest Conc. 66±7.7particles kg^{-1}	Horton et., (2017)
5.	Great Lakes River Tributaries USA	Surface water	Dissection microscope	0.3mm – 5mm	Max. Conc. 3.2 particles m^{-3}, Med. Conc. 1.9particles m^{-3}	Baldwin et al. (2016)
6.	River Rhine, Germany	Surface water	FTIR	300µm - <5000µm	Av. Conc. 0. 005particles.L^{-1}	Mani et al. (2015)
7.	Manas River Basin, China	Surface water	Fluorescent microscope, SEM, µ-FTIR	0.1mm – 1.0mm	21±3 - 49±3 items. L^{-1}	Wang et al. (2020)
8.	29 Japanese Rivers	Surface water	Stereoscopic microscope,	0.3mm-	1.6pieces.m^{-3}&	Kataoka et al.,

			FTIR	0.5mm	0. 44mg.m^{-3}	(2019)
9.	Bloukrans River, South Africa	Sediments	Stereo microscope	<63µm-<1000µm	Conc. range. 5.5±2.5% to 109±28.2%	Nel et al., (2019)
10.	Flemish River	Sediments	Stereomicroscope	<5 mm		Slootmaekers et al., (2019)
11.	Wei River	Water, sediments	Metallographic microscope, SEM	<5mm	3.67 – 10. 7itemL^{-1}	Ding et al. (2019)

S/N = serio number; SEM = scanning electron microscopy, µ-FTIR = micro Fourier transform infra red spectroscopy

1.3 Microplastics as vectors of chemical contaminants

Surface characteristics, chemical makeup, carbon chains, functional groups, size, shape, and crystallinity of MPs greatly influence their interaction with contaminants and subsequently act as a vector in aquatic environments (Lee et al., 2014; Q. Chen et al., 2019; Llorca et al., 2020; Luo et al., 2022). The crystallinity of MPs affects their physical properties, such as density and permeability. This, in turn, affects their hydration and swelling behaviour, which affects the accessibility of adsorption sites for contaminants (Klein & Fischer, 2019). The following subsections provide a detailed review of MPs as vectors of water's persistent organic pollutants (POPs), metals and pharmaceutical compounds.

1.3.1 Microplastics as Vectors of POPs

Persistent organic pollutants (POPs) are synthetic organic compounds resistant to environmental degradation via chemical, biological, and photolytic processes. Consequently, they persist in the environment for extended periods (Kodavanti et al., 2014). Studies have established that MPs can be adsorbents for different types of hydrophobic organic pollutants (HOP) listed in the Stockholm Convention of POPs (Llorca et al., 2020). Hydrophobic organic pollutants exhibiting polarities (expressed as log K_{ow}) in the range of 3.3 to 9 are most often reported in the literature as associated with MPs and Polychlorinated Biphenyls (PCBs)(Lee et al., 2014). Among these pollutants are perfluoroalkyl substances (PAFSs) (Llorca et al., 2018), polycyclic aromatic hydrocarbons (PAHs), hexachlorocyclohexane (HCHs), pesticides (i.a., DDT, OCP), even bisphenol analogues (BPAs) (Torres et al., 2021). Partitioning of these hydrophobic organic compounds (HOCs) between environmental waters and plastics typically follows the order LDPE ≈ HDPE ≥ PP > PVC ≈ PS (O'Connor et al., 2016).

Mato et al. (2001) reported evidence of MPs accumulating PAHs, PCBs, phthalates, and pesticides with a factor concentration of up to 10^6 times greater than the surrounding ambient waters. These MPs interact with POPs in the environment because they possess high, specific surface areas and are hydrophobic (Mato et al., 2001). Over time, these MPS support the propagation of diatoms, hydroids, filamentous algae, and tarry residues that increase the accumulation of pollutants through the vector phenomenon (Wang et al., 2016). Table 1.3 is a summary of the literature reporting chemical contaminants adsorbed onto MPs. This is not an exhaustive list.

Table 1.3: The concentrations of chemical contaminants on microplastics in different sample types in freshwater systems.

S/N	MP type	Chemical group	Contaminant type	Indicator of a vector of the MPs	Type of experiment	Sample matrix	Mechanism of interaction (when reported)	Concentration reported on MPs	References
1	PE, PS	POPs	PAHs	The concentration of affiliated PAHs	*In situ* study	water		Conc. PAHs on MPs: 3400-119,000ng. g^{-1}	Mai et al. (2018)
2	HDPE PP	POPs	PCBs, PBDEs, DDTs	The concentration of affiliated POPs	*In situ* experiment	Water		Conc. POPs on MPs (ng. g^{-1} – pellet) PBDEs 10-133, PCBs 3-60, DDTs 0.1 – 7	Pozo et al., (2020)
3	-	POPs	PAH, PCBs	The concentration of MPs - affiliated POPs	*In situ* experiment	Water		Conc. POPs on MPs (ng. g^{-1} pellet) PAH 1454-6000, PCBs 0.8-104.6	Gorman et al. (2019)
4	PE, PS, PP, PE, PVC	Pharmaceuticals	Antibiotics	K_d of POP between MP and water system	Laboratory experiment	Water	Hydrogen bonding, hydrophobic interaction, Van der Waals force,	K_d 7.36±0.257 to 756±48.0LKg^{-1}	Li et al. (2018)

							electrostatic interaction.		
5	Beached MPs, Virgin MPs	HMS	Al, Cr, Cu, Fe, Mn, Sn, Ti	The concentration of MPs - affiliated HMs	*Insitu* experiment			Conc. Fe 22.78mg.kg^{-1}, Al 45.27mg.kg^{-1}. Metal concentration on pellets collected from beaches is greater than on virgin pellets.	Vedolin et al., (2018)
6	Virgin PS, aged PVC	HMS	Cu, Zn	Concentration difference of the HM on MPs and seawater	*In situ* study	Water		[Cu] in seawater 1.67±0.29 [Cu] on PS 1100.97±273.23 ppm [Cu] on PVC 1320.72±268.60 ppm [Zn] in seawater 0.53±0.09 ppm [Zn] on PS 160.27±97.00 ppm [Zn] on PVC 102.09±21.47ppm	Brennecke et al., 2016)
7	PS, PE, PET	POP	PCBs	MPs can adsorb PCBs in a	Laboratory experiment	Sediment/ water	π-π interactions, Van der	PCBs adsorbed onto MPs between 20% to	Llorca et al., (2020)

				water/sediment system.			Waal forces	60%	
8	**PET, HDPE, PVC, LDPE**	**Pharmaceuticals**	**Atenolol, Ibuprofen, Sulfamethoxazole**	**Value of sorption coefficient (K_d) based on the mass of MPs**	***In situ* experiment**	**Water**		**The sorption coefficient on all five plastics was Approx. 0. 034$L.Kg^{-1}$ atenolol, 0. 025$L.Kg^{-1}$ ibuprofen and Sulfamethoxazole**	**Magadini et al., (2020)**

S/N= serial number; POPs = Persistent Organic Pollutants; PAHs = Polycyclic Aromatic Hydrocarbons; PCBs = Polychlorinated biphenyls. DDTs = Dichloro Diphenyl Trichloroethane; PBDEs = Polybrominated diphenyl ethers; BDE = Brominated diphenyl ether; HOCs = Hydrophobic Organic Chemicals; HMs = Heavy Metals; PE = Polyethylene or polyethene; PS = Polystyrene; PET = Polyethylene terephthalate; PVC = Polyvinyl chloride; HDPE = High density polyethylene; LDPE = low density polyethylene

Tanaka et al. (2013) measured polybrominated diphenyl ethers (PBDEs) in the abdominal adipose of 12 birds that had ingested contaminated plastic particles and in the tissues of six lantern fish and one squid (Tanaka et al., 2013). In all the birds, PBDEs were in the range 0.3–186ng/g–lipid weight based on a medium concentration of 1.5ng/g lipid, leading to the conclusion that ingestion of the contaminated plastics explained the detection of PBDEs and that the transfer of the derived chemicals to biological tissues may have occurred. Similar observations were made earlier by Teuten et al. (2009). Uptake of contaminants requires their desorption from the microplastics, which depends on diffusion and partitioning factors. Mayer et al. (2007) used a microscale diffusion mass transfer of PAHs through water, air, surfactant solution, humic acid solution, aqueous soil, and the digestive fluid of a deposit-feeding worm. In all the tested experiments, the mass transfer of PAHs was more significant through the tested media than through water. Therefore, contaminants of chemical nature adsorbed on MPs are likely to be transferred more rapidly to aquatic organisms through digestive fluids rather than water, indicative of the significance of the vector role of MPs.

1.3.2 Microplastics as vectors of metals

Metals are present in the environment from naturally occurring and anthropogenic sources (Liu et al., 2021). At elevated concentrations, metals can be highly toxic (Naqash et al., 2020). Owing to large surface-area-to-volume ratios, microplastics can act as vectors of metals, and such metals can become concentrated on the surfaces of microplastics (Brennecke et al., 2016). In Western Europe, for example, concentrations of 0.8, 1.3, 20, and 4.4μg g-1, respectively, for cadmium (Cd), chromium (Cr), lead (Pb), and antimony (Sb) were recorded on microplastic surfaces (Turner et al., 2019). In another study in south-western England, Massos and Turner (2017) detected Cd, Pb metals on surfaces of microplastics. Similar studies of adsorbed metals on microplastic surfaces have been reported from the North Atlantic region (Prunier et al., 2019), South America (Vedolin et al., 2018), and the Persian Gulf (Dobaradaran et al., 2018).

Other studies that have investigated the association of metals with MPs include Rochman et al. (2014), who measured the accumulation of nine targeted metals – Al, Cr, Mn, Fe, Co, Zn, Cd, and Pb – to five microplastics PET, HDPE, PVC, LDPE, and PP within 12 months. The results revealed that the accumulation of Cr exceeded 100 ng.g^{-1}. The study also found a complex mixture of metals on plastic debris, including those listed as priority pollutants by the United States Environmental Protection Agency (US EPA): Cd, Ni, Zn, and Pb.

The factors explaining why MPs are vectors for metals in aquatic systems include photo-oxidative weathering, increasing surface polarity, and charge (Turner & Holmes, 2015). Holmes et al. (2012) indicated that plastic pellets rapidly adsorb trace metals, presumably because of bivalent cation and oxyanion interactions with charged regions of the plastics or between neutral metal-organic complexes and hydrophobic plastic surfaces. The overall vector processes are controlled by biofilm accumulation on plastic surfaces, giving MPs greater surface area. However, the long-term dynamic change of metal load on microplastics during biofilm development remains largely unknown (Naqash et al., 2020). Several factors that affect the transport of contaminants within a polymer matrix need to be considered as they cause differences in intra-organismal adsorption and desorption behaviour (Hartmann et al., 2017; Pereao et al., 2020). Overall, the environmental-mediated risk of the behaviour of chemical contaminants associated with MPs has not been explored. Yet this association could change the density of MPs and also favour the formation of biofilms that influence how river organisms are likely to ingest microplastics (Hartmann et al., 2017).

1.3.3 Microplastics as vectors of pharmaceuticals

The extensive use of pharmaceutical products has led to their ubiquity in the aquatic environment (Oberg & Leopold, 2019; Atugoda et al., 2021). Approximately 160 pharmaceutical compounds from commonly used medication groups, such as antibiotics, anti-inflammatory drugs, and medicines for heart disease, have been detected in open surface waters (Oberg & Leopold, 2019). The co-occurrence of pharmaceutical compounds with MPs in aquatic ecosystems has led to them being adsorbed on the surfaces of MPs. Several factors regulate the sorption of pharmaceutical compounds by MPs, and these include the Kow (octanol-water partition coefficient; Ebele et al., 2017), polymeric properties of the MP, and matrix conditions, for example, salinity and dissolved organics (Atugoda et al., 2021). The interaction of MPs with pharmaceuticals and other emerging contaminants has recently been reviewed (Vieira et al., 2021). However, evidence suggests more research is needed to understand the mechanisms underpinning the interaction between MPs and pharmaceutical compounds (Atugoda et al., 2021).

Elizalde-Velázquez et al. (2020) investigated the sorption of three common non-steroidal anti-inflammatory drugs (NSAIDs) – ibuprofen, naproxen, and diclofenac – onto PE, PP, and PS. The NSAIDs were highly adsorbed onto the microplastics in acidic conditions, with PE particles exhibiting the highest affinity for all three pharmaceutical compounds and with hydrophobicity governing the interactions. J. Li et al. (2018) studied the adsorption of five antibiotics on five MPs in freshwater and seawater systems. The authors established the most substantial adsorption capacity with polyamide for the antibiotics with a distribution coefficient (Kd) value ranging from 7.36±0.257 to 756± 48.0 L kg^{-1} in freshwater systems, attributing it to the porous structure of MPs and hydrogen bonding. These results indicated that the commonly observed polyamide (PA) particles could serve as vectors of antibiotics in aquatic environments. Razanajatovo et al. (2018) researched the sorption and desorption of Sulfamethoxazole (SXM), propranolol (PRP) and sertraline (SER) (anti-depressant) β-blocker drugs by PE MPs. The concentration of the drugs was 86.78, 133.49, and 329. 97µg.g^{-1} for SXM, PRP, and SER, respectively, with an initial concentration of less than 1mg.L^{-1}. At the same time, the desorption percentages of the two hydrophobic pharmaceuticals for the PE microplastics were 8% PRP and 4% SER, respectively, after 3 hours.

Other studies that have covered the vector role of MPs for pharmaceuticals and their possible bioaccumulation include Wu et al. (2016), who investigated the adsorbed concentration of carbamazepine (anti-convulsant) on PE microplastics in µg L^{-1} and X. Liu et al. (2019)who studied the sorption of steroidal hormone 17β-estradiol (E2) on a wide range of MP types. The MPs included linear-low-density polyethene (LLDPE), LDPE, medium-density polyethene (MDPE), and poly methyl methacrylate (PMMA). They reported maximum adsorption of 1.65µg.g-1 with PVC MPs with an environmental factor of E2 on MPs of up to 10^1–10^4. The results agreed with a previous review by Mato et al. (2001) that predicted that the concentration of PCBs and DDE on plastic pellets could reach 10^5 to 10^6(Mato et al., 2001).

Because of an overwhelming number of possible combinations, the interactions between microplastics and pharmaceuticals are still debated; nonetheless, they must be addressed, as evidence of microplastics acting as sorbents and, subsequently, vectors for pharmaceutical drugs are available. What is clear is that (i) limited studies have covered MPs as vectors of pharmaceuticals; for instance, NSAIDs, a widely used class of pharmaceutical drug, is absent in the literature studies retrieved, and limited studies have considered ibuprofen, diclofenac and naproxen; (ii) the majority of studies have been carried out under laboratory conditions; few studies have used environmentally relevant conditions, and therefore, the effects of environmental conditions on sorption affinities of microplastics for these compounds remain unknown; (iii) research on sorption capacities of aged, field microplastics and microplastics made of polar polymers (PA and polyacrylic) as a function of pharmaceutical concentration are either non-existent or limited. Although elsewhere, research has begun to explore MPs as vectors of pharmaceuticals and whether these pharmaceuticals can be released into biological systems, studies currently need to be conducted in South Africa or the rest of the African continent. The current research is, therefore, the first to study the vector effect of MPs in South Africa. For these reasons, this study investigates MPs as vectors of common pharmaceutical compounds in South Africa, two antibiotics: sulfamethoxazole (SXM) and ciprofloxacin (CIP); and two endocrine-disrupting chemicals, 17β-Estradiol (E2) and 4-(2, 6-dimethyl-2-heptyl) phenol ($C_{18}H_{24}O$). These compounds have been reported as occurring in the Bloukrans and Swartkops Rivers in South Africa (Farounbi & Ngqwala, 2020; Vumazonke et al., 2020). The study further advances the knowledge in the field by examining the adsorption kinetics and the effects of seasonality and land use on the adsorption of pharmaceuticals by microplastics.

1.4 Seasonality and spatial factors of microplastics as vectors of chemical pollutants

The assessment of freshwater pollution, both seasonal and spatial, has gained significance as these characteristics are critical determinants of the distribution and abundance of microplastics and chemical pollutants in freshwater ecosystems (Stanton et al., 2020; Xia et al., 2020). For example, H. Liu et al. (2022) observed a significant elevation of MP load during the wet season (7314 ± 4640 MP/kg) relative to the dry period (4873 ± 3136 MP/kg). Spatially, the primary factors that contribute to freshwater contamination are land cover and proximity to human activities; microplastics and organic chemical pollutants usually originate from several terrestrial sources, such as wastewater treatment plants and informal settlements (Deng et al., 2020; Xia et al., 2020). Seasonality also plays a significant role in the interaction of MPs and chemical contaminants in aquatic environments (Suet et al., 2021).

Seasonal variations, specifically in aquatic environments, lead to water chemistry, algal biomass, and river water discharge variations. These factors affect the solubility of both microplastics and chemical contaminants in water systems, subsequently affecting adsorption (Suet et al., 2021). For example, elevated temperatures might render organic chemical pollutants more soluble, increasing their propensity to adhere to microplastics (Guo et al., 2018; Y. Zhang et al., 2021). Adsorption can be impacted by variations in salinity, pH, and the amount of dissolved organic matter that comes with seasonal changes (Guo et al., 2018; Suet et al., 2021). Both chemical pollutants and microplastics can enter water systems through seasonal rainfall runoff (Suet et al., 2021). In addition to their direct impact on the adsorption process, changes in seasonal rainfall can also modify the amount of dissolved organic matter in water (Mohammadi et al., 2022). The pollutants and microplastics involved, the local climate, and the kind of water system will all impact the overall effect of seasonality on the adsorption of chemical contaminants onto microplastics (Kittipongvises et al., 2022).

A study by Mohammadi et al. (2022) found that the association of MPs and Dibutyl phthalate (DBP) and Di(2-ethylhexyl) phthalate (DEHP) was impacted by seasonality. In summary, the current literature available indicates that chemical interactions play a significant role in the microplastic adsorption of pollutants because the chemical bonds on microplastics are linked to both the surrounding medium's pH and salinity, and these are altered by seasonal changes and physical properties of microplastics, colour, and age (Eder et al., 2021; Suet et al., 2021). These findings suggest that seasonality can significantly affect the adsorption of chemical contaminants onto microplastics in water ecosystems. For these reasons, the current study investigates the effects of selected plastic properties and spatial-temporal variability on the adsorption of selected pharmaceutical pollutants on microplastics.

1.5 Analytical methods for quantifying chemical contaminants associated with microplastics

Gas chromatography-mass spectrometry (GC-MS) is the commonly reported spectrometric method used to quantify OCP, DDT, hexachlorobenzene, PBDEs, PAHS, and hexachlorocyclohexane (HCHs) associated with MPs. The gas chromatography-electron capture detector (GC-ECD) detects higher-brominated PBDEs. Using these spectroscopic techniques, some authors (e.g. Frias et al., 2010; Pozo et al., 2020; Yeo et al., 2020) reported PCB concentrations sorbed on MPs in river systems in countries like Japan at 126.13 ng/g, Portugal at 14.52 ng/g, Chile at 39.90 ng/g, and Hong Kong at 159.67 ng/g as evidence for the adsorption of

PCBs onto MPs in the environment. In places like Hong Kong, PAH concentrations ranged from 0.13 ng/g to 121.13 ng/g (Lo et al., 2019). Of pesticide concentrations adsorbed, DDT seems to be the most highly adsorbed POP, with a concentration of 156.01 ng/g reported (Lo et al., 2019).

For metals associated with MPs, the most frequently used quantification techniques have been transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR) and energy-dispersive spectroscopy (EDS) (Melo-Agustín et al., 2022). These three analytical techniques combine powerful magnification, quick elemental analysis, quantitative elemental data and a raster scanning of MP surfaces (Li et al., 2020). For instance, in the North Atlantic subtropical gyre, Prunier et al. (2019) used a TEM analysis to show the presence of mineral particles on the surfaces of the plastic debris. Godoy et al. (2019) studied the sorption of the metals Cd, Co, Cu, Cr, Ni, Pb, and Zn, onto five different microplastic particles: PE, PET, PP, PS, and PVC. The study reported significant Pb, Cr, and Zn adsorption on PE and PVC, where PET showed the least adsorption capacity using energy-dispersive spectroscopy. However, in other studies, authors reportedly used other analytical techniques. For instance, Vedolin et al. (2018), using inductively coupled plasma optical emission spectroscopy (ICP-OES), investigated the adsorption for Al, Cr, Cu, Fe, Mn, Sn, Ti, and Zn on collected environmental plastic particles. The results indicated Fe ($227.78 mg.g^{-1}$) as the highest concentration, followed by Al ($45.7 mg.g^{-1}$). Guo and Wang (2019b) investigated the adsorption of Sr^{2+} onto three kinds of microplastics – PET, PE, and PVC – using a novel film-pore mass transfer (FPMT) model. The results indicated that the external mass transfer step determined the sorption of Sr^{2+} onto MPs.

1.6 Microplastics as vectors of chemical contaminants: ecological and human health risks

The main concern about MPs acting as vectors of chemical contaminants is that if MPs can adsorb/absorb chemicals from the environment, they can then transport these chemicals to other organisms, with several adverse effects (Shen et al., 2019; Arienzo et al., 2021). For example, aquatic organisms which are generalist feeders may mistake MPs for their prey because of their small size and colour (Ma et al., 2020). The adsorbed chemicals may be released into the organism's tissues, adversely affecting stress-related defence, the endocrine disruption system, cytoskeletal dynamics, and energy metabolism (Ma et al., 2020). Fish intake of MPs at a specific concentration of organic pollutants may damage the function of the endocrine system in adult fish (Rochman et al., 2014). For example, at a concentration of 20μg/l, 17α-ethylenediethylene glycol (EE2) and microplastics strongly destroy zebrafish (Chen et al., 2017). Studies have further shown that a combination of MPs and organic pollutants can bring about deoxygenation in the intestines of fish (Batel et al., 2018).

Humans can ingest MPs in several ways, such as through drinking water, as many communities, particularly across Africa, obtain their water directly from river systems(Ahmed & Wiese, 2019) and through the consumption of seafood and aquatic organisms. When the adsorbed/absorbed chemicals are released into the human body systems, they can lead to several health issues, among them cancer, issues with reproduction, and developmental delays (Mato et al., 2001; Teuten et al., 2009; Hiltunen et al., 2017).

However, current studies have mostly been laboratory studies using clean organisms exposed to contaminated MP placed in clean water, which creates conditions that promote chemical transfer to the tested organisms (Koelmans et al., 2016). Under more environmentally relevant exposure scenarios, modelling studies have suggested that the MP-exposure route for organic contaminants is negligible concerning the combined food and water intake (Koelmans et al., 2013). Furthermore, the body burden of these chemicals may decrease if they have opposing concentration gradients between plastic and biota lipids (Xu et al., 2018a). Therefore, more field experimentation is necessary where the influence of different environmental factors, including temperature, pH, salinity, dissolved organic matter (DOM) and other coexisting ions that can alter the surface properties of microplastics (e.g., surface charges or hydrophobicity) is explored about the MP-exposure route for chemical contaminants to aquatic organisms (Menéndez-Pedriza & Jaumot, 2020; Suet et al., 2021).

1.7 Rationale and significance of the study

Microplastics have been shown to act as adsorptive vectors for organic chemical pollutants in aquatic environments. The fact that microplastics could act as vectors, potentially accumulating and disseminating chemicals, poses a serious ecological and human health risk. Despite the potential risk posed by the so-called vector effects of microplastics, research that explores this area of research is mainly inconclusive, especially compared to other natural routes of exposure to chemicals in the environment (Gouin et al., 2011; Beckingham & Ghosh., 2017)

Microplastic research in quantification and analysis is now relatively well-established (Campanale et al., 2020; Yang et al., 2020). However, more needs to be done to understand how microplastic properties, seasonality, physico-chemical conditions, land use type and chemical types influence the adsorption of chemicals onto microplastics. This is particularly true in Africa, where microplastic research is emerging (Campanale et al., 2020; Owowenu et al., 2023). Investigating how microplastic properties such as size, crystallinity, shapes, etc influence adsorption is critical to understanding and managing risks posed by the so-called vector effect. Seasonality has been implicated in influencing microplastic transport, but the extent to which it influences organic chemical adsorption by microplastics remains largely unknown. Therefore, this study addresses essential research gaps about the influence of seasonality, land use types, microplastic properties and water physicochemical variables on the adsorption of organic chemical pollutants.

Like many rivers in developing countries, South African rivers such as the Swartkops and Bloukrans Rivers are experiencing intensive deterioration in water quality due to chemical pollution (Atugoda et al., 2021; Thiele et al., 2021). Chemicals include pharmaceuticals and endocrine-disrupting compounds usually originating from land-use activities, especially effluents from WWTWs, unplanned informal and urbanised human settlement activities and poorly managed agricultural practices (Department of Water Affairs, 2011). With the ubiquity of MPs in the river systems and current evidence suggesting that MPs can act as adsorbents and vectors for organic chemical pollutants, the interaction between MPs and organic chemical pollutants deserves urgent research attention, particularly in South Africa, where the risk posed by microplastics remains largely unexplored. Therefore, this study aims to investigate the interactions between microplastics and antibiotics, and endocrine disrupting compounds. This study makes an essential contribution by applying an integrated in-situ – laboratory-based approach to understand better the MPs vector role for selected organic pollutants in the Swartkops and Bloukrans River ecosystems.

1.8 Aim and Objectives

1.8.1 Aim

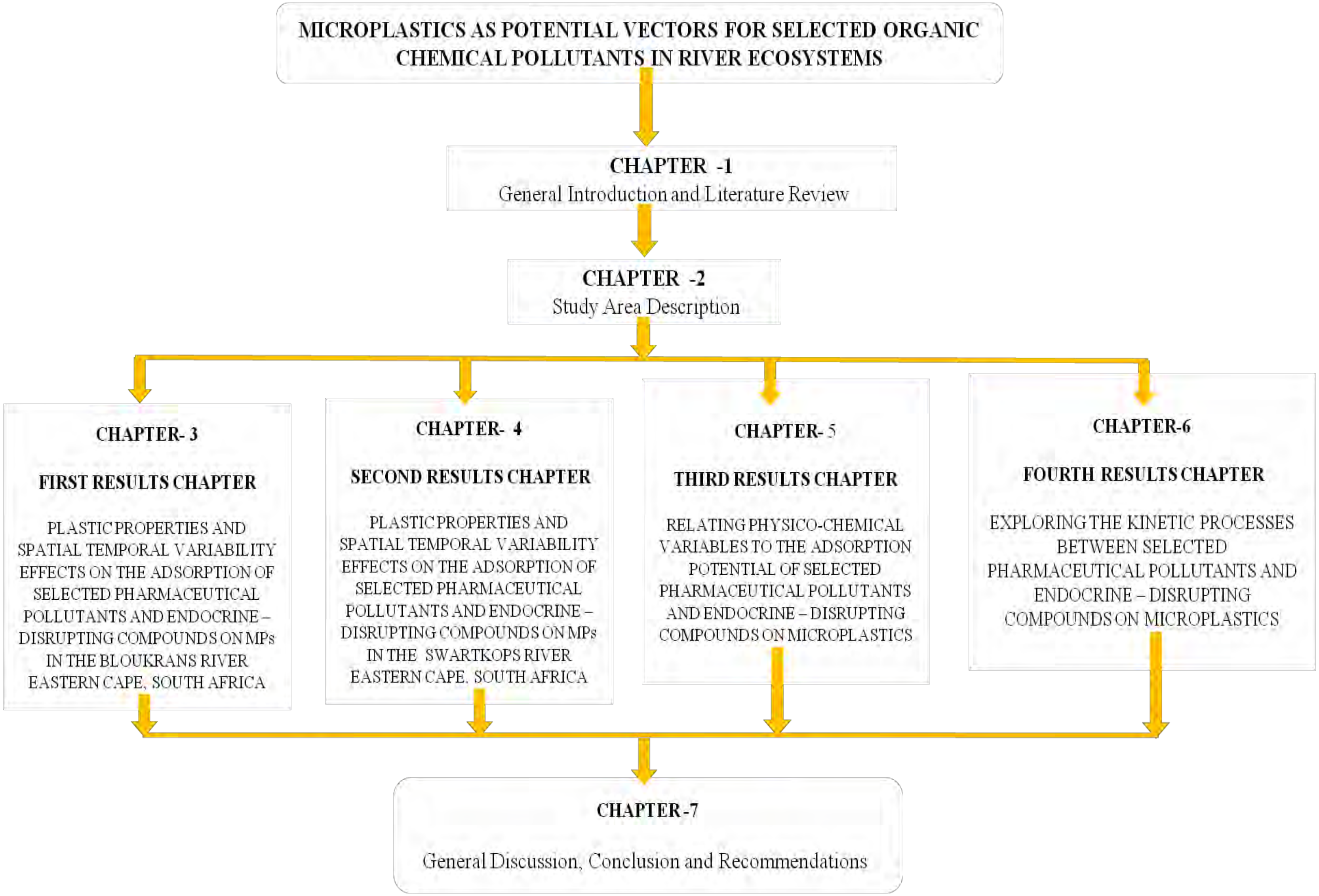
The aim of the study was to investigate microplastics as potential vectors of selected organic pollutants in the Swartkops and Bloukrans Rivers.

1.8.2 Objectives

The overall aim of this study was achieved through the following objectives:

1) To investigate the effects of selected plastic properties and spatial-temporal variability on the adsorption of selected pharmaceutical pollutants and endocrine-disrupting compounds on microplastics.
2) To determine the influence of selected physico-chemical variables on the adsorption of pharmaceutical pollutants and endocrine-disrupting compounds on microplastics.
3) To explore the kinetic adsorption processes between the selected pharmaceutical pollutants and endocrine-disrupting compounds on microplastics.

1.9 Thesis Overview



CHAPTER 2: Study Area Description

2.1 Introduction

This chapter describes the experimental study areas and provides a biophysical description of the Bloukrans and Swartkops River systems, their climate, geology and soils, vegetation, water quality, and hydrology. The selected experimental study sites with their geospatial coordinates and the selected land-use types are also described. The chapter ends with map descriptions of the experimental study sites on the two river systems.

2.2 Study Area Description

2.2.1 Bloukrans River

The Bloukrans River is a tributary of the Kowie River in the Eastern Cape Province of South Africa (Figure 2.1).The catchment area covers about 220 km^2, with a total river flow length of about 40 km. The river runs through an agricultural area characterised by irrigated crop fields and dairy farms (Dalu et al., 2019). The headwaters drain most of the town of Grahamstown/Makhanda. The Bloukrans used to be a seasonal river system but constant sewage leakages and the city's ageing and unmaintained wastewater infrastructure, it has turned into a perennial river system (Dalu et al., 2019; Mangadze et al., 2019).

2.2.2 Swartkops River

Swartkops River, also known as Zwartskops River (Afrikaans) in the Eastern Cape, falls within South Africa's most geo-hydrologically significant and most important artesian groundwater basin – the Port Elizabeth-Uitenhage artesian basin (Figure 2.1). The river flows for approximately 240 kilometres from its source in the Zuurberg Mountains to its mouth at Algoa Bay and acts as an important source of water for human use, agricultural, domestic, commercial, and industrial use in the Eastern Cape Province of South Africa (Odume et al., 2022). Pollution loading of the river derives from the Uitenhage-Dispatch-Port Elizabeth areas, from farming activities west of Swartkops-Brak River confluences, and from informal settlements (Odume et al., 2022).

2.3 Biophysical context of the Bloukrans River and Swartkops River catchments

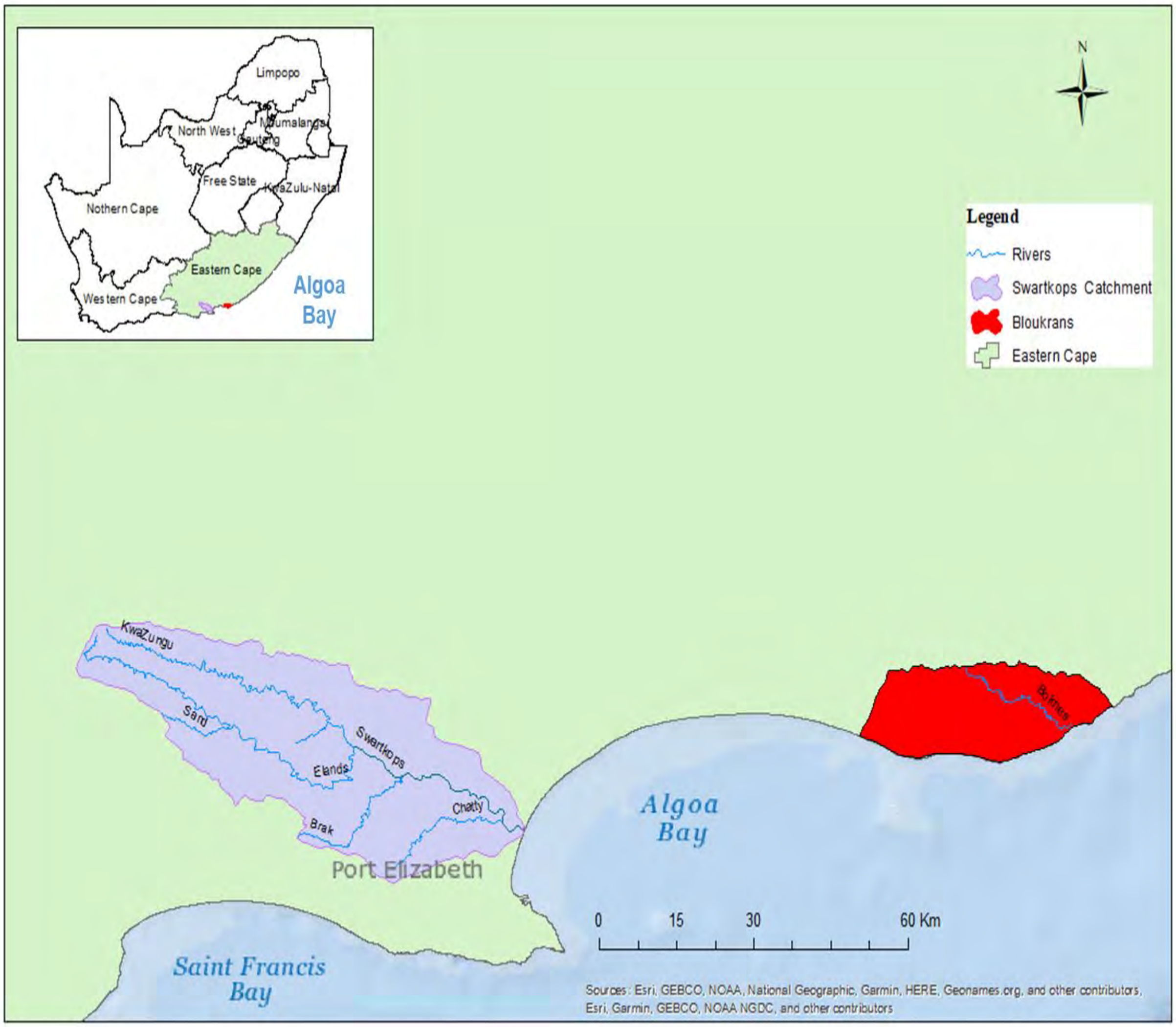


Figure 2.1: Map of South Africa (upper left) indicating the province where the study was conducted and the river catchments, the Swartkops and Bloukrans Rivers. Slate-blue for Swartkops River catchment (bottom left) and Red for Bloukrans River Catchment (bottom right).

2.3.1 Climate of Bloukrans River catchment

The river is located within the warmer temperature climate region of South Africa (Dalu et al., 2017). The air temperature ranges between 1.5 ^{o}C (minimum, winter) and 43 ^{o}C (maximum, summer); the average annual precipitation is< 650 mm, with the peak in rainfall occurring in the summer season being December to February (Dalu et al., 2017). The climatic conditions of the river catchment indicate that discarded waste in the environment could easily decompose because of favourable temperatures (South Africa Weather Services (http://www.weathersa.co.za) database: Dalu et al., 2019).

2.3.2 Geology and soils of Bloukrans River catchment

The geology of the Bloukrans River is complex and varied. The river flows through a number of different geological formations, including the Drakensberg Group, the Stormberg Group, and the Karoo Super group (Daniel, 1974). The Drakensberg Group is a group of volcanic rocks formed about 250 million years ago; the Stormberg Group consists of sedimentary rocks formed about 200 million years ago, while the Karoo Super group is a group of sedimentary rocks of about 180 million years (Rust, 1991).

The soils of the Bloukrans River are also complex and varied, reflecting the diverse geology of the area. In the upper reaches of the river the soils are mainly shallow and stony, especially towards Grahamstown, while the soils in the lower reaches are deeper and more fertile. The soils in the gorges and canyons are often very thin due to erosion by the river. The valley soils, formed from the weathering of the underlying rocks, are more fertile. The Bloukrans River soils are important for agriculture, as they support a variety of crops, including maize, wheat, pineapples and chicory grapes. They also provide grazing (animal farming), as they support a variety of grasses and other plants. Both the geology and soils of the Bloukrans River have a significant impact on the river's ecosystem(Hydrological Characeristics and Properties of Soils in Southern Africa, Report, 1985).

2.3.3 Vegetation of Bloukrans River catchment

The vegetation of the Bloukrans River in Grahamstown is diverse and includes a variety of plant species, from grasses and shrubs to trees(River Health Programme, 2007), well adapted to the river's climate and geology, which is characterised by hot, dry summers and cool, wet winters. The vegetation provides habitat for a variety of wildlife, including birds, mammals, and reptiles(Daniel, 1974).

2.3.4 Water quality and hydrology of Bloukrans River catchment

Increasing concern about the water quality of the Bloukrans River stems from pollution from agriculture, sewage, and industry surrounding the river catchment (Mangadze et al., 2019). The river is also affected by climate change, which causes more frequent, severe droughts

(Smetherham et al., 2019). The Department of Water and Sanitation (DWS) monitors the quality of the Bloukrans River for a variety of parameters, including pH (reported be on average6.5 – 8.0, which falls within acceptable range), dissolved oxygen (DO, above 5mg/l), nutrient, salinity, which is recorded as low, but reported to increase during heavy rains with increased sewage runoff from the Grahamstown area(DWA, 2012).

The hydrology of the Bloukrans River is characterised by a Mediterranean climate, with hot, dry summers and cool, wet winters. The river flow is seasonal, with the highest flows occurring in the summer months, December to January, with a mean annual runoff of approximately 100 million cubic meters (http://www.weathersa.co.za database). Human activities, such as water abstraction and dams also affect the river. Climate change, which is causing more frequent and severe droughts, so reducing the river's flow, is a major threat to the water quality and hydrology of the Bloukrans River. Extreme rainfall events, also caused by climate change, can increase pollution in the river (Smetherham et al., 2019).

2.4 Social-economic context of the Bloukrans River catchment

The Bloukrans River is located in a rural area with a low population density of more than~120,000 and an average population growth rate of 7.33% (Weaver et al., 2017).This is a relatively high growth rate and is reportedly increasing pressure on the already stretched natural resources of the area where the main economic activities along the river are agriculture and livestock grazing. Grahamstown/Makhanda is the commercial town in the catchment with many informal settlements on its outskirts (Weaver et al., 2017). Like a number of rural towns in South Africa, Grahamstown/Makhanda faces the challenge of unregulated plastic waste disposal and management that has resulted in logistical challenges in plastic waste collection (Verster & Bouwman, 2020). Approximately only 1% of households have formal waste collection at least once a week and 75.1% of households use informal refuse waste dumps because waste management services are irregular and ineffective (Rodseth et al., 2020).

Local waste recycling, key among them plastic waste recycling businesses, operate in Grahamstown; however, reportedly owing to limited capital, they operate with difficulty, neglecting the greater percentage of generated wastes. Of the waste generated in the townships and farm settlements, 75% reportedly ends in landfills which are usually washed by seasonal rain runoffs into the Bloukrans River (Bouwman et al., 2018). The river catchment faces the further challenge of faeces from the many domestic animals – dogs, cows, donkeys – that live side by side with the townspeople (Dalu et al., 2016). Although the Belmont Valley wastewater treatment works (WWTWs) along the Bloukrans River is responsible for treating the wastewater of the city, the intake exceeds the plant's capacity, and a quantity of untreated sewage is released into the Bloukrans River. Poor management of the municipal landfill has further aggravated the situation, with storm runoff and poor drainage systems reportedly containing leached-out substances and contaminants that have impacted ground water in this area (Dalu et al., 2019).

The health and functionality of the entire Bloukrans River system are threatened by refuse and waste, including plastics, from the informal, urban settlement, from illegal dumpsites around Grahamstown/Makhanda, and from the untreated wastewater effluent discharges from the Belmont wastewater treatment works, and agricultural farmlands (Smetherham et al., 2019). All these land-use activities have exerted more pressure on the already deteriorating water quality of Bloukrans River.

2.5 Biophysical context of the Swartkops River catchment

2.5.1 Climates

The average annual rainfall varies between 100 mm in the upper region (headwaters) to 500 mm on the dry north area near the coast. The river receives high rains during the summer months, December to February(Honours, 1997). The average evaporation for the catchment is approximately 1700 mm, being highest during summer months, November to March (Hamandawana et al., 2020).

2.5.2 Geology and soils

The geology of the Swartkops River catchment in South Africa is complex and varied, reflecting the region's long geological history (Roering, 1984). The catchment is located within the Cape Fold Belt which is mostly sedimentary in origin, dominated by sandstone, shales, and limestone in the river bed load in the upper reaches of the Swartkops River and its tributaries (Hamandawana et al., 2020). These areas have been subjected to a variety of geological processes, such as folding, faulting, and erosion. The central part of the Swartkops River consists mainly of Cretaceous to Jurassic Uitenhage Group deposits with river alluvium in the valley and floodplain areas. The south-central region is composed of Nanaga Formation aeolianites of the Algoa Group (Roering, 1984).

The soils of the Swartkops River catchment are also diverse, reflecting the underlying geology and climate (Roering, 1984). The dominant soil types are sandy clay loams and clay loams, soils that are typically well-drained and moderately fertile. However, there are also areas of sandy soils, which are drier, and clay soils, which tend to water logging (Maclear, 1996).

2.5.3 Vegetation

The Swartkops River supports a diverse range of vegetation, reflecting the varied ecosystems along its course(Adams et al., 2019). The upper reaches are characterised by succulent thicket with its aloes, a shrub land biome native to the Cape Floristic Region (Taylor & Morris, 1981), and known for its high biodiversity and unique plant life, with many species found nowhere else in the world. As the river flows downstream, the vegetation transitions to a mixture of grasslands, with woodlands along the riverbanks and in scattered patches that provide shade and habitat for a variety of plants and animals. Common tree species include *Vachelliakarroo*, *Combretum molle* and *Salix mucronate.* Wetlands provide important habitats for water birds and other aquatic species(Taylor & Morris, 1981).

2.5.4 Water quality and hydrology

The main pollutants affecting the Swartkops River derive from agriculture, urban and industrial runoff. Agricultural runoff is the major source of nutrients, such as nitrogen and phosphorus (Adams et al., 2019) which can cause algal blooms, depleting oxygen levels in the water and harming aquatic life. Urban runoff contains a variety of pollutants, including plastic fragments, sewage, oil, chemicals, and heavy metals. Industrial effluent can contain a variety of chemicals, some of which are toxic to aquatic life. As a result of these pollutants, the water quality of the Swartkops River is often poor (Odume et al., 2022) and the river is classified as eutrophic, meaning that it has high levels of nutrients. The river also has high levels of faecal coliform bacteria, which are an indicator of sewage pollution (Taljaard et al., 2022).

The Swartkops River is a seasonal river, with a flow that varies greatly throughout the year. The river flows at its highest during the rainy summer season, and at its lowest during the dry winter season (http://www.weathersa.co.za database). The average annual runoff of the Swartkops River is about 180 million cubic meters. However, the river is becoming increasingly stressed due to climate change and increasing water demand. As climate change reduces rainfall in the catchment area and reduces the amount of water available for the river, increasing demand for water from agriculture, industry, and domestic use is putting pressure on the river's water resources (Odume et al., 2022).

2.6 Social-economic context of the Swartkops River catchment

The Swartkops River and its surroundings form a large and diverse area encompassing a wide range of social and economic conditions(Adams et al., 2019), progressing through a variety of communities, from small rural villages to large urban settlements and serving a population of approximately 1.2 million people. The catchment is home to a variety of economic activities, including agriculture, industry, and tourism, thus the Swartkops River is categorised as an urban river (Nyawo, 2017).

The economic activities along the Swartkops River are also diverse, ranging from subsistence agriculture to large-scale industrial enterprises. One of the key social-economic issues in the Swartkops River catchment is poverty. Although the Swartkops River is an important ecological and socio-economic asset, one which provides water for small-scale irrigation, the health and functionality of the entire river system are being threatened by deteriorating water quality (Odume et al., 2022). Raw sewage from the informal settlements, agricultural farmlands, and municipal waste dumpsites, including plastic waste, are treated by three wastewater treatment works: Drift-sands Wastewater Treatment Works, Kelvin Jones Wastewater Treatment Plant, and the Fish-water Flats Wastewater Treatment Works. These treatment plants discharge wastewater effluent into the river. Approximately 4.9% of the population make use of dumpsites in their neighbourhood (Rodseth et al., 2020); an indication of the Swartkops River acting as dumpsite for both industrial and domestic chemical and plastic waste.

2.7 Sampling sites

As shown in Table 2.1, four sites in the same ecoregions differentiated by the land-use type in each of the Bloukrans and Swartkops River catchments were selected for this study.

Table 2.1: Geospatial information and land use types of the sampling site.

Site Name/Land Use	River Name	Latitude	Longitude
B1-Municipal/informal-settlement refuse area.	Bloukrans	33°18′51.84′′S	26°33′6.192′′E
B2-Wastewater treatment work	Bloukrans	33°19′5.16′′S	26°34′5.52′′E
B3-Agriculture impacted area	Bloukrans	33°19′25.32′′S	26°35′59.928′′E
B4-Control site	Palmiet	33°22′10.92′′S	26°28′32.988′′E
S1-Wastewater treatment works	Swartkops	33°47′11.4′′S	25°26′2.04′′E
S2-Municipal/informal-settlement refuse area.	Swartkops	33°47′32.28′′S	25°24′28.08′′E
S3-Agriculture impacted area	Swartkops	33°45′08.28′′S	25°20′33′′E
S4-Control site	Swartkops	33°43′47.172′′S	25°19′01.092′′E

Site B1 **(Bloukrans River –Upper reaches)**

This site is situated on the upper reaches of the Bloukrans River on the outskirts of Grahamstown/Makhanda, near the Fort England Psychiatric Hospital. The site is polluted mainly with raw sewage from the informal settlements located on the outskirts of the city, with visible sewage pipes feeding directly into the river. Solid waste, grey water and urban debris coming directly from the city were visible at the site. The surrounding vegetation on this site was mainly grasses (*Eragrostiscurvula* being the most common) and shrubs (*Rhus lancea*: tall, evergreen shrub; *Searsialeptodictyon*: a low, spreading shrub). The site was selected as an example of a highly impacted municipal-household refuse area (Figure 2.2).

***Site B2* (Bloukrans River – Wastewater works impacted site)**

This site is located downstream of the discharge point of the Belmont Valley Wastewater Treatment Works (WWTW) and receives treated wastewater effluent discharged from the municipal treatment works located in the Belmont Valley. The surrounding vegetation is mainly shrubbery. This site was selected as an example of a site impacted by wastewater treatment works (Figure 2.2)

***Site B3* (Bloukrans River – Agriculture-impacted site)**

This site is further downstream of Site 2 and is surrounded by large-scale commercial agricultural and livestock farming. An extensive irrigation system supports agricultural activities within the vicinity of the site. The main pollution source at Site 3 is run-off from the cultivation activities nearby, and run-off from the road which is the main pass-way for the cattle grazed in the surrounding farmlands (Figure 2.2). The site was selected as an example of an agriculture-impacted site.

***Site B4* (Palmiet River – control site)**

Site 4 is located in the small Palmiet River outside the town of Grahamstown/Makhanda. Although it is not located in the same Bloukrans catchment area (Figure 2.2), it was the least impacted and thus chosen as the control site. Surrounding vegetation is mainly grasses (*Hyparrheniahirta*: a tall, tufted grass), shrubs (*Eriocephalusericoides*: a small, evergreen shrub), and trees (*Combretum molle*: a medium-sized, deciduous tree).

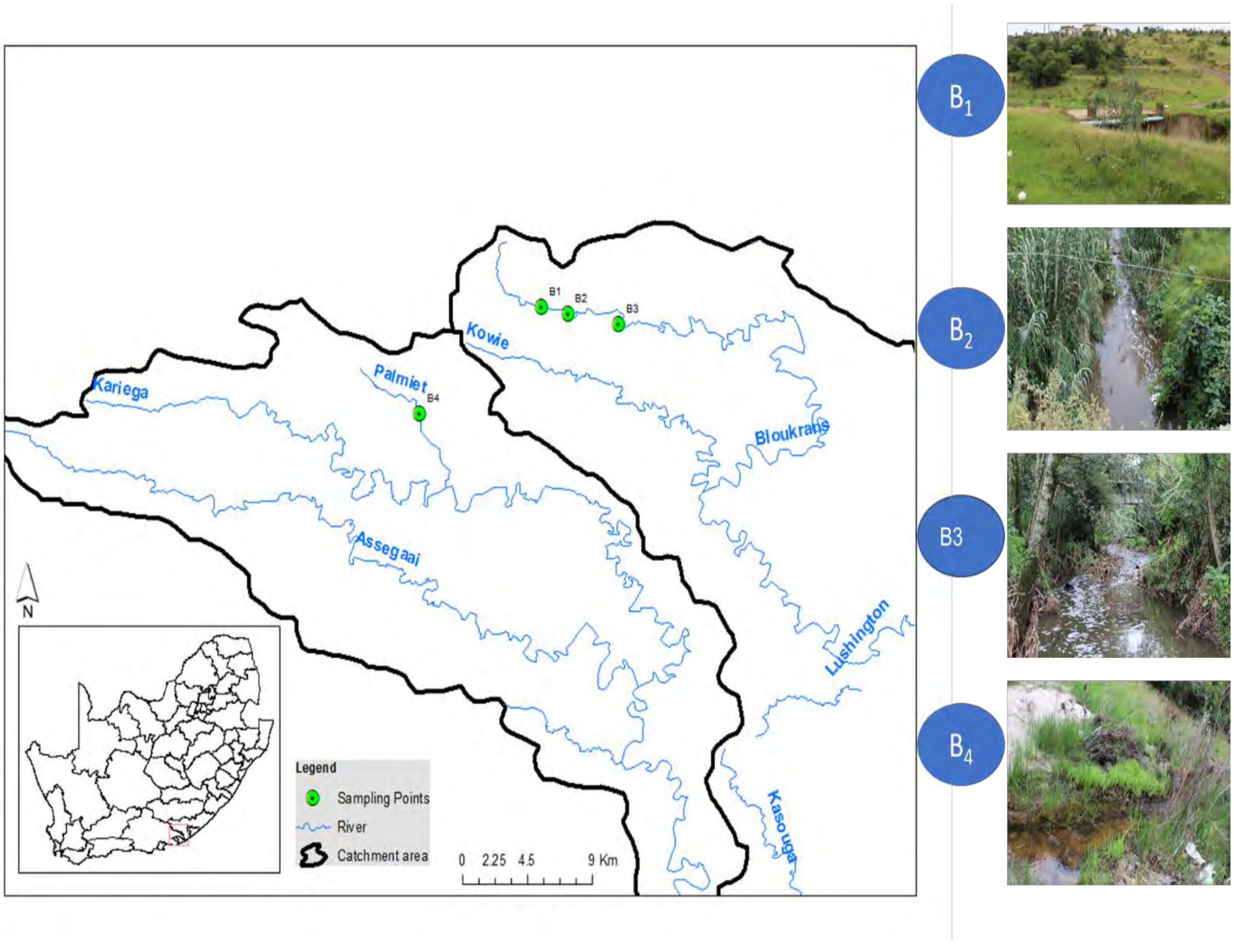


Figure 2.2: Map of Bloukrans River catchment showing the location of the experimental sampling sites along the Bloukrans and Palmiet Rivers. Site B1 (Bloukrans River upstream site of the WWTW), Site B2 (downstream of discharge point of the WWTW site), Site B3 (Bloukrans River agriculture impacted site), Site 4 (Palmiet River, control site).

Site S4 **(Swartkops River – control site)**

Site S4 was situated in the upper reaches of the river near a game reserve and, as the least impacted, was chosen as control site (Figure 2.3). The vegetation around the site was mainly characterised by fynbos, a shrub land biome (Renosterveld types), and woodland (*Vachelliakarroo* and *Combretum molle* types in the hill side).

Site S3 **(Swartkops River – Agriculture-impacted site)**

This site was located in the upper reaches of Uitenhage town, where surrounding impacts included free-ranging livestock, and other agricultural practices. It was thus selected as an example of an agriculture-impacted site (Figure 2.3).

***Site S2* (Swartkops River – within Uitenhage town)**

Site S2 was situated in the town of Uitenhage, upstream of the discharge point of Kelvin Jones WWTW. Surrounding impacts include industrial and municipal waste, run-off from roads and informal settlements. It was selected as an example municipal/informal settlement-impacted site (Figure 2.3).

***Site S1* (Swartkops River – Wastewater works-impacted site)**

Site S1 is situated further downstream of the discharge point of the Kelvin Jones WWTW, but within the industrial town of Uitenhage where surrounding impacts include industrial and wastewater effluent discharges, run-off from roads and the rail network, and free-ranging livestock. The site was selected as a WWTW-impacted site (Figure 2.3).

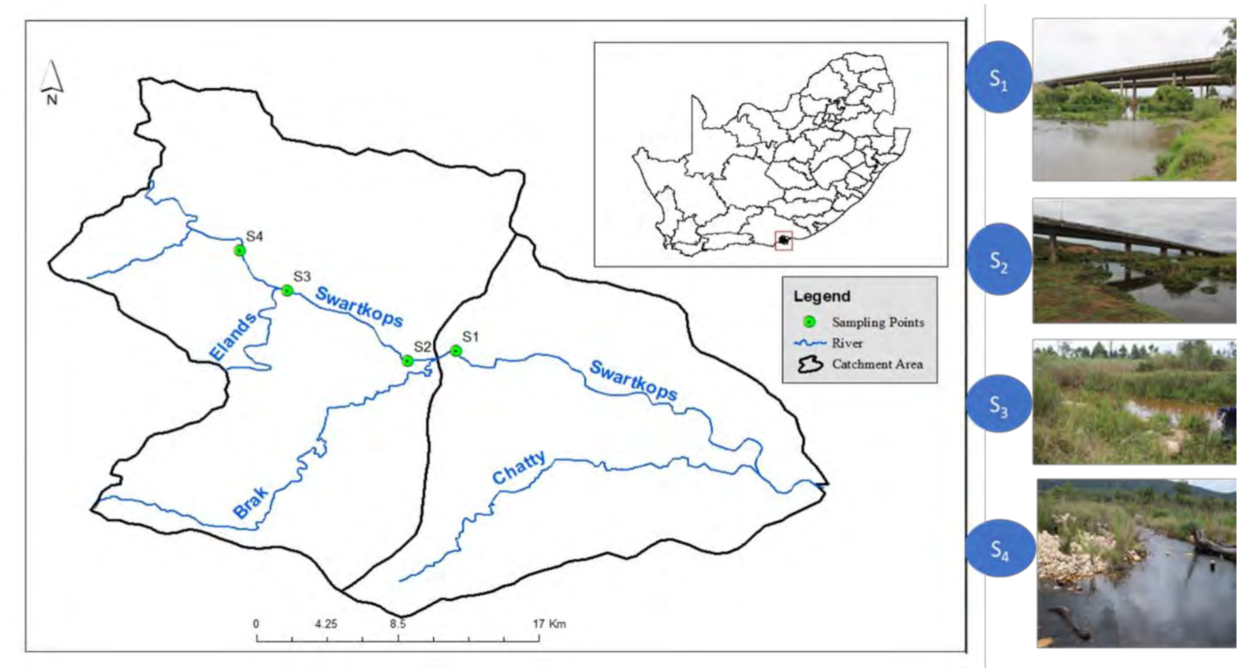


Figure 2.3: Map of Swartkops River catchment showing the location of the experimental sampling sites along the Swartkops River. Site S1 (WWTW-impacted site), Site S2 (within Uitenhage town), Site S3 (Agriculture-impacted site), Site S4 (control site).

CHAPTER 3: Effects of Selected Plastic Properties and Spatial-Temporal Variabilities on the Adsorption of Selected Pharmaceutical Pollutants and Endocrine-Disrupting Compounds on Microplastics in the Bloukrans River, Eastern Cape, South Africa

3.1 Introduction

The interaction between microplastics (MPs) and chemical pollutants in water systems is usually mediated by several factors, including MP properties such as size, shape, surface properties, **hydrophobicity** and polymer type(Hartmann et al., 2017; Wang et al., 2018; Atugoda et al., 2021). However, the key among these properties is the size of MPs, a crucial factor in influencing the adsorption of pollutants in water systems onto MPs(Hartmann et al., 2017). As microplastic size decreases, both the specific surface area of MPs and the Brownian motion increase, resulting in a higher capacity to adsorb pollutants, leading to potential environmental and human health concerns(Atugoda et al., 2021; Guan et al., 2020).

Several studies have demonstrated a positive correlation between MP size and adsorption capacity. A study by Velzeboer et al. (2014) investigated the adsorption of polychlorinated biphenyls (PCBs) onto polyethene (PE) microplastics of varying sizes. They found that PCB adsorption increased significantly with decreasing microplastic size. Another study by Zhan et al. (2016) examined the adsorption of 3,3′,4,4′-tetrachlorobiphenyl (PCB)onto PP MPs of different sizes. They observed a similar trend, with smaller PP microplastics exhibiting higher adsorption capacity. However, current literature suggests that studies investigating this interaction between hydrophilic organic pollutants and MPs remain limited (Razanajatovo et al., 2018; Atugoda et al., 2020).

The enhanced adsorption of pollutants by smaller microplastics can have several ecological consequences. As vectors, MPs can facilitate the transport of pollutants to organisms, potentially leading to bioaccumulation and adverse effects on their health (de Souza Machado et al., 2018; Fabra et al., 2021). The presence of pollutants adsorbed onto microplastics can also contribute to the overall load of pollutants in water systems, potentially affecting water quality and further posing risks to human and ecosystem health (Wu et al., 2019; Sun et al., 2020; Gao et al., 2021). Therefore, further investigation of the influence of MPs' size on pollutant adsorption is crucial for assessing the potential risks of microplastic pollution in aquatic environments. This knowledge can inform the development of effective mitigation strategies, such as reducing microplastic inputs into waterways and developing technologies to remove microplastics and associated pollutants from water systems (Vedolin et al., 2018; Godoy et al., 2019; Yu et al., 2019).

In addition to MPs properties, spatial factors like location, land-use types, hydrodynamic conditions, water flow and turbulence, and temporal factors like seasonal variations can play an essential role in influencing the adsorption of pollutants by MPs in water systems (Van et al., 2012). These factors can affect the distribution, abundance, and interactions of MPs with pollutants, ultimately influencing the overall adsorption process in the environment (Guo & Wang, 2019; Fu et al., 2021). Microplastic concentrations and types vary across different locations due to varying sources attributed to land-use differences and environmental conditions (Dris et al., 2018). This heterogeneity can produce spatial disparities in microplastic-organic pollutant interactions.

Seasonal changes in environmental conditions, such as temperature, precipitation, and algal blooms, are crucial factors that can alter microplastic properties and pollutant behaviour and so affect adsorption dynamics (Filella & Turner, 2018). However, information in the literature about the seasonal effects of MP-facilitated adsorption of organic chemical pollutants could be more extensive (Fu et al., 2021; Santana-Viera et al., 2021). Besides, most contemporary studies have been laboratory-based experiments that cannot sufficiently depict the influencing factors in complex natural environments (Sun et al., 2020; Y. Zhang et al., 2021). Therefore, *in situ*, investigation of the complex interplay of spatial and temporal dimensions of microplastic adsorption of pollutants of emerging concern, especially pharmaceuticals and hormone disruptors, can provide a more comprehensive understanding of the potential impacts of microplastic pollution on aquatic ecosystems and human health (Ebrahimi et al., 2022).

In this study, the adsorption of sulfamethoxazole, ciprofloxacin, 17β- Estradiol, and 4-(2, 6-dimethyl-2-heptyl) phenol were examined under field experimental conditions in the Bloukrans River. Two size ranges of Polyethylene Terephthalate (PET) and Polypropylene (PP) MPs were utilised for the experiment. The first size range was Type 1 (2mm<-≤5mm), and the second, regarded as Type 2, was 0.5mm<-≤2mm). The experiments accounted for both temporal and spatial variabilities. Therefore, this chapter aimed to investigate the effects of selected plastic properties and spatial-temporal variabilities on the adsorption of selected pharmaceutical pollutants and endocrine-disrupting compounds on microplastics in the Bloukrans River. This objective partly fulfils the first objective of this study, as stated in Chapter 1.

3.2 Materials and Methods

3.2.1. Microplastic sourcing

PET and PP fragments were sourced from the Grahamstown Recycling Centre (GRC), crushed into small pieces of approximately <5 mm using a metallic blender, and subsequently sorted to yield particles of two size ranges: Type1 (2 mm<-≤ 5mm), and Type 2 (0.5mm<-≤2 mm) using stainless metallic sieves. The MPs were pre-washed with 75% ethanol and sterile ultrapure water to disinfect and remove residual contaminants on the surface.

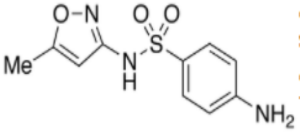
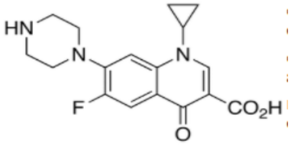
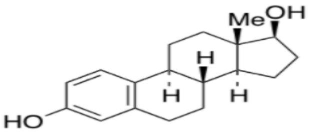
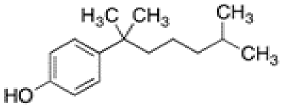
3.2.2. Microplastic characterisation

The Fourier transform infrared spectrophotometer (A Perkin Elmer FT-IR two universal ATR) was used to confirm the basic structural units of both PP and PET MPs polymer used in this study. Scanning electron microscopy-energy dispersive X-ray analysis (SEM-EDX) (Quanta 250, FEI, USA) was used to inspect the surface morphology of the MPs before and after retrieval to establish the potential adsorptive characteristics of the MPs. Secondary electron images (SE), Backscattered Electron images (BE), and Energy Dispersive X-ray data (EDS) were collected in high vacuum on a Tescan Vega 2 Scanning Electron Microscope with a W-filament. Energy Dispersive X-ray data was collected on an Oxford INCA Penta-FET-X3 Si (Li) detector, at 20.00kV. Samples were coated with sputtered gold.

3.2.3 Selection of targeted compounds, standards, and reagents used for this study

Target analytes were selected based on previous studies in the Bloukrans River (Farounbi & Ngqwala, 2020; Vumazonke et al., 2020). The selection was further based on their detection frequency in freshwaters elsewhere in South Africa (Ncube et al., 2021). The selected target analytes and their respective internal standards are presented in Table 3.1. Sulfamethoxazole, ciprofloxacin, 17β-Estradiol, 4-(2, 6-dimethyl-2-heptyl) phenol and their isotopically labelled internal standards in their crystalline form were purchased from Toronto Research Chemicals Inc. (North York, Ontario, Canada). Acetonitrile (HLPC grade) and methanol (HLPC grade) were obtained from Sigma Aldrich, Gauteng, South Africa. Milli-Q water was used to prepare aqueous solutions for all chemicals. All standard stock solutions were prepared in methanol at a concentration of 1000 mg/l.

Table 3.1: Class, molecular formulas, and molecular masses of targeted organic compounds selected for the current study and the respective internal standards used for their quantification.

Compound (abbreviation) [CAS]	**Class**	pk_a	**Molecular formula**	**Structural formula**	**Molecular mass**	**Internal Standard**
Sulfamethoxazole (SXM) [723-46-6]	Sulphonamides	3.92	$C_{10}H_{11}N_3O_3S$		253.05211	Sulfamethoxazole-d4
Ciprofloxacin (CIP) [85721-33-1]	Fluoroquinolone antibiotics	6.0 - 8.8	$C_{17}H_{18}FN_3O_3$		313.1332	Ciprofloxacin-d8
17β-Estradiol (E2) [50-28-2	Oestrogen	10.71	$C_{18}H_{24}O_2$		272.1776	17β-Estradiol-16,16,17-d3
4-(2,6-dimethyl-2-heptyl) Phenol($C_{18}H_{24}O$) [521947-27-3]	Metabolite of nonylphenol.		$C_{18}H_{24}O$		220.1822	4-(2,6-dimethyl-2-heptyl)Phenol-13C6

3.2.4 Experimental design

Microplastic deployment

In-situ deployment of the MPs followed a methodology used by Magadini et al., (2020) with adjustments. The PP and PET MPs pellets in two size categories Type 1(2mm<-≤5 mm), and Type 2(0.5mm<-≤2 mm) were used. Prior to deployment, the PP and PET MPs and all glassware were washed with ethanol (75%) and air dried. The MP particles were placed into stainless-steel mesh infuser bags with an approximate pore size of 100µm. The mesh bags containing the MPs were then tightly sealed and label-tagged according to the retrieval period. Three mesh infuser bags per site (Figure 3.1) containing the respective MP (PP or PET separately) were placed into one big, artificially made stainless steel mesh bag, labelled, and sealed. In total each of the four sites along the two river systems (see section 2.7) had six big stainless steel mesh bags. The deployment was done once in each of the two seasons summer (S) (20th, January 2022) and autumn (A) (7th, April 2022).

The bags were submerged approximately 60 cm below the water surface at each experimental site (detailed deployment experimentation design see Figure 3.1). The GPS coordinates, date, surface water temperature, time, river width, water depth and water flow rate were recorded at the time of each deployment.

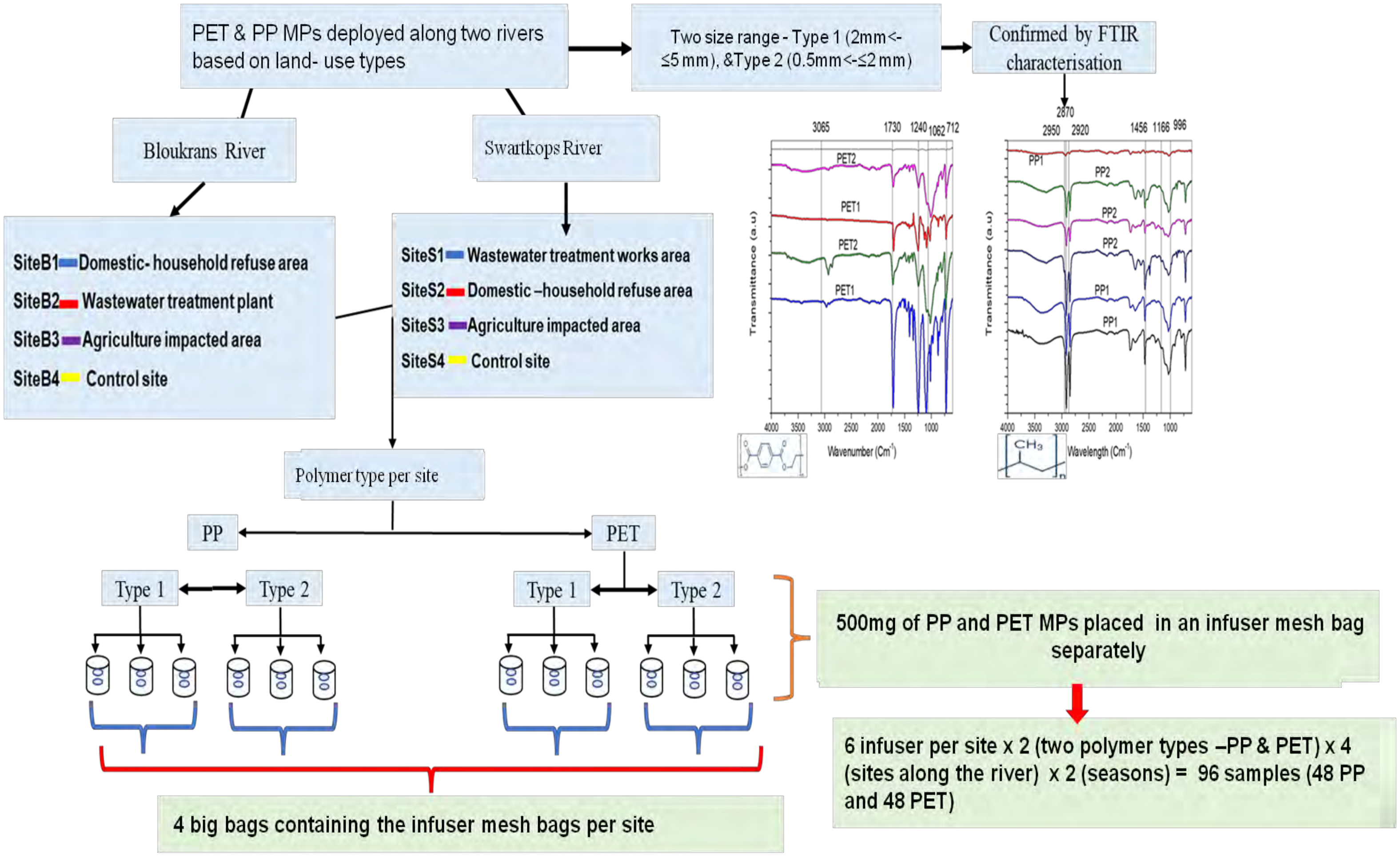


Figure 3.1: Summary of the experimental design setup for MP deployment along the Bloukrans River. The set-up graphically represents each of the four experimental study sites on the river.

Microplastics retrieval

At periodic intervals of 7, 14 and 35 days calculated based from the day of deployment in each of the seasons (Figure 3.2), a total of four mesh bags, two containing each polymer (PP or PET MP) of the respective size was retrieved per site. The retrieved mesh bags containing PP and PET separately were placed into cooler boxes and transported back to the water quality laboratory of the Institute for Water Research (IWR), Rhodes University. Samples were analysed within 24 – 48 hours after each retrieval.

3.2.5 Treatment of the microplastic particles after retrieval

At the laboratory, the MPs were removed from the stainless-steel mesh bags and washed with 100 mL of the filtered surface water from the respective study site to dislodge any environmental particles attached. Each sample was immersed in Milli-Q water for 30 minutes. The samples were then transferred into separate vials containing 5 mL of 1:1 methanol: acetonitrile solution for 10 hours to extract the analytes of interest from PP and PET particles. A 1 mL portion of each extract was centrifuged using the universal 320R, at 12,500 revolutions per minute (RPM) for 30 minutes. The supernatant extract was filtered through a 0.2µm syringe filter. The sample processing steps after retrieval are summarised in Figure 3.2 below.

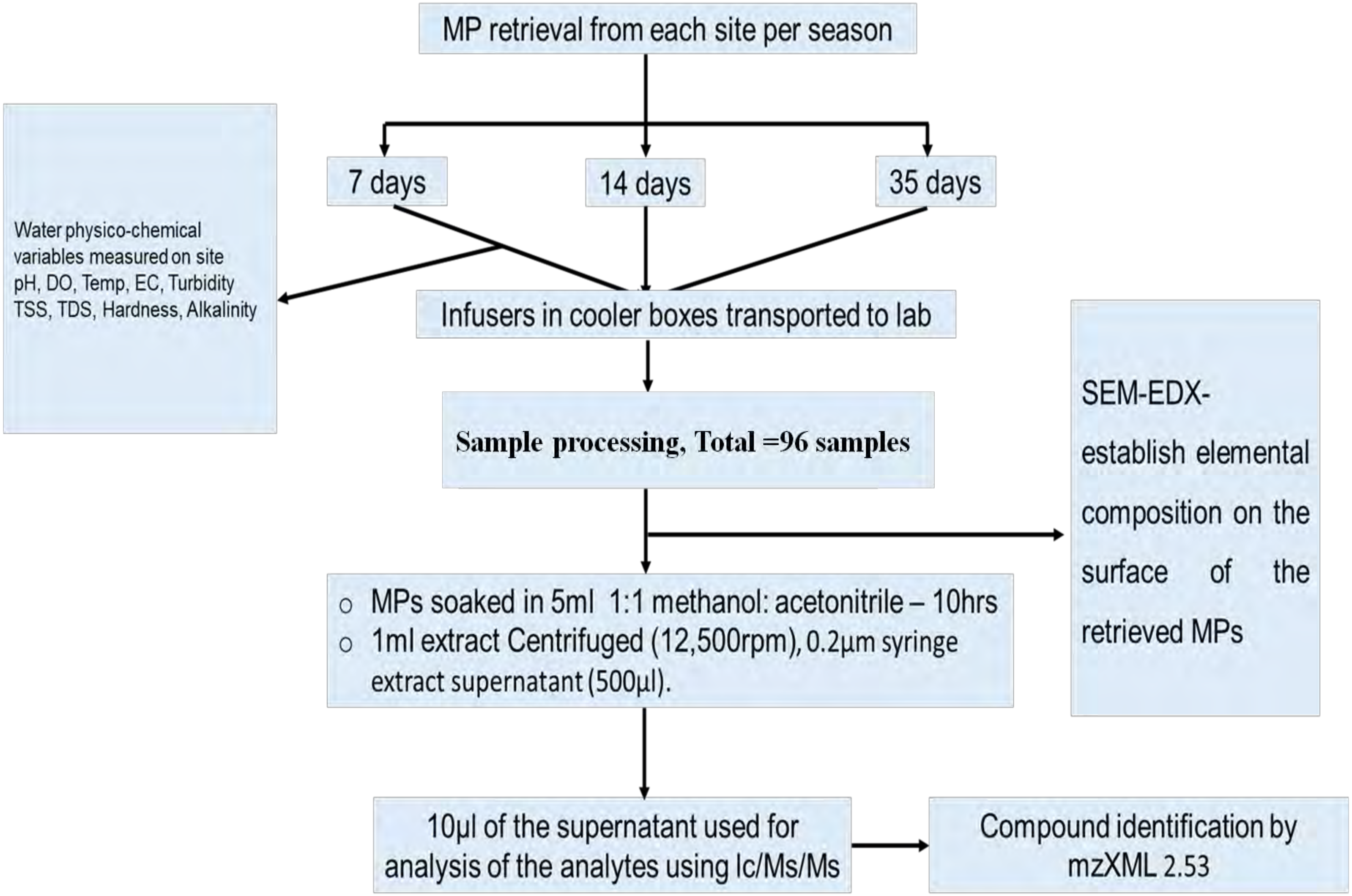


Figure 3.2: Summary of the sample processing steps after the PP and PET MPs retrieval from the Bloukrans River study sites.

3.2.6 Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis and data acquisition

The analysis started with compound optimisation where the analysis method was validated for each targeted analytes regarding recovery, the limit of detection (LOD) and the limit of quantification (LOQ) using the linear regression analysis. The LOQ and LOD were determined based on the signal–to–noise (S/N) ratios of the residual standard deviation (SD) of the y-intercept of the regression line of the calibration curve and the slope of the regression line (Appendix R). Recoveries for the targeted analytes at the two spiking concentration levels (50 ng and 100 ngL^{-1}) for the spiked blanks ranged from 65% to 98%. The linearity was observed in the concentration range studied (100 µgl^{-1} to 1000 µl^{-1}) with a correlation coefficient greater than 0.998 for all the targeted analytes (Appendix R). Additionally, a spectra rate: 12.00Hz, Mass range start (20 – 20000m/z, Mass range End (50 – 40000m/z), Ion Energy 3.0eV and a grouping length of 5 were applied.

Subsequently the analysis was done in a multiple reaction monitoring mode using Dionex Ultimate3000 UHPLC (Thermo Fisher Scientific, Sunnyvale, CA, USA) at a speed core-HPLC SP18-030526 polar C18 column (100×3.0mm, 2.6µm) (Fortis Technologies, Neston, CH64-3UG, United Kingdom). The binary mobile phase comprised acetonitrile and water, each adjusted with 0.1% formic acid (V/V). A method used by Nantaba et al. (2020) was adopted without modification. Each analysis started with a 5-minute equilibration of the column at 15% acetonitrile. The mobile phase gradient was ramped at a flow rate of 0.4 mL/min (held for 5 minutes) starting from 15% to 50% acetonitrile in 2 minutes (held for 2 minutes), then ramped to 80% acetonitrile in 3 minutes and finally to 15% in 3 minutes (held for 3 minutes). The injection volume for each sample was 5 µL, and the column and auto sampler temperature was maintained at 40 ^{o}C and 4 ^{o}C, respectively.

Mass spectrometric analysis of the targeted adsorbed compounds was carried out using a triple quadrupole mass spectrometer (Bruker Compact QToF, Bremen, Germany) equipped with an electron spray ionisation source in the positive mode only (ESI^{+}). The ESI source parameters were set in a positive mode: the nebuliser pressure was set to 3.0 bar, and the capillary voltage was 2000V. The temperature and flow rate of the drying gas was set at 220 ^{o}C and 9.0 L/min, respectively. The scan range of 50 m/z to 1000 m/z was analysed, MS^{2} spectra were recorded in data-acquisition mode, selecting the three most intense precursor ions for the acquisition of MS^2 spectra at a collision energy of 3.0 eV and transfer time of 80 µs (Appendices C and D).

3.2.7 Compound identification and data processing.

After the acquisition, the raw data were converted to mzXML format using the Bruker Compass software (Bruker, Bremen, Germany). The resulting mzXML files were processed in MZmine 2.53 using a method described by Pluskal et al. (2010) with modification. Mass lists were created using the mass detection module with a noise level of 1000 counts for MS1 and 100 counts for MS2 m/z tolerance with mass tolerances of 10 ppm. The chromatogram builder module created peak lists with a minimum retention time (min), a minimum peak height of 3000 counts and an m/z tolerance of 20 ppm. The peak lists were deconvoluted using the local minimum search algorithm with the following parameters: chromatographic threshold 0.01%, search minimum in retention time range 0.2 minutes, absolute minimum height 3000 counts, and peak duration range 0.05–3 minutes. For the MS^2scan, the m/z range was set to 0.01 and the retention time range to 0.2. Next, the deconvoluted peaks lists were aligned using the join aligner module with an m/z tolerance of 20 ppm, a retention time tolerance of 0.1 minutes, weighting m/z and a retention time set to 75 and 25, respectively. The aligned peaks list was then subjected to gap-filling and filtered to exclude all features present without associated MS^2 spectra, and peak IDs were resent, followed by the export of a .csv table and .mgf file.

3.3 Statistical Data Analysis

The data were subjected to relevant univariate and multivariate statistical analyses to indicate statistical differences and elucidate relations and trends. The summary statistics were performed using the R programming environment (R Core Team 2022). Before analysis, the data were captured and prepared in Excel (Microsoft 2010 Office) and exported to R software package Version R 4.3.0. The normality of the data was tested using the Shapiro-Wilk normality test. Non-parametric tests were chiefly used because most data violated the assumption of normality. The Kruskal-Willis test was used to compare the concentration of the same organic compound between study sites, followed by Tukey’s honestly significant difference test (Tukey’s HSD) for multiple comparisons. Statistical significance was set at $P < 0.05$ for all the tests.

3.3.1 Box and whisker plots

Box and whisker plots were used to visualise the spatial-temporal variability of the concentrations of adsorbed organic chemical pollutants based on (i) land-use type;(ii) MPs size range and(iii) seasons.

3.3.2 The Kruskal-Willis test

The Kruskal-Willis multiple comparison test was used to determine the significant differences of organic chemical pollutant concentration adsorbed in terms of study sites (land-use type: B1, B2, B3, B4 for Bloukrans River, then S1, S2, S3, S4 for Swartkops River), microplastic size ranges (Type1 and Type2), and seasonal differences (summer and autumn). R software package version R 4.3.0 was used.

3.3.3 Two-factor analysis of variance (ANOVA)

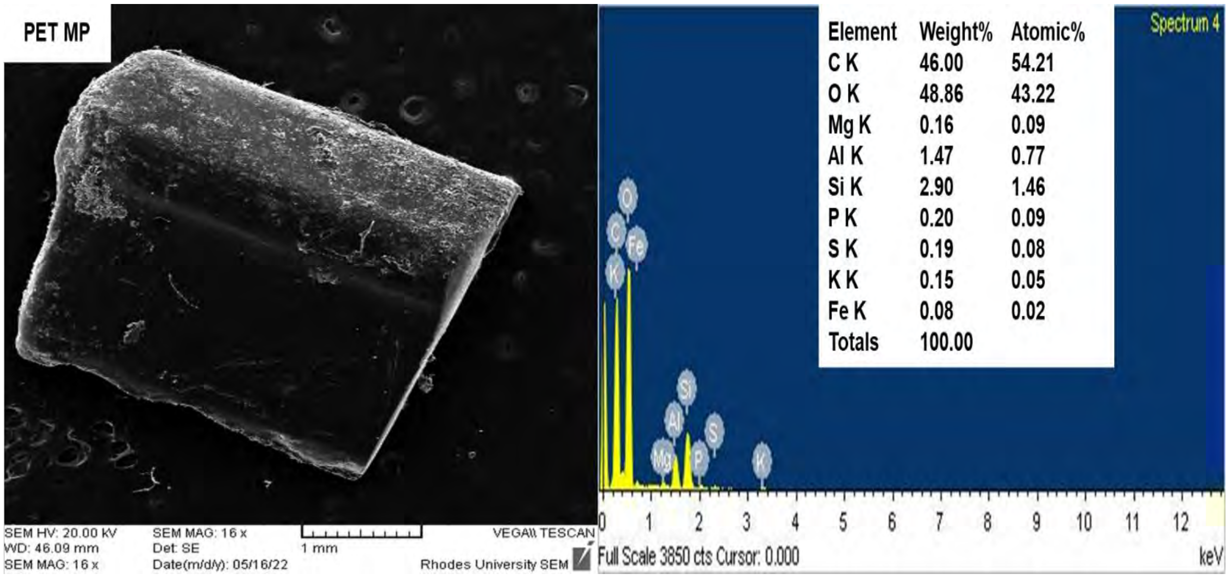
As a parametric test, the analysis of variance Two-way ANOVA was used to test for differences in the means of the measured concentration of the same organic chemical compound adsorbed on the MPs between the four study sites, using both sites and days (period) and them sites and seasons as factors. This was followed by Tukey's HSD for multiple comparisons to indicate land-use types and seasons that differed. Statistical significance was set at $P < 0.05$ for all the tests. ANOVA was conducted using R software package Version R 4.3.0.

3.4 Results

3.4.1 Microplastic particle characterisation

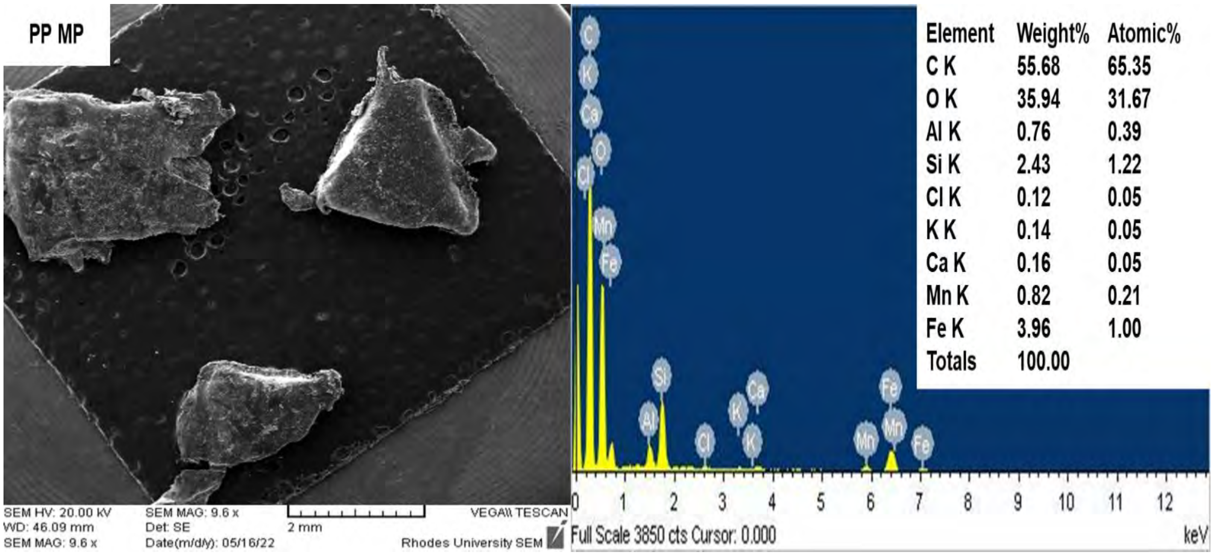
The FTIR spectrum of both PET and PP (Figure P1-Appendix P) confirmed the characteristic bands at 3065 cm^{-1} for C-H aromatic stretch, 1730 cm^{-1} for C=O stretch, 1240 cm^{-1} for C-O-C for asymmetric stretch, 1062 cm^{-1} for C-C stretching and 712 cm^{-1} for OCH symmetric bends for PET and 2950 cm^{-1}, 2870 cm^{-1} for CH_3 asymmetric and symmetric stretches, 2920 cm^{-1} $–CH_2$ asymmetric stretch and 1456 cm^{-1} for CH_3 umbrella mode for PP.

The SEM-EDX of the retrieved MPs shown in Figures 3.3–3.4 indicated that the surface of the retrieved MPs exhibited several micropores with cracked surfaces, indicating some change in the surface morphology of the MPs after retrieval. Before deployment, the EDX micrographs of the MPs indicated carbon and oxygen as the main elements. However, other elements can be seen after retrieval, among them, Fe, K, Al, Mg, P, S, and Si as seen in the PET and PP EXD micrographs.



Element	Weight%	Atomic%
C K	46.00	54.21
O K	48.86	43.22
Mg K	0.16	0.09
Al K	1.47	0.77
Si K	2.90	1.46
P K	0.20	0.09
S K	0.19	0.08
K K	0.15	0.05
Fe K	0.08	0.02
Totals	100.00	

Figure 3.3: SEM-EDX monographs of the retrieved PET surface morphology and elemental compositsion.



Element	Weight%	Atomic%
C K	55.68	65.35
O K	35.94	31.67
Al K	0.76	0.39
Si K	2.43	1.22
Cl K	0.12	0.05
K K	0.14	0.05
Ca K	0.16	0.05
Mn K	0.82	0.21
Fe K	3.96	1.00
Totals	100.00	

Figure 3.4: SEM-EDX monographs of retrieved PP MPs. Surface morphology and elemental composition.

3.4.2 Spatial-temporal variability of the adsorbed organic chemical pollutants

Spatial differences in the concentrations adsorbed (based on land-use differences).

Results for Sites B1 to B4, for PET Type2 MPs, showed the highest adsorbed concentrations of ciprofloxacin were at B3 (15603.96 ngL^{-1}) followed by B2(6769.847ngL^{-1}). For sulfamethoxazole, the highest adsorbed concentration was at B3 (609.1765ngL^{-1}) followed by B1(168.6046ngL^{-1}). 17β-estradiol was highest at B1 (49.2182ngL^{-1}) followed by B2 (94.4620ngL^{-1}).The highest adsorbed concentration of 4-(2, 6-dimethyl-2-heptyl) Phenol was at B1 (69.7775ngL^{-1}), followed by B2 (55.9102ngL^{-1}), and the lowest at B3 (28.5129ngL^{-1}) (Figure 3.5 and Appendix: TableS1). The adsorbed concentration for ciprofloxacin was lowest at B1 (3829.641ngL^{-1}), and for sulfamethoxazole at B2 (94.4620ngL^{-1}). Generally overall, very low concentrations were detected at the control site, B4.

For PET Type 1, sulfamethoxazole was highest at B3 (446ngL^{-1}), ciprofloxacin at B2 (3182.428ngL^{-1}), 17β-estradiolat B1 (24.8066 ngL^{-1}), and 4-(2, 6-dimethyl-2-heptyl) Phenol at B1 (26.8457ngL^{-1}), respectively (Figure 3.5).By comparison, B3 recorded the highest adsorbed concentration of the organic chemical compounds (Figure 3.5). The lowest mean concentration of sulfamethoxazole, ciprofloxacin, 17β-estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol was at B2 (36.8212ngL^{-1}), B1 (1203.628ngL^{-1}), B2 (10.7043ngL^{-1}) and B1 (9.0764ngL^{-1}) (Table S1 – Appendix S).

For PP Type2 MPs, the highest concentration of ciprofloxacin was recorded at B2 (30285.19± 28783.78ngL^{-1}) followed by (11119.6001ngL^{-1}) at B3. For sulfamethoxazole, 17β-estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol, the mean highest concentration adsorbed by PP MPs Type2 recorded were all at B3: (3380.214ngL^{-1}), B3 (100.3635ngL^{-1}), B3 (378.8924ngL^{-1}), respectively (Figure 3.6 and Appendix S:Table S2).

For PP Type1 MPs, the highest mean concentration recorded for ciprofloxacin was at B3 (1937.146ngL^{-1}), sulfamethoxazole at B3 (64.1593ngL^{-1}), 17β-estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol at site B1 (64.1593ngL^{-1}) and (62.7282 ngL^{-1}), respectively (Figure 3.6).

Overall, the highest mean adsorbed concentration of the organic compounds for both Type1 and Type2 for PET and PP MPs was recorded at B3 (Figures 3.5, 3.6). Statistically, the mean concentration adsorbed at Sites B1, B2, B3 and Site B4 differed significantly from each other

($P<0.05$) based on both the ANOVA test (Figures3.7–3.10) and Tukey multiple comparisons (Tukey HSD post-hoc-test). See TableT1-Appendix-T)

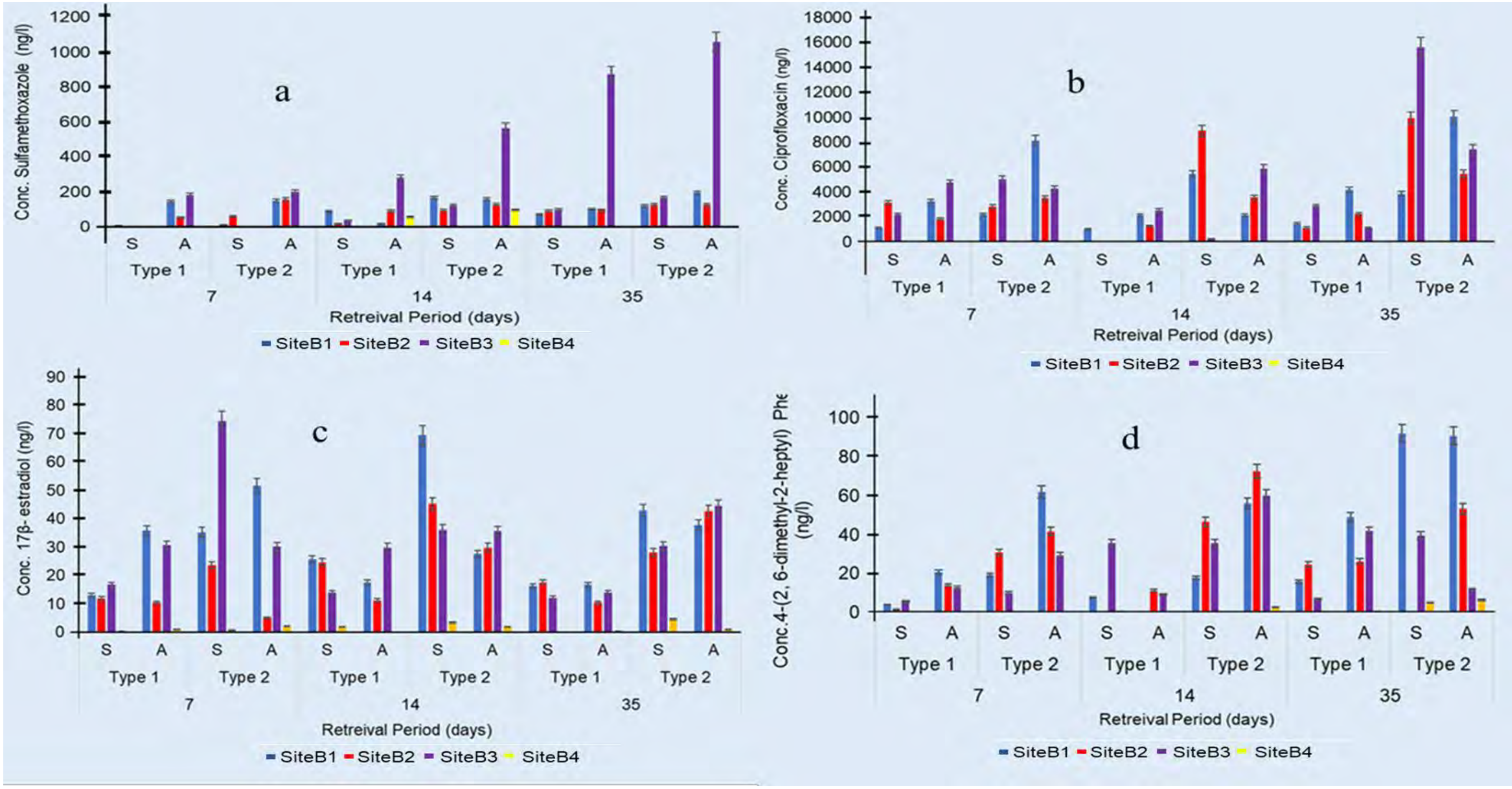


Figure 3.5: Spatial, temporal, and seasonal variability of adsorbed concentration of Sulfamethoxazole (a), Ciprofloxacin (b), 17β-Estradiol (c) and 4-(2, 6-dimethyl-2-heptyl) Phenol (d) onto PET Type1 and Type2 MPs at the four study sites: B1, B2, and B3 in the Bloukrans River, and B4 on Palmiet River over two seasons, S = summer and A = autumn during the study period. Standard error (bars).

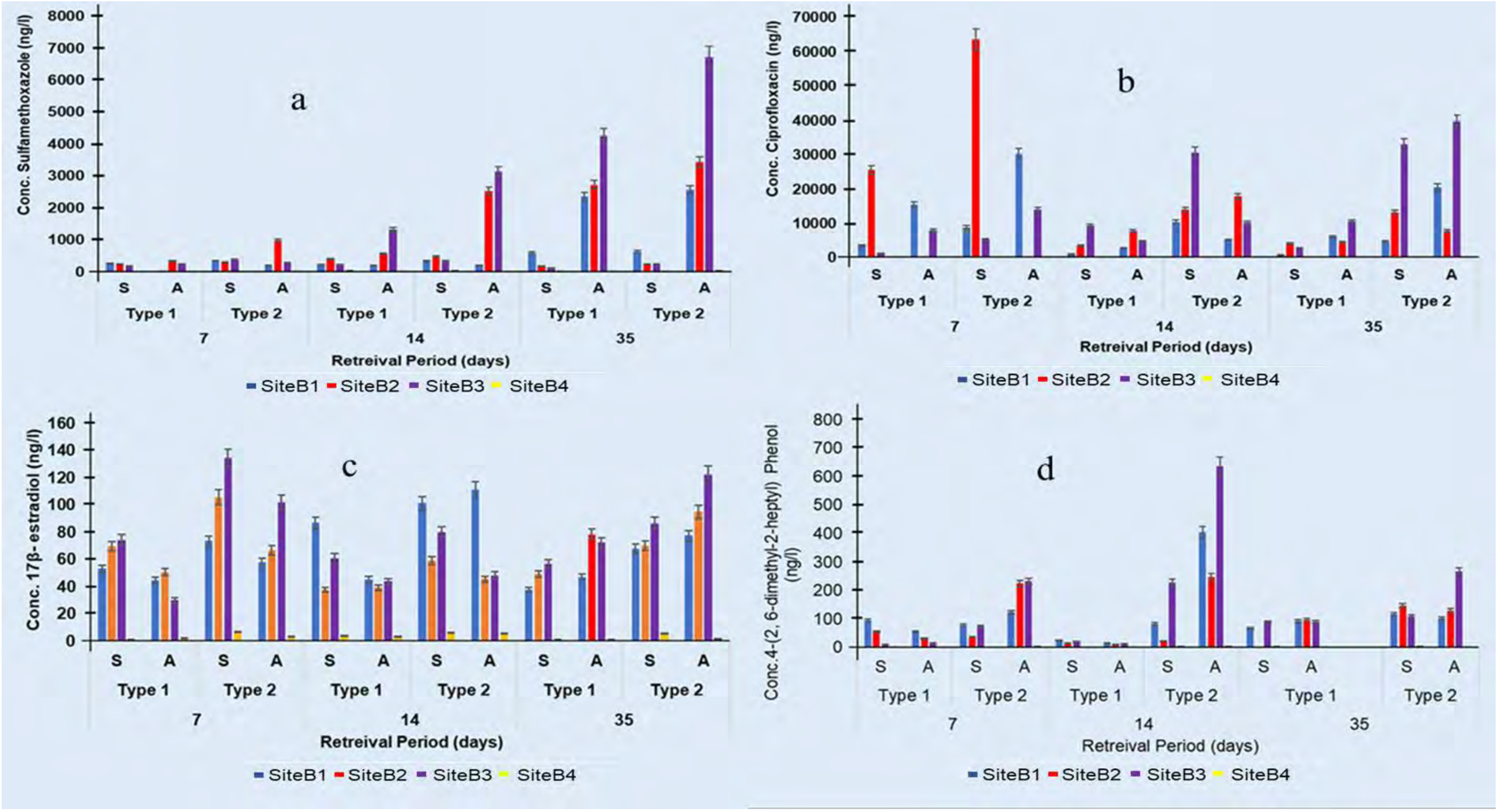


Figure 3.6: Spatial, temporal, and seasonal variability of adsorbed concentration of Sulfamethoxazole (a), Ciprofloxacin (b), 17β-Estradiol (c) and 4-(2, 6-dimethyl-2-heptyl) Phenol(d) onto PP Type1 and Type2 MPs at the four study sites: B1, B2, and B3 in the Bloukrans River, and B4 on Palmiet River over two seasons, S = summer and A = autumn during the study period. Standard error (bars).

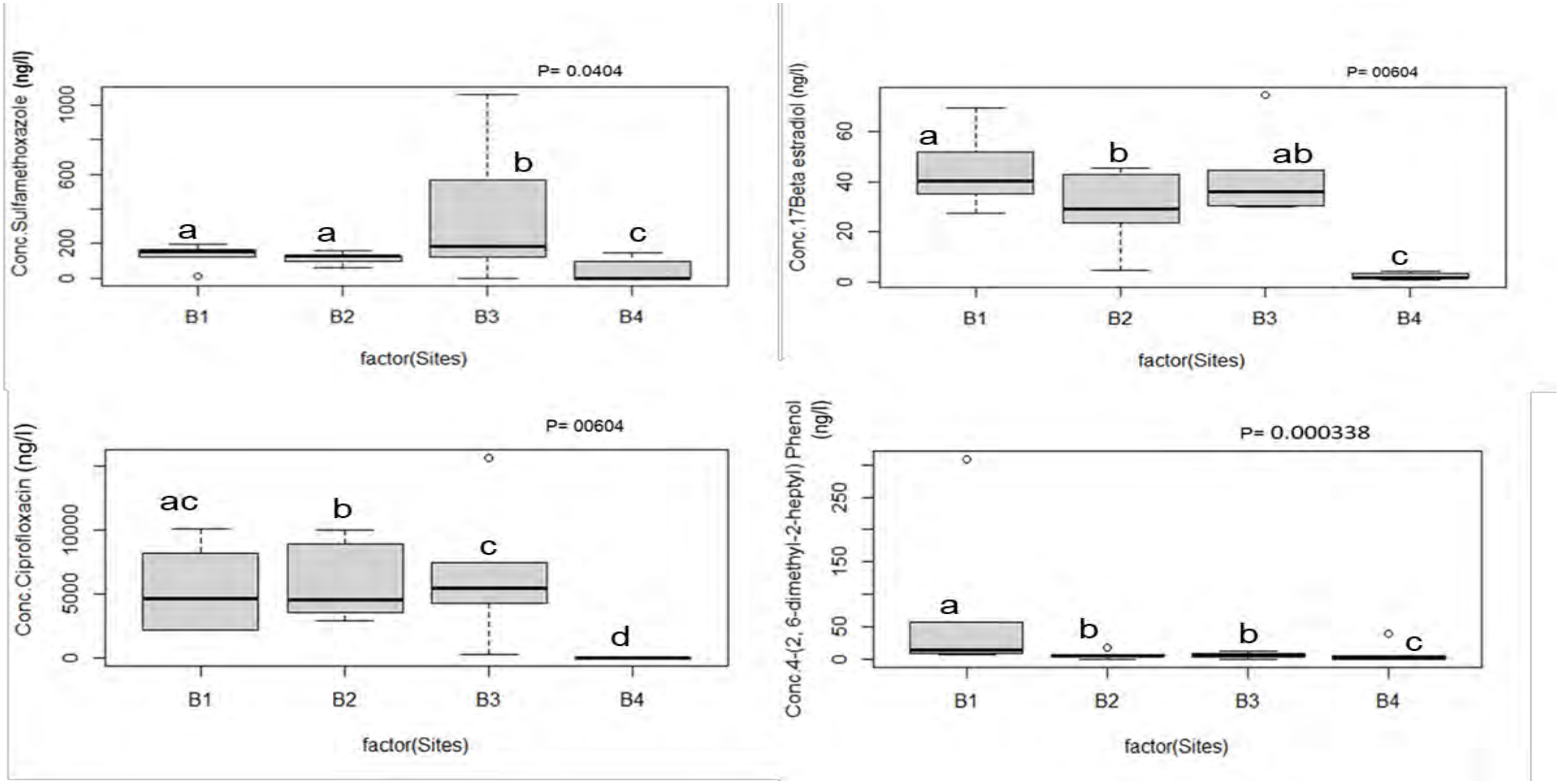


Figure 3.7: ANOVA (boxplots)-factor (sites) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl)Phenol adsorbed on the PET Type2 MPs at the four study sites in the Bloukrans River (B1, B2, B3) and Palmiet River (B4) during the study (January 2022 to May 2022,summer to autumn). The same alphabet letter on the bars indicates no statistically significant difference (*P*> 0.05), whereas different alphabet letters show statistically significant difference (*P*< 0.05).

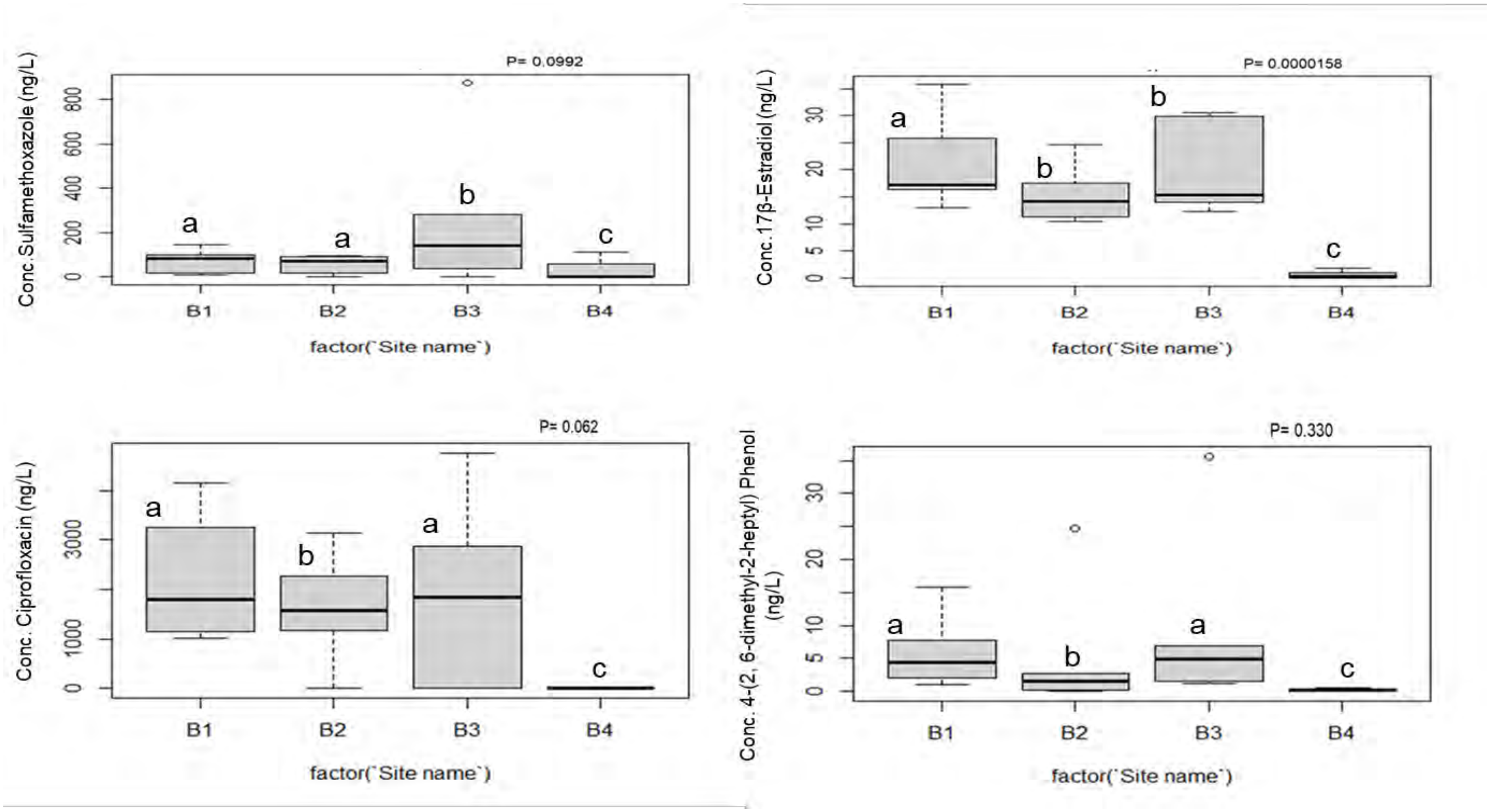


Figure 3.8: ANOVA (boxplots)-factor (sites) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl)Phenol adsorbed onto PET Type1 MPs at the four study sites in the Bloukrans Rivers (B1, B2, B3) and Palmiet Rivers (B4) during the study (January 2022 to May 2022 – summer to autumn). The same alphabet letter on the bars indicates no statistically significant difference (*P* > 0.05), whereas different alphabet letters show statistically significant difference (*P*< 0.05).

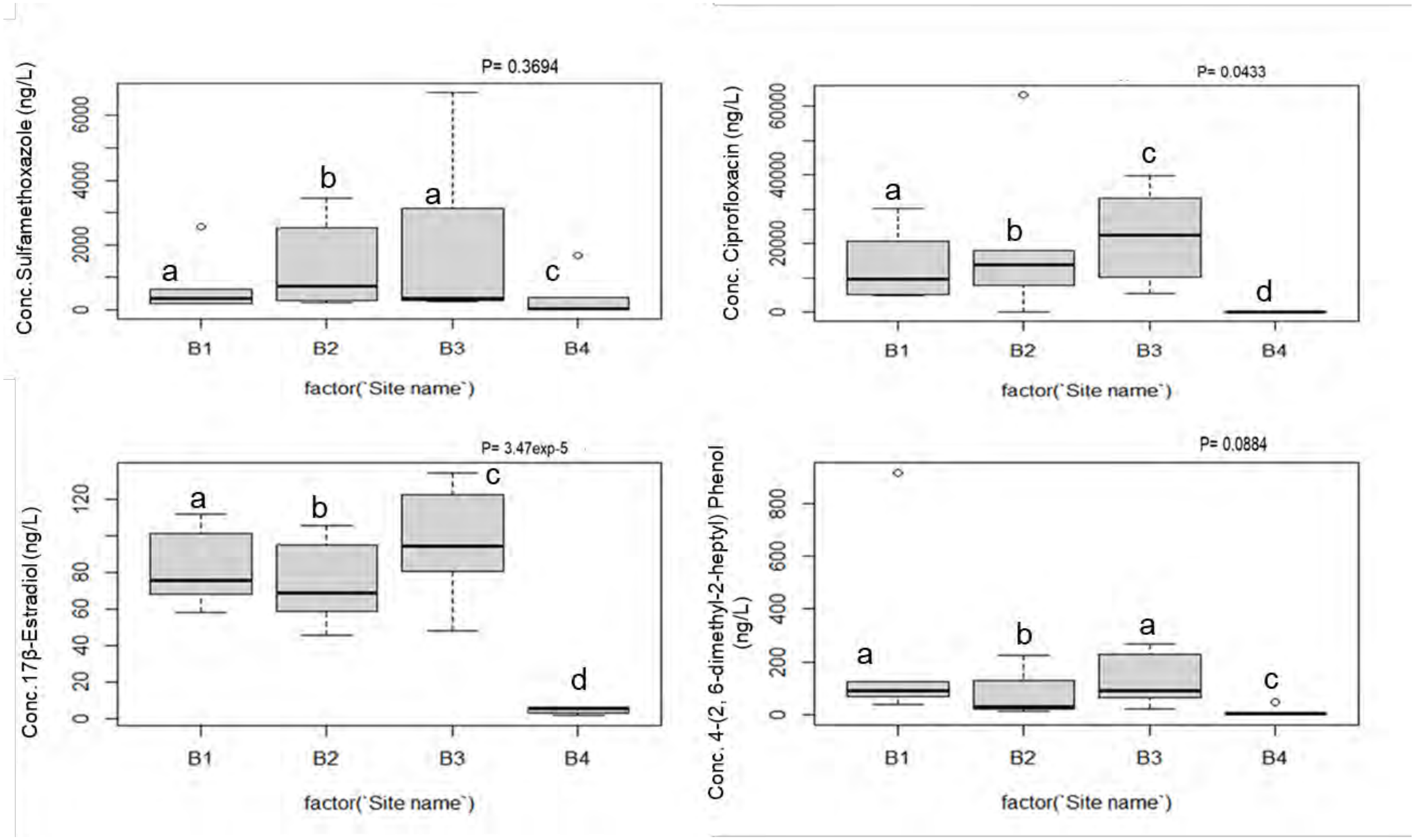


Figure 3.9: ANOVA (boxplots)-factor (sites) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl)Phenol adsorbed onto PP Type2 MPs at the four study sites in the Bloukrans Rivers (B1, B2, B3) and Palmiet Rivers (B4) during the study (January 2022 to May 2022 – summer to autumn). The same alphabet letter on the bars indicates no statistically significant difference ($P > 0.05$), whereas different alphabet letters show statistically significant difference ($P < 0.05$).

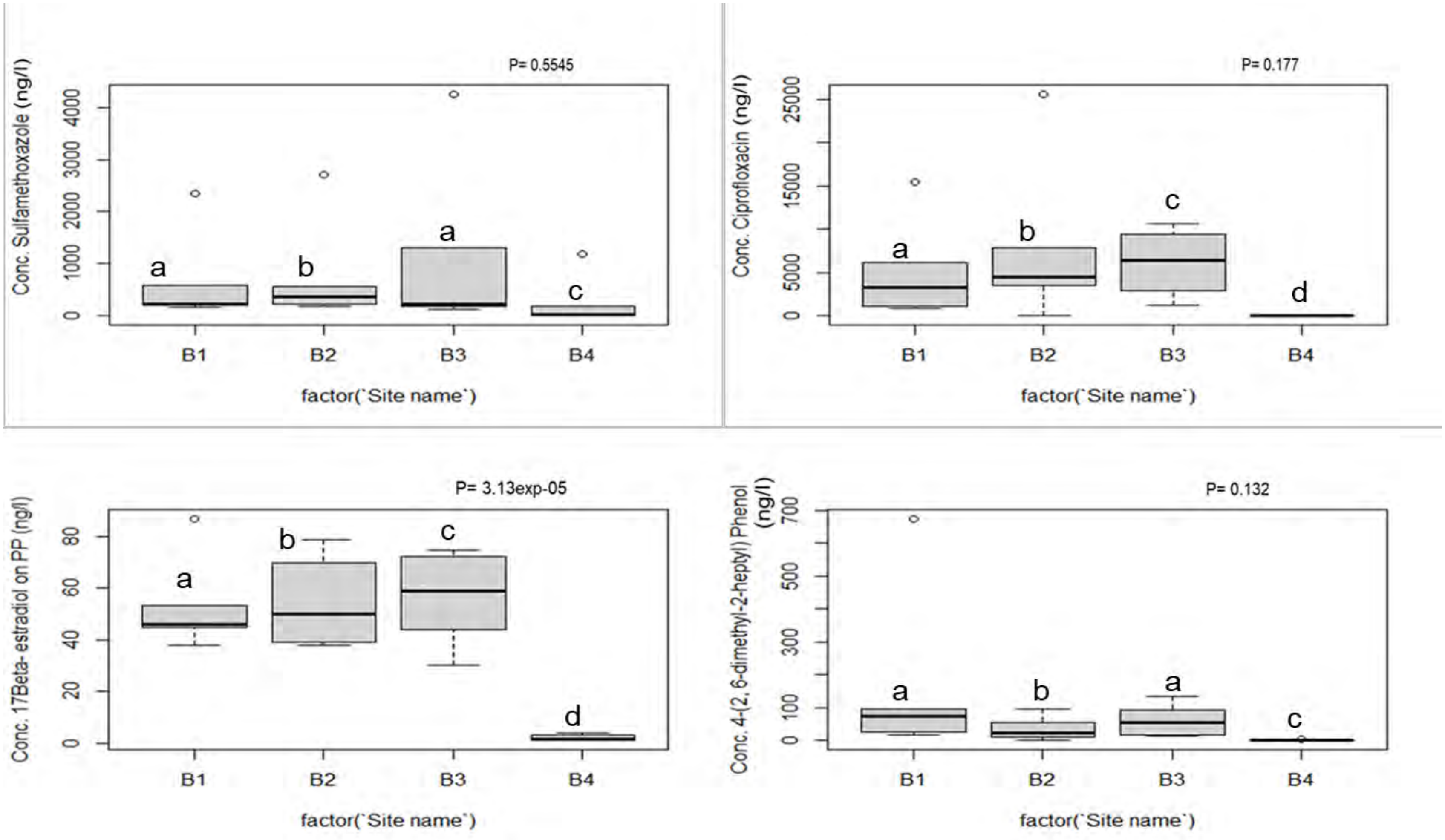


Figure 3.10:ANOVA (boxplots)-factor (sites) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl)Phenol adsorbed onto PP Type1MPs at the four study sites in the Bloukrans River (B1, B2, B3) and Palmiet River (B4) during the study (January 2022 to May 2022 – summer to autumn). The same alphabet letter on the bars indicates no statistically significant difference (*P*> 0.05), whereas different alphabet letters show statistically significant difference (*P*< 0.05).

Temporal differences in concentration adsorbed: based on deployment period and seasonality.

Temporal patterns both in terms of period of deployment and seasonality were examined to better understand the equilibrium interaction between MPs and the organic chemical compounds.

For the period of deployment the trend is shown in Figures3.11–3.14. Generally, the adsorbed concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol at all the study sites was significantly higher after 35 days than all other days during the two seasons ($p< 0.05$) (Figures3.11–3.14).

The Tukey multiple comparisons test (Tukey HSD post-hoc-test; see Appendix T, Table T) indicated that the adsorbed concentration of Sulfamethoxazole, Ciprofloxacin, 17β- Estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol, was significant ($p< 0.05$) after 37days compared with adsorbed concentration after 7 days at all study sites. Similarly, the adsorbed concentration between 35days and 14 days was also generally significant ($p< 0.05$). However, no significant differences were observed when comparing adsorbed concentration after 14days and 7days, indicating that sorption equilibrium was only reached after more than a month's deployment period.

The trend of the adsorbed concentration for all the tested chemicals was non-linearity over the study's period of deployment (Figures 3.11–3.14) in all the instances attributed to the temporal changing environmental factors, including ionic strength, pH, and dissolved organic matter (DOM) that can facilitate adsorption and desorption processes of pollutants from the MPs to occur co-currently (Menéndez-Pedriza & Jaumot, 2020).

We used a maximum deployment period of 35days in this study based on the findings of Rochman et al. (2013),who established that equilibrium time decreases with Kow for compounds with Kow >5 (Rochman et al., 2013)

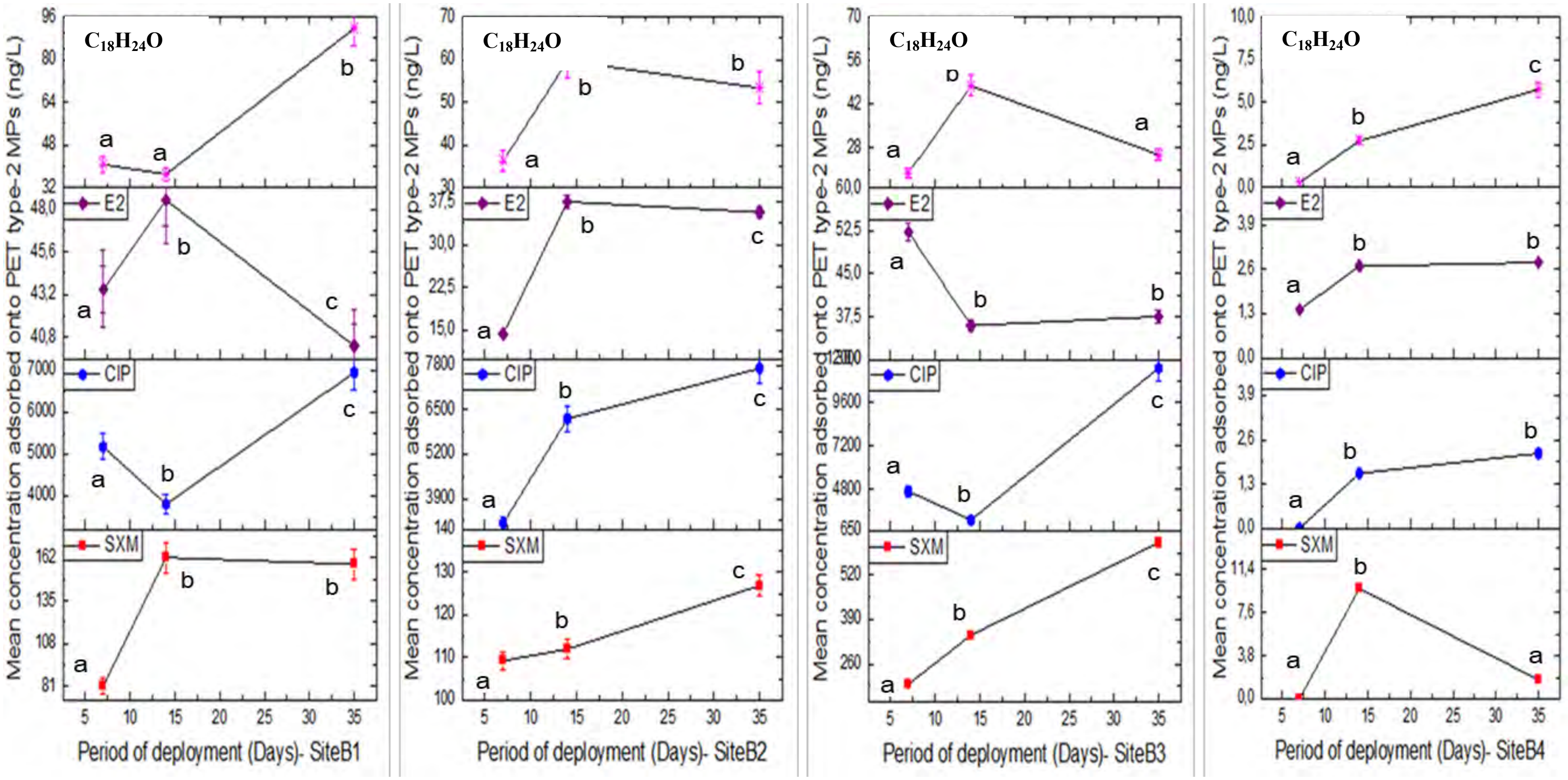


Figure 3.11: Time series (temporal) of mean (centre point) and standard deviation (bar) of adsorbed concentration trends of Sulfamethoxazole (SXM), Ciprofloxacin (CIP), 17β-Estradiol (E2) and 4-(2, 6-dimethyl-2-heptyl)Phenol ($C_{18}H_{24}O$) onto PET Type2 MPs at the four sampling sites in the Bloukrans River (B1, B2, B3) and Palmiet River (B4) over a deployment period of 35 days during the study period (January 2022 to May 2022 – summer to autumn). Time series with different alphabet letters across the sites indicate statistically significant differences ($P < 0.05$), whereas series having the same alphabet letter in common across the sites indicate no statistically significant difference ($P < 0.05$).

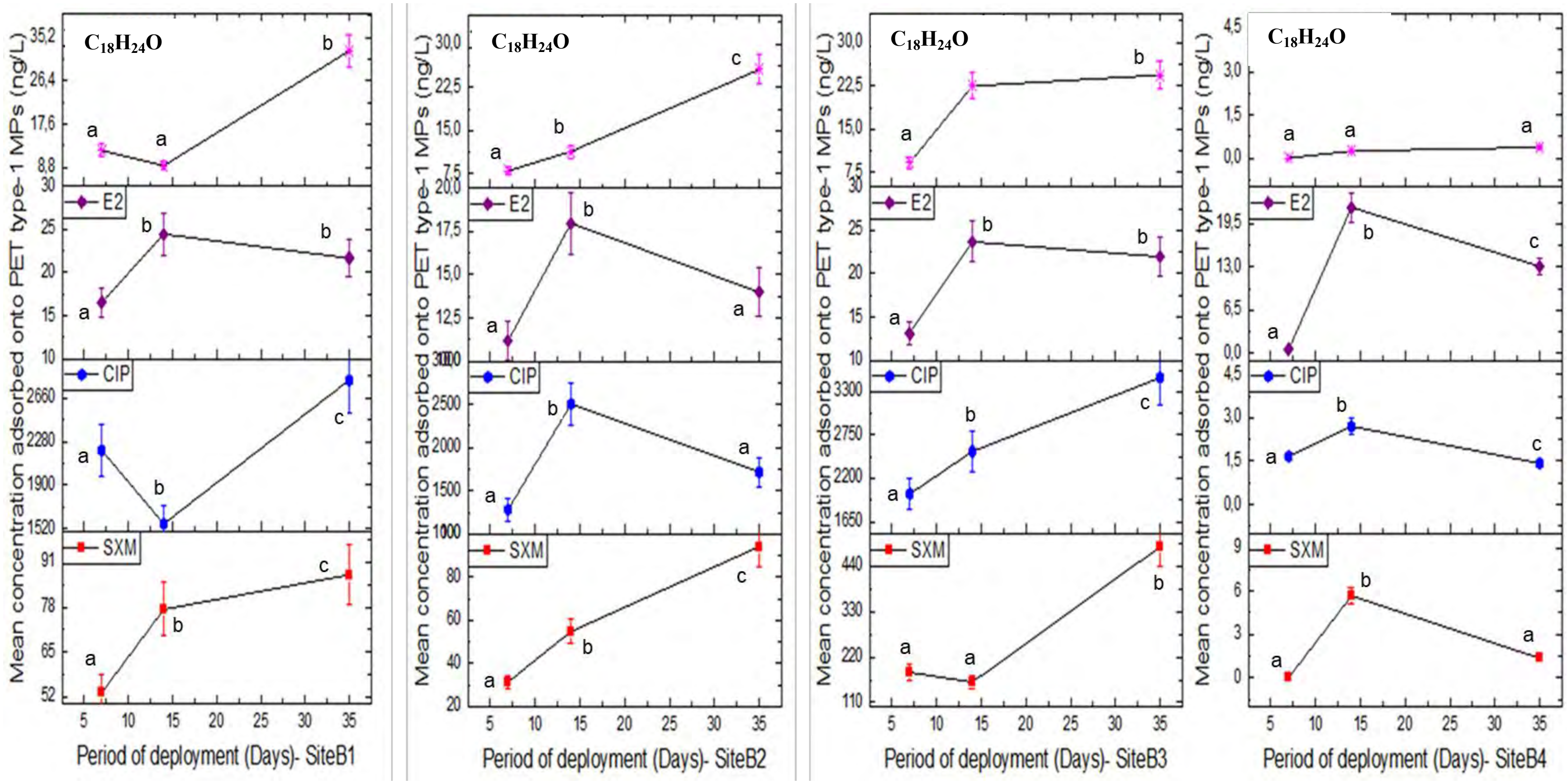


Figure 3.12: Time series (temporal) of mean (centre point) and standard deviation (bar) of adsorbed concentration trends of Sulfamethoxazole (SXM), Ciprofloxacin (CIP), 17β-Estradiol (E2) and 4-(2, 6-dimethyl-2-heptyl)Phenol ($C_{18}H_{24}O$) onto PET Type 1 MPs at the four sampling sites in the Bloukrans River (B1, B2, B3) and Palmiet River (B4) over a deployment period of 35 days during the study period (January 2022 to May 2022 – summer to autumn). Time series having completely different alphabet letters across the sites indicate statistically significant differences ($P < 0.05$), whereas series having the same alphabet letter in common across the sites indicate no statistically significant difference ($P < 0.05$).

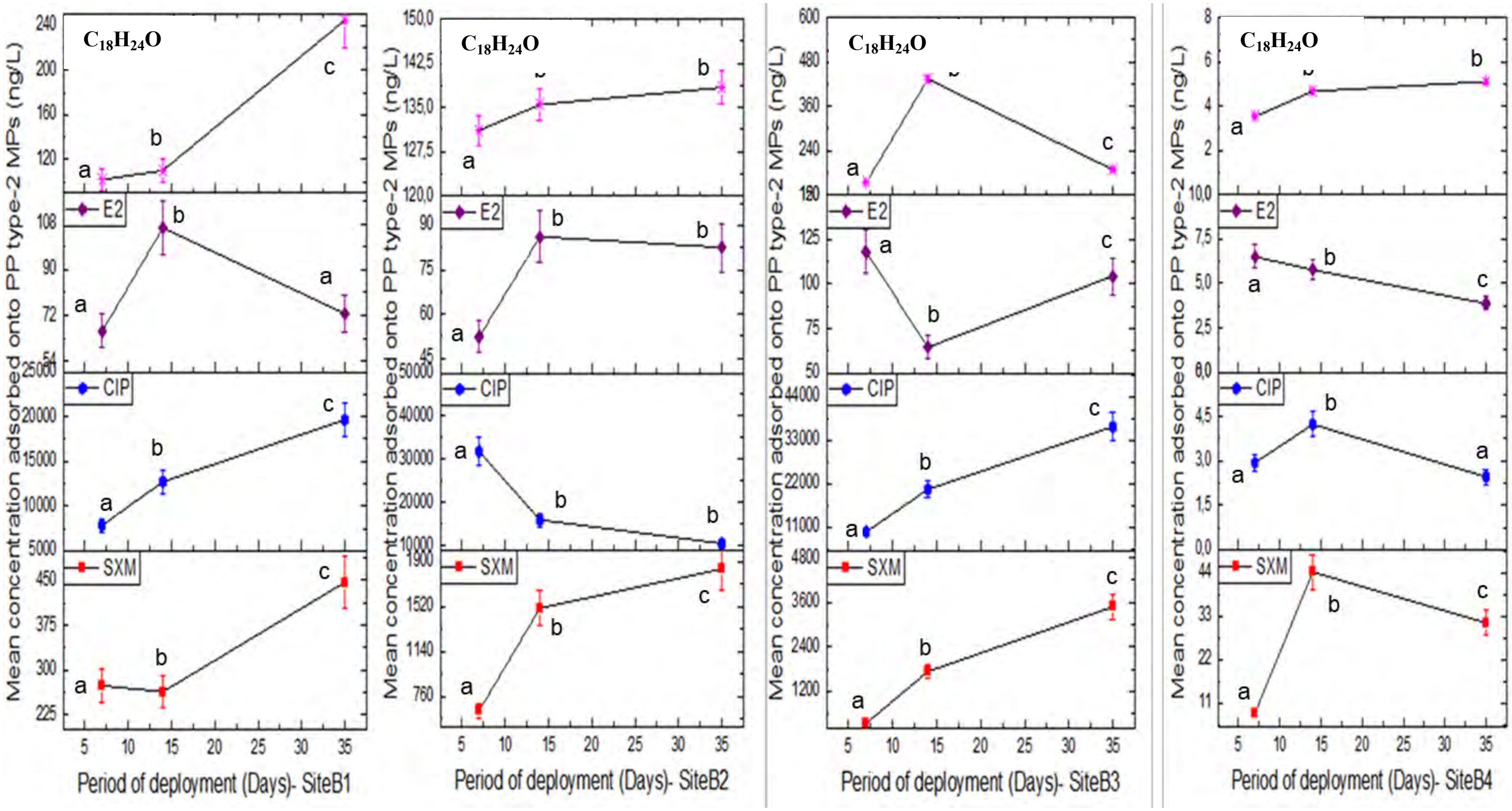


Figure 3.13: Time series (temporal) of mean (centre point) and standard deviation (bar) of adsorbed concentration trends of Sulfamethoxazole (SXM), Ciprofloxacin (CIP), 17β-Estradiol (E2) and 4-(2, 6-dimethyl-2-heptyl)Phenol ($C_{18}H_{24}O$) onto PP Type2 MPs at the four sampling sites in the Bloukrans River (B1, B2, B3) and Palmiet River (B4) over a deployment period of 35 days during the study period (January 2022 to May 2022 – summer to autumn). Time series having completely different alphabet letters across the sites indicate statistically significant differences ($P < 0.05$), whereas series having the same alphabet letter in common across the sites indicate no statistically significant difference ($P < 0.05$).

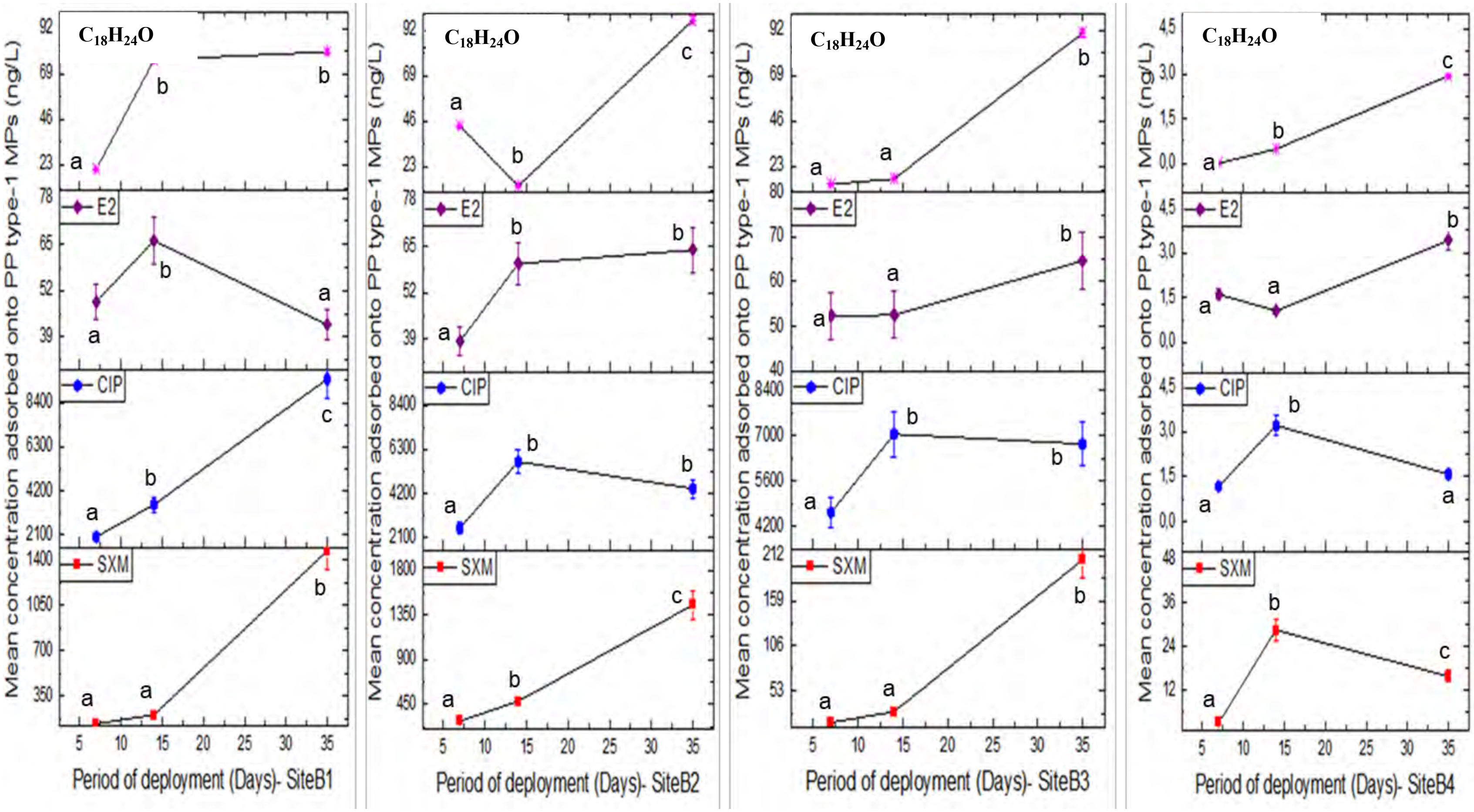


Figure 3.14: Time series (temporal) of mean (centre point) and standard deviation (bar) of adsorbed concentration trends of Sulfamethoxazole (SXM), Ciprofloxacin (CIP), 17β-Estradiol (E2) and 4-(2, 6-dimethyl-2-heptyl)Phenol ($C_{18}H_{24}O$) onto PP Type1 MPs at the four sampling sites in the Bloukrans River (B1, B2, B3) and Palmiet River (B4) over a deployment period of 35 days during the study period (January 2022 to May 2022 – summer to autumn). Time series having completely different alphabet letters across the sites indicate statistically significant differences (*P*< 0.05), whereas series having the same alphabet letter in common across the sites indicate no statistically significant difference (*P*< 0.05).

Seasonal variations of the concentration adsorbed.

Differences were observed in the concentrations of the adsorbed organic chemicals based on seasonal differences, that is, summer and autumn. For instance, the concentrations of Sulfamethoxazole:1937.1461ngL^{-1} and Ciprofloxacin: 8168.1072ngL^{-1} in autumn were approximately one order of magnitude higher than the recorded concentrations adsorbed of the same organic chemicals in the summer season, that is, Sulfamethoxazole: 171.5380ngL^{-1} and Ciprofloxacin: 1870.7311ngL^{-1}(TablesS1,S2;Appendix S).

For 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol, the concentration differed between the two seasons. However, the Kruskal-Willis test and Tukey's honestly significant difference test (Tukey's HSD) for comparisons that were used to indicate the difference between the sampling seasons in terms of the adsorbed concentration of the same pharmaceutical were only significant ($P < 0.05$) for17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl), phenol ($P = 0.0482$ PET MPs Type1; Figure 3.16) and 17β-Estradiol($P = 0.0423$, PP MPs Type2; Figure 3.17). Generally, differences in the adsorbed concentration of the pharmaceuticals on the two MPs over the two seasons were not significant (Figures3.15–3.18).

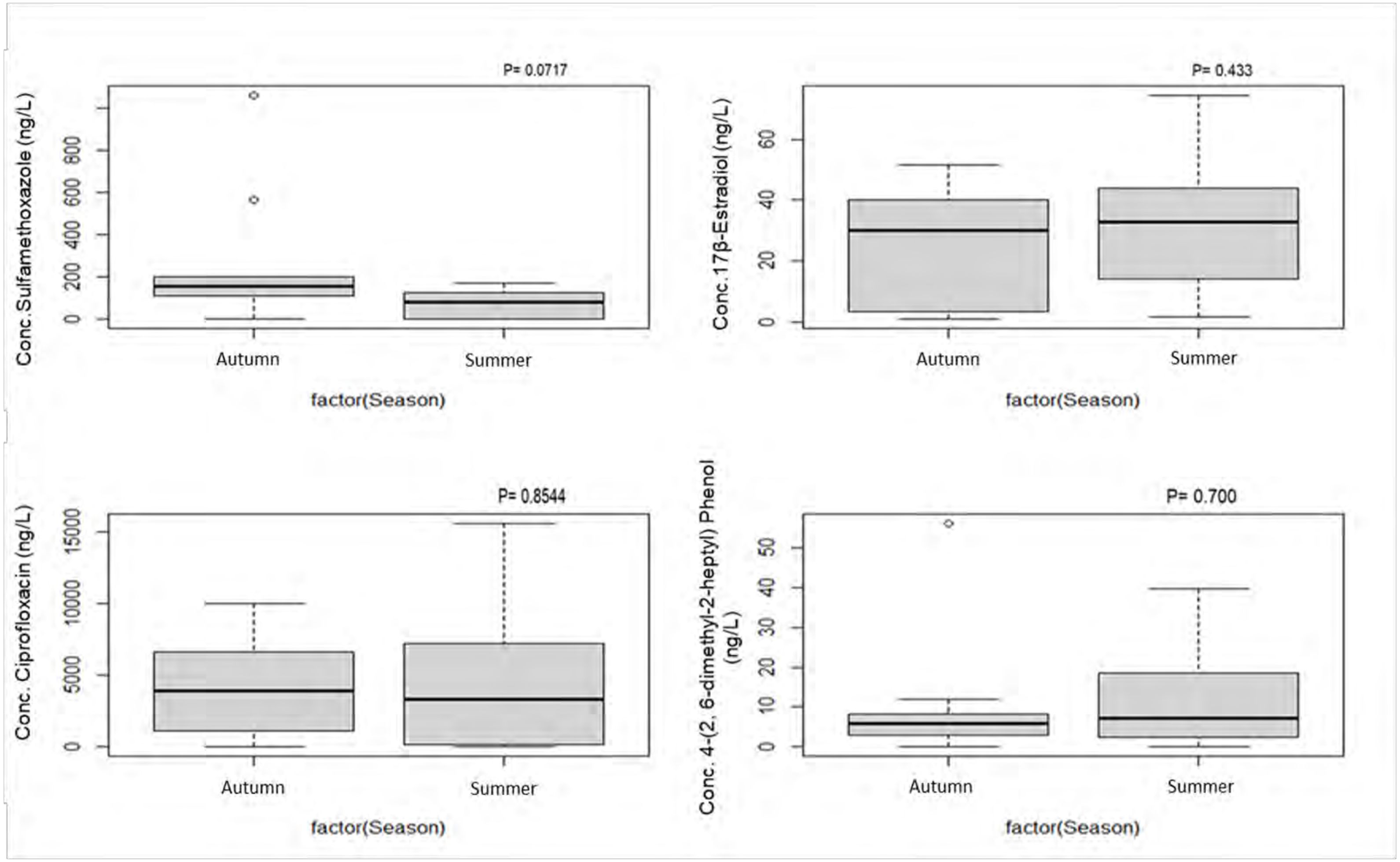


Figure 3.15: ANOVA (boxplots)-factor (seasons): Seasonal variability for the mean concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol adsorbed onto PET Type2 MPs in the Bloukrans River (January 2022 to February 2022 – summer; March 2022 to May 2022 – autumn).

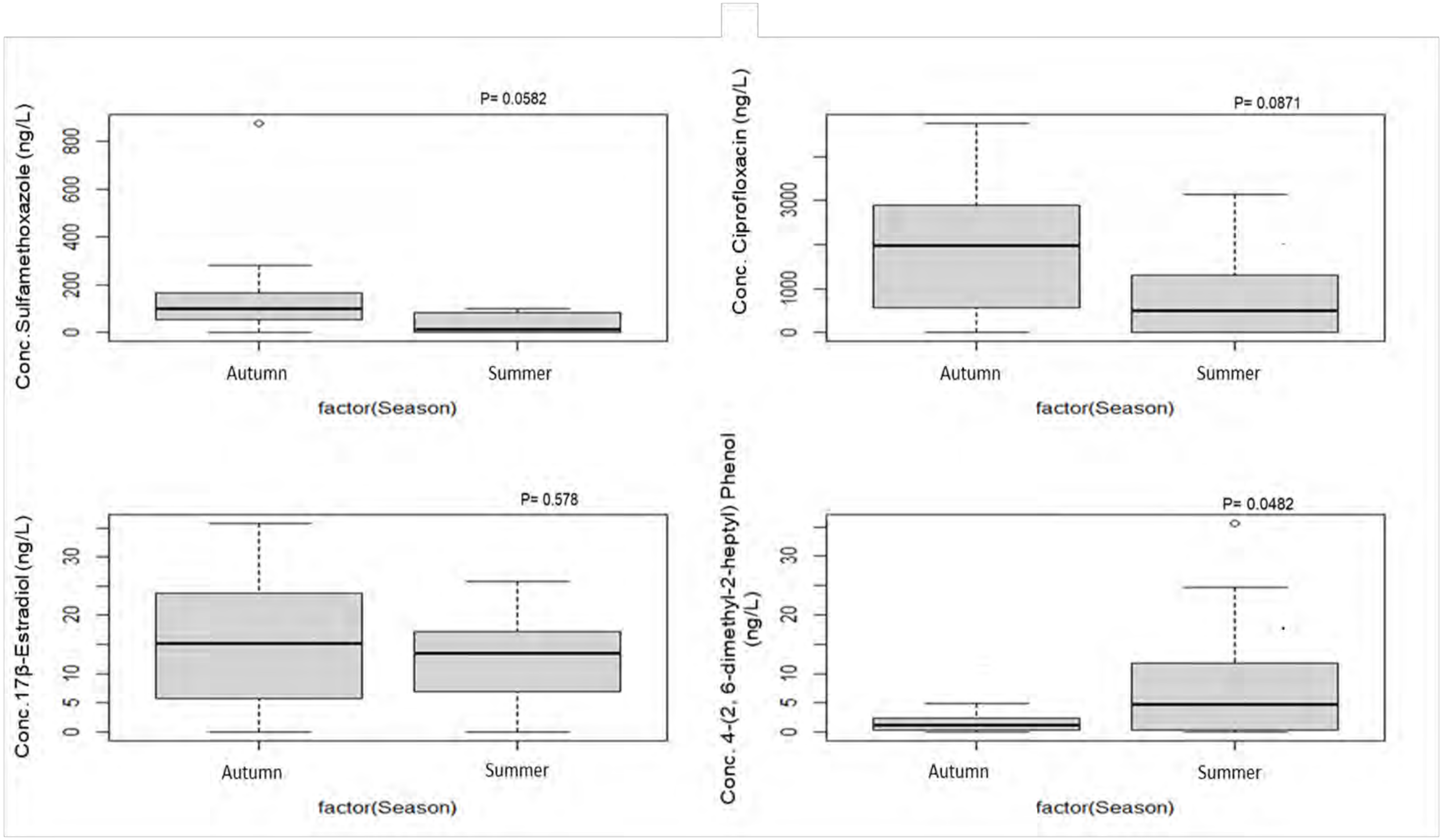


Figure 3.16: ANOVA (boxplots)-factor (seasons): Seasonal variability for the mean concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol adsorbed onto PET Type1 MPs in the Bloukrans River (January 2022 to February 2022 – summer; March 2022 to May 2022 – autumn).

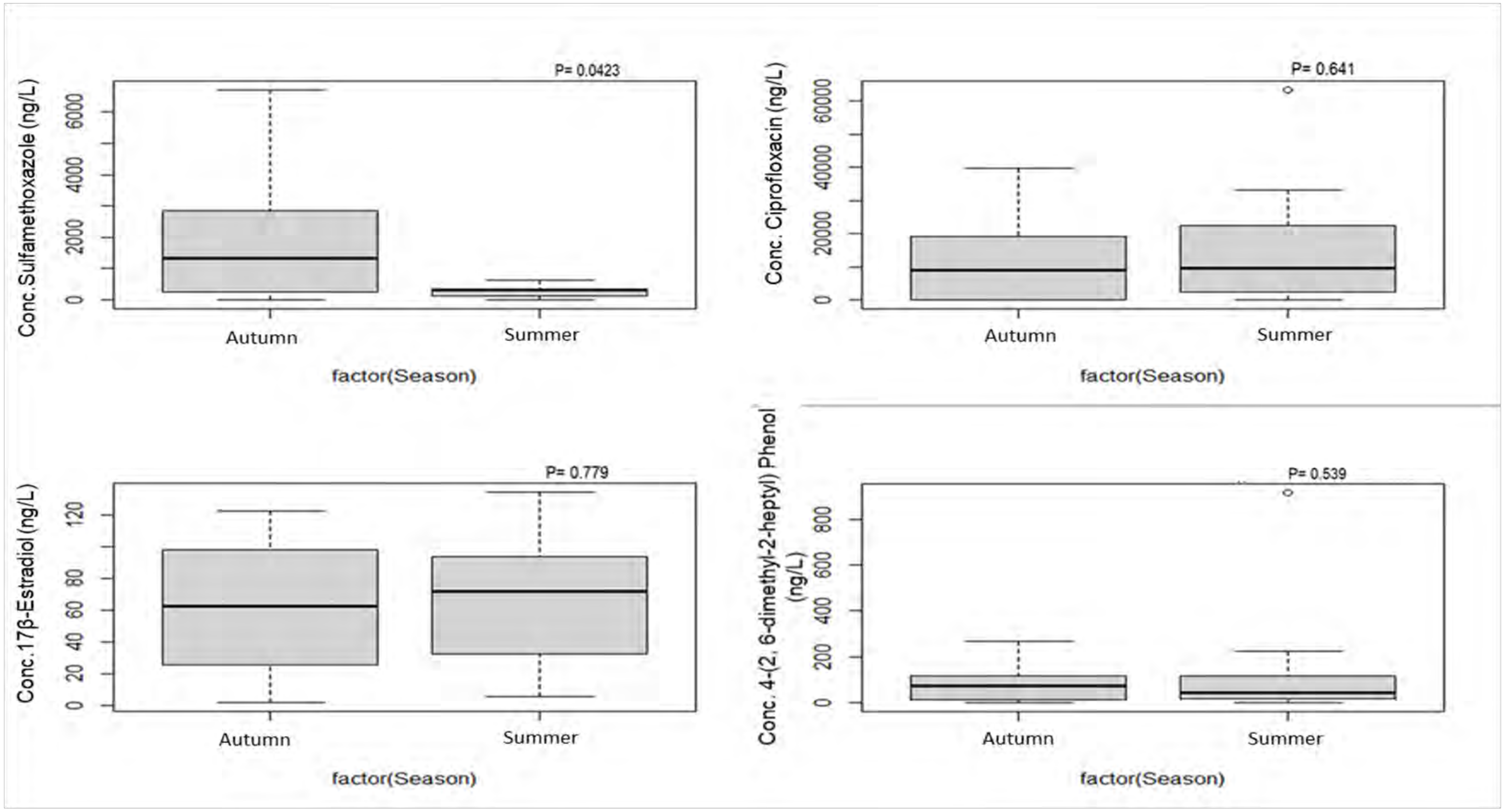


Figure 3.17: ANOVA (boxplots)-factor (seasons): Seasonal variability for the mean concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol adsorbed onto PP Type2 MPs in the Bloukrans River (January 2022 to February 2022 – summer; March 2022 to May 2022 – autumn). The same alphabet letter on the bars indicates no statistically significant difference (*P* > 0.05), whereas different alphabet letters show statistically significant difference (*P*< 0.05).

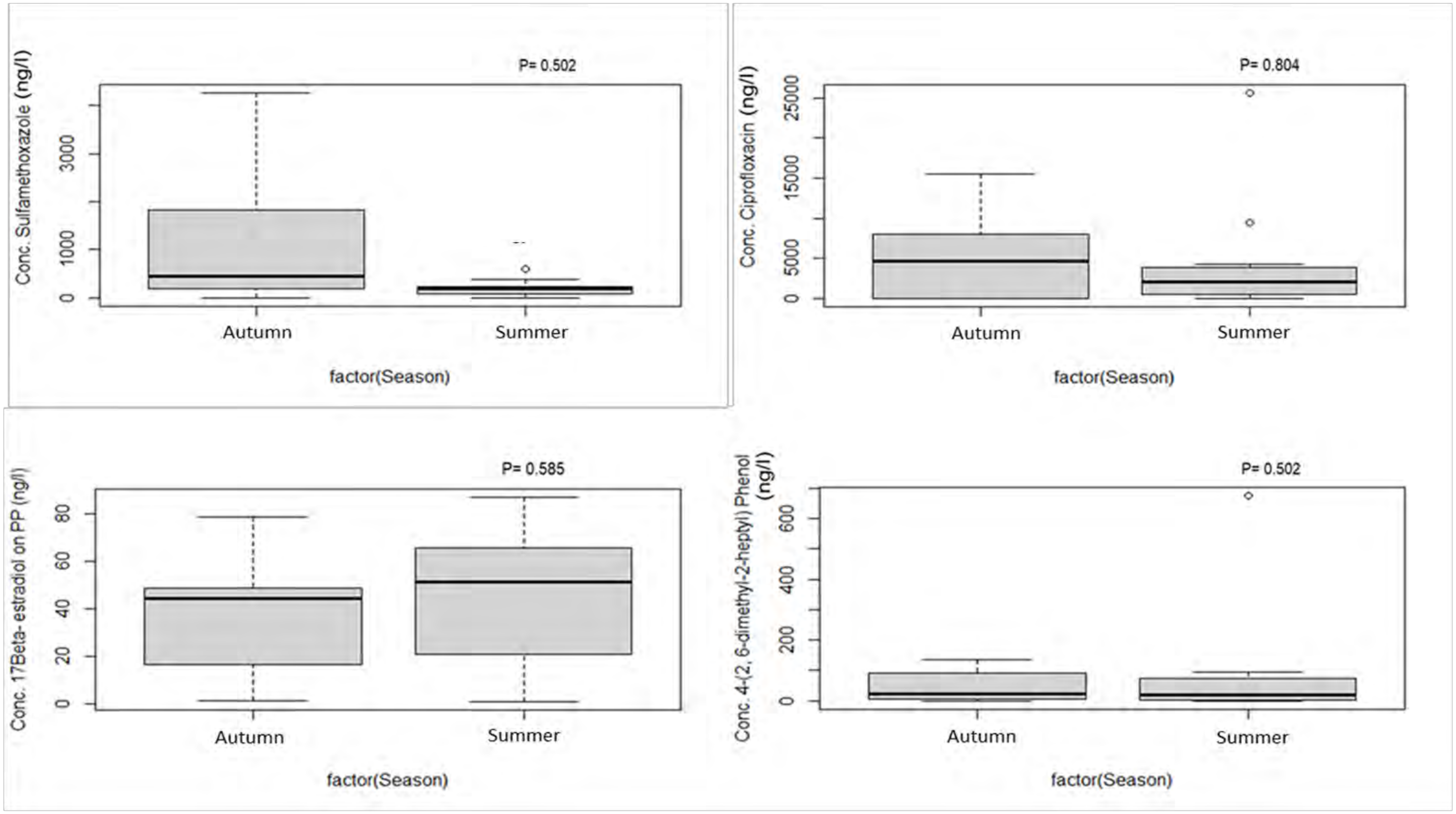


Figure 3.18: ANOVA (boxplots)-factor (seasons): Seasonal variability for the mean concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol adsorbed onto PP Type1 MPs in the Bloukrans River (January 2022 to February 2022 – summer; March 2022 to May 2022 – autumn).

3.4.3 Relationship between microplastic particle size and concentration of adsorbed chemical pollutants

The influence of microplastic size Type 2 (0.5mm<-≤2mm) and Type 1(2mm<-≤5mm) on the adsorption process was assessed (Figures: 3.19 – 3.21) and determined that size had an influence on the adsorption process.

The adsorbed concentration of sulfamethoxazole (94.4620 ngL^{-1}, B2 summer), ciprofloxacin (7232.4112ngL^{-1}, B2 summer) on Type2 PET MPs (0.5mm<-≤2mm) was approximately two and five orders of magnitude higher, respectively, than the recorded adsorbed concentration of the same compounds on the Type1MPs (2mm<-≤5mm): sulfamethoxazole (36.8212ngL^{-1}, B2 summer) and ciprofloxacin (1437.6061ngL^{-1}, B2 summer) - Appendix S:Table S1. Similarly, on Type 2 (0.5mm<-≤2mm), 4-(2, 6-dimethyl-2-heptyl) Phenol (32.3239ngL^{-1}) was twofold higher than that recorded on Type1 (2mm<-≤5mm) (18.0546ngL^{-1}) at the same site B2, in the same season summer. A similar trend was observed with the adsorbed concentration of 4-(2, 6-dimethyl-2-heptyl) Phenol (25.8657ngL^{-1}) on Type 2 (0.5mm<-≤2mm) as compared with (8.8733ngL^{-1}) (Appendix S: Table S1).

For PET MPs size differences, statistically significant differences ($P < 0.05$) were observed for adsorbed concentration of ciprofloxacin. In the case of sulfamethoxazole, 17β-estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol, no significant differences in the adsorbed concentration by the two PET MPs size ranges ($P > 0.05$, Figure 3.19).

In the case of PP MPs, the following observations were made: the adsorbed concentration of sulfamethoxazole (3380.214ngL^{-1}; B3 Autumn) ciprofloxacin(21326.16ngL^{-1}; B3 Autumn) on Type 2 (0.5mm<-≤2mm) were approximately one and two orders of magnitude higher, respectively, than the recorded adsorbed concentration of the same compounds on Type1 (2mm<-≤5mm), sulfamethoxazole (1937.146ngL^{-1}; B3 Autumn) and ciprofloxacin (7753.592ngL^{-1}; B3 Autumn) (See Appendix S:Table S2).

Findings for17β-estradiol were: (48.7525ngL^{-1}) on Type1, compared with (90.9184ngL^{-1}) on Type2. In the case of 4-(2, 6-dimethyl-2-heptyl) Phenol, measurements on Type1 were (40.2513ngL^{-1}) compared with (378.8924ngL^{-1}) at B3 in the same season. Overall, higher adsorbed concentrations were recorded on Type2 (0.5mm<-≤2mm) smaller PP MPs than on

Type 1 (2mm<-≤5mm), bigger PP MPs. Statistically at $P = 0.005$, there were significant differences ($P > 0.05$) in the adsorbed concentrations of sulfamethoxazole, ciprofloxacin, and 17β-Estradiol (Appendix S: Table S2). For 4-(2, 6-dimethyl-2-heptyl) Phenol there were no significant differences in the concentration on the two PP MPs size ranges ($P < 0.05$, Figure 3.21).

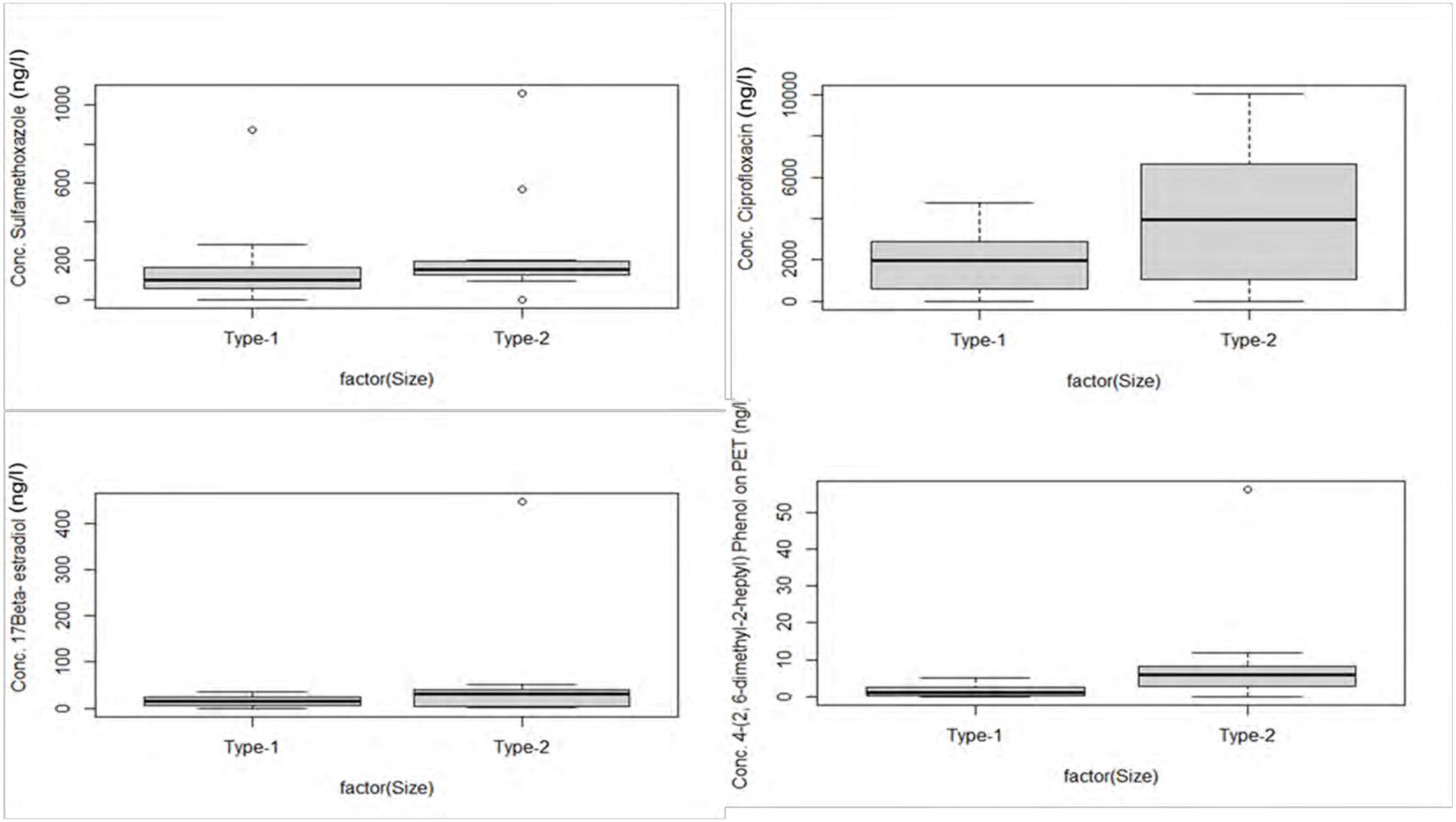


Figure 3.19: ANOVA (boxplots)-factor (MP – size range) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol adsorbed onto on the PET MPs, Type1 (2mm<-≤5mm), and Type 2 (0.5mm<-≤2mm), during the autumn season in the Bloukrans River (March 2022 to May 2022).

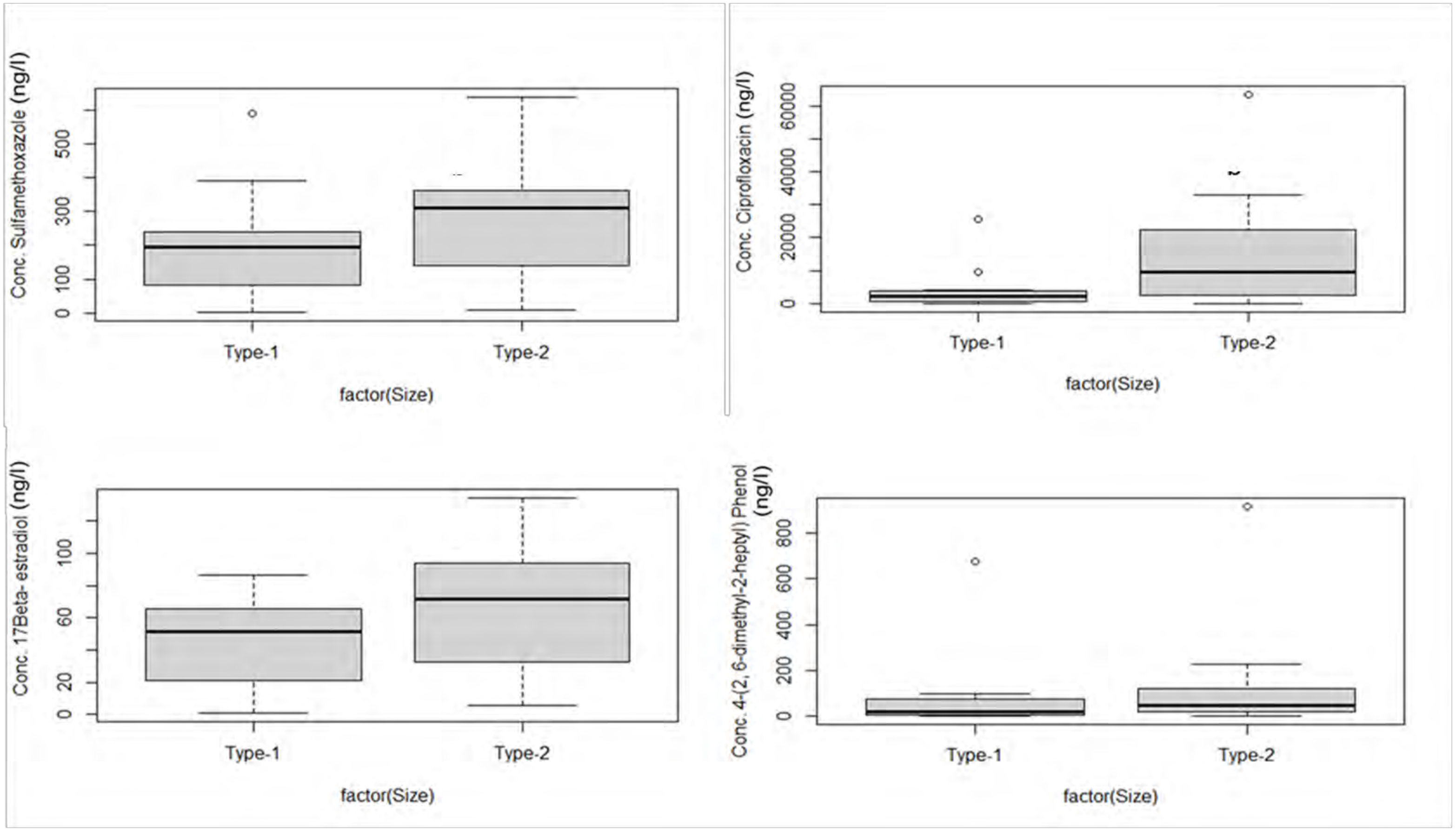


Figure 3.20: ANOVA (boxplots)-factor (MP – size range) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol adsorbed onto on the PP MPs, Type1 (2mm<-≤5mm), & Type 2 (0.5mm<-≤2mm), during the summer season in the Bloukrans River during the study (January 2022 to February 2022).

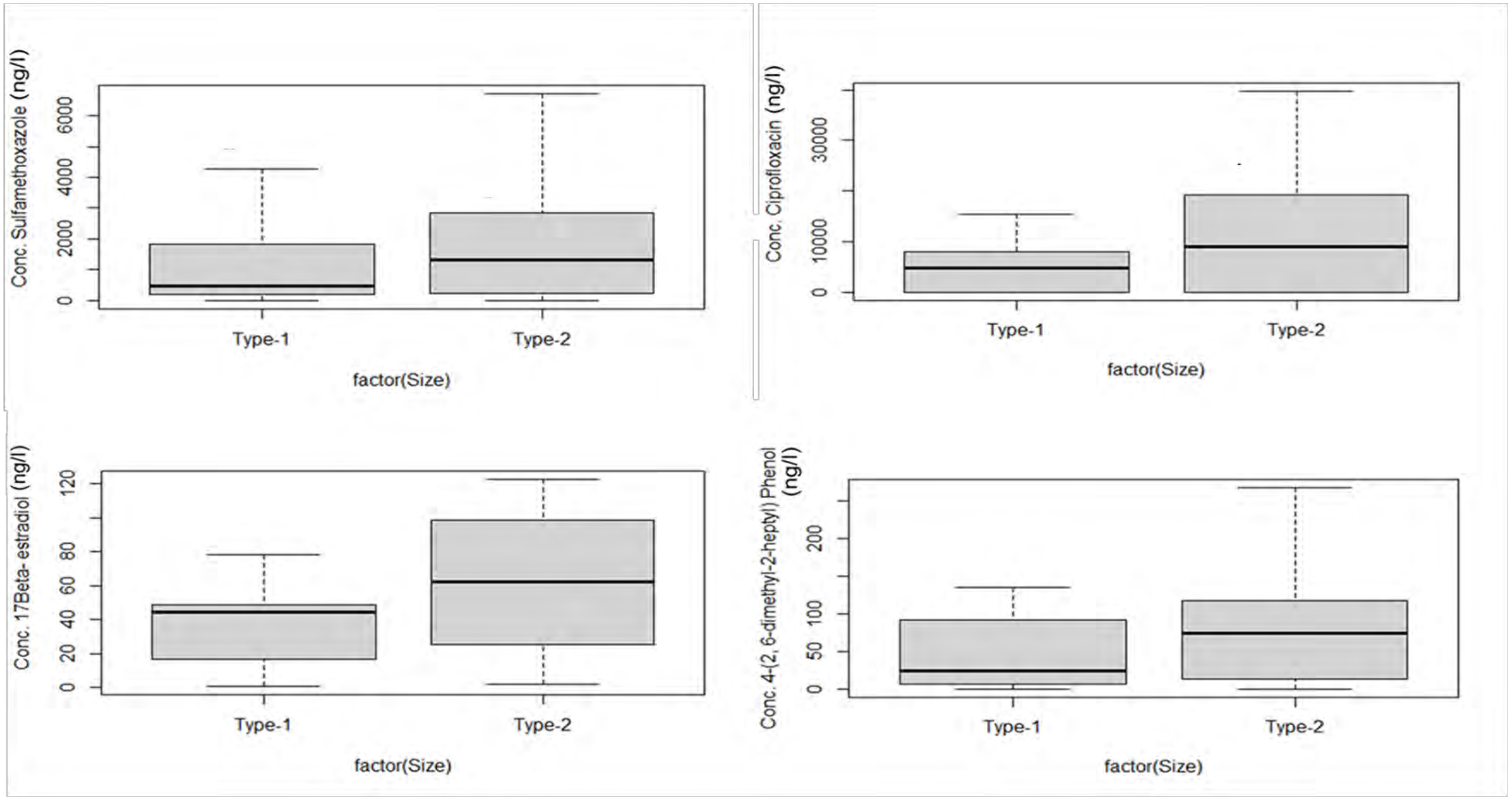


Figure 3.21: ANOVA (boxplots)-factor (MP – size range) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol adsorbed onto on the PP MPs, Type1 (2mm<-≤5mm), and Type 2 (0.5mm<-≤2mm), during the autumn season in the Bloukrans River during the study (March 2022 to May 2022).

3.5 Discussion

Sulfamethoxazole, ciprofloxacin, 17β-estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol were all detected on the deployed MPs, that is, PET and PP at the study sites during the two seasons. The MPs' adsorptivity of chemical pollutants could be explained by the chemical properties of MPs, including size that influences the MP surface area, coupled with the high-fat solubility and low water solubility of organic compounds targeted (Santana-Viera et al., 2021). Additionally, polar functional groups, like carbonyl groups in PET MPs, coupled with the non-porous nature of PP, have been identified in the literature as contributing to forming H-bonding that facilitates the adsorption process (Santana-Viera et al., 2021; Santos et al., 2021).

The smaller-sized MPs (MPType2) were correlated with higher adsorbed concentrations of the organic chemicals; that is, the adsorption capacity was in the order MPType2 > MP Type1. The highest concentrations of sulfamethoxazole, ciprofloxacin, 17β-estradiol and 4-(2, 6-dimethyl-2-heptyl)Phenol on PP Type2 recorded was 3380.2141ngL^{-1}, 30285.1911ngL^{-1}, 24.8066ngL^{-1}and 90.9184ngL^{-1}, respectively, compared to PP Type1 MPs where the highest concentration was, 1937.1461ngL^{-1}, 11119.6123ngL^{-1}, 56ngL-1, 62ngL^{-1}, respectively. The same trend was observed with PET MP size ranges (Table S1-S2 – appendix S). These results were consistent with studies elsewhere, especially under laboratory conditions (H. Li et al., 2021; X. Zhang et al., 2019). The high adsorptive trends of small MPs have been ascribed to the enlarged surface area of the MPs (Mo et al., 2021; X. Liu et al., 2022). This implies that the smaller MPs and the pollutants could easily be transported within the ecosystem and thus increases the threat to aquatic organisms.

The different adsorbed concentrations of individual organic compounds at the different study sites resulted from specific site characteristics (land-use types), such as surrounding land use, proximity of wastewater treatment works, animal husbandry and crop farming activities, and river hydrology (e.g., water flow). Generally, the concentration of the adsorbed organic compounds was significantly different between different land-use types considered in this study (Figure 3.5 – Figure 3.10). This finding highlights the critical role spatial factors play in influencing the adsorption of pollutants by microplastics in water systems.

The specific site characteristics can affect the interactions of microplastics with pollutants, ultimately influencing the adsorption processes. Among the organic compounds detected on the retrieved MPs (on both PET and PP), Ciprofloxacin was present at greater concentrations than sulfamethoxazole, 17β-estradiolor4-(2, 6-dimethyl-2-heptyl) Phenol at all the sites along the Bloukrans River (Figure 3.5–3.6). The concentration of ciprofloxacin recorded in the targeted sites ranged from 287.0400 to 10033.597 ngL^{-1} and could be explained by the differences in hydrophobicity (log Kow and *p*Ka) of the targeted organic compounds as a critical factor affecting the adsorption process. Studies have shown that fluoroquinolones have higher adsorptive properties than sulphonamides, as they can form ionic structures owing to their low *p*Ka values(Harrower et al., 2021, and references therein).

High concentrations of Sulfamethoxazole detected on MPs retrieved downstream of agricultural- and WWTWs impacted sites could be explained by the possible application of this antibiotic in animal medicine and its introduction to the river water as a component of runoffs. This agrees with the notion that animal husbandry and human waste, with WWTWs, are critical sources of fluoroquinolones in surface waters, representing the origin of the organic compounds adsorbed onto the MPs. Sulfamethoxazole is resilient to degradation and thus lasts in the environment. The detection of 17β-Estradiol on the retrieved MPs may be due to municipal waste deposited directly into the river (with the highest concentration recorded near the municipal/informal-settlement-impacted site B1). These observations suggest that a significant amount of Grahamstown's household wastewater does not pass through sewers or that free cattle grazing along the Belmont Valley contributes to the evidence of the hormone in the retrieved MPs.

From the perspective of temporal differences, the slight differences observed in concentrations adsorbed during the study seasons, summer-autumn and the period of deployment (over 35 days), could be accounted for by seasonality: during the cold season (autumn), the residence time of MPs and chemical pollutants is extended in river systems. It could influence adsorptivity(C. Li et al., 2020) (Figures3.11–3.14. In the rainy (summer) seasons, more extreme flows exacerbate the MP-pollutant interactions as the chemical pollutants that had previously been trapped in the sediments are re-suspended; however, this could favour desorption in addition to adsorption (Hurley et al., 2018; Talbot & Chang, 2022). Nevertheless, several studies have shown that there is always a higher concentration of chemical pollutants that could lead to higher adsorptivity in the rainy season (high-flow) compared to the dry season (low-flow) (He et al., 2013; F. Li et al., 2020; Harrower et al., 2021). The low adsorptivity during cold conditions has been explained by the stability of the pollutants during wet seasons, as they are not likely to break down because temperatures tend to be low (Harrower et al., 2021). This could explain the non-significant ($P> 0.05$) differences in the adsorbed concentration of the organic chemicals between the two seasons observed in the data analysis.

The complex nature of different environmental water systems that can vary on an hourly or daily basis can affect the partitioning of the organic compounds between water and the microplastics, producing varied adsorption concentration rates over a given periodic range (Kinigopoulou et al., 2022), hence the non-linear increase of MP-adsorbed loads of chemicals from 7 days to 35 days (Figures3.11–3.14).

3.6 Conclusion

This chapter focused on the adsorption behaviour of sulfamethoxazole, ciprofloxacin, 17β-estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol by PET and PP microplastic particles using an *in situ* experiment along the Bloukrans River. All four targeted compounds were present on the retrieved MPs, with adsorption of the organic chemical pollutants by MPs significantly correlated with the type of land use in the Bloukrans River catchment. Along the river, the concentration of adsorbed pollutants varied in response to impacts of the nearby land-use types, such as municipal waste-impacted sites, wastewater treatment works and agriculture-impacted sites, acting as chemical pollutant point sources.

Downstream site B3 recorded relatively higher mean concentrations of the pollutant, most likely due to the cumulative effect from upstream polluted sites of the river channel (B1 and B2). This means that MPs' adsorptivity for pollutants and associated ecological risks are exacerbated in severely polluted areas.

Although no significant differences in adsorbed concentration of the pollutants between seasons were observed, there were noticeable differences in concentrations adsorbed over the period of deployment, an indication of the interplay of both spatial and temporal factors in influencing the complex and dynamic adsorption patterns, with pollutant adsorption varying across different locations and periods. This highlights the importance of considering spatial and temporal dimensions when assessing microplastic-pollutant interactions in aquatic environments. However, more importantly, it indicates that integrating spatial and temporal considerations into microplastic research while developing and designing monitoring programmes allows the environmental risks associated with microplastic pollution to be evaluated and for regionally targeted mitigation strategies developed.

The adsorption capacity of the targeted chemical pollutants on both PET and PP microplastics was significantly influenced by the size of the MPs deployed ($P < 0.05$), underscoring the significant role microplastic size plays in influencing the adsorption of pollutants in water systems. As microplastic particles decrease in size, their specific surface area, which measures the surface area per unit volume, increases. This increase in specific surface area provides more binding sites for pollutants to attach to, leading to enhanced adsorption capacity (Mo et al., 2021). However, because the deployed microplastics and the river water phases were not in equilibrium, adsorption processes may not only have been affected by the physicochemical properties of MPs (especially size) but also by other factors, such as molecular weight of the chemical pollutants and salinity of the environmental, which could influence the whole adsorption process. Nevertheless, these results indicate the partitioning behaviour (adsorption processes) in the natural dynamic environmental river water system.

The role of microplastic size in influencing the adsorption of pollutants in water systems has several implications for pollutant dynamics and potential environmental impacts, including increasing the potential exposure of organisms to pollutants, with microplastics acting as vectors for contaminants. First, as organisms ingest microplastics, the pollutants accumulate in their tissues, leading to bioaccumulation. Second, adsorption contributes to the overall load of pollutants in water systems, potentially affecting water quality and posing risks to human and ecosystem health. This knowledge can inform the development of effective mitigation strategies, such as reducing microplastic inputs into waterways and developing technologies to remove microplastics and associated pollutants from water systems.

CHAPTER 4: Effects of Selected Plastic Properties and Spatial-Temporal Variability on the Adsorption of Selected Pharmaceutical Pollutants and Endocrine-Disrupting Compounds on Microplastics in the Swartkops River, Eastern Cape, South Africa

4.1 Introduction

The Swartkops River lies in the urbanised and industrialised part of the Eastern Cape (Chapter 2, Section 2.2.1.2) (Binning & Baird, 2001; Department of Environment Forestry and Fisheries, 2020). Like many other urban areas, the sources, pathways, contamination and receiving compartments of microplastics still need to be clarified (DFFE, 2018, 2020; Verster & Bouwman, 2020). The river catchments cover large urbanised areas of the Nelson Mandela Metropolitan Municipality that include Uitenhage, kwaNobuhle, Dispatch and extend into the Port Elizabeth/Gqeberha municipal areas (Nyawo, 2017; Odume et al., 2022).

Over the years, Swartkops River water quality has progressively degraded as a result of contamination due to high-density anthropogenic activities, chiefly agriculture practices, industry activities from the Uitenhage municipality, plastic recycling plants, WWTWs, informal and urban settlements, with the most significant contributor to the river pollution being industrialised and municipal waste discharge (Nyawo, 2017; Mgaba et al., 2022; Odume et al., 2022). Odume et al., 2022 observed that the Swartkops River faces the urban river syndrome (that is, the accelerating decline of the river ecology)and made numerous recommendations on the need to protect the water resources from this river system(Odume et al., 2022).

Microplastic’s ability to adsorb/absorb chemical pollutants, as explained in Chapter 3, can be influenced by a complex interplay of many factors, especially in an urban river system, and these factors could mediate this interaction differently (Karapanagioti & Werner, 2019). The unique characteristics of different river systems can impact both the presence and behaviour of microplastics and their associated contaminants (Kataoka et al., 2019; Al-Zawaidah et al., 2021). For instance, urban rivers are characterised by lower flow (low gradient rivers) (Kataoka et al., 2019), which can allow microplastics to settle and interact with pollutants for more extended periods, facilitating adsorption (Karapanagioti & Werner, 2019). Urban river systems are also prone to abundant organic matter that can compete with pollutants, reducing their adsorption onto microplastics(McCormick et al., 2016; Wang et al., 2021). Conversely, studies have indicated that organic matter can act as a vector of both microplastics and pollutants in water systems(Al-Zawaidah et al., 2021; Fu et al., 2021; Wang et al., 2021).

With spatial-temporal factors influencing river characteristics, like organic matter content, sediment composition, flow dynamics, biological activity, river channel morphology, water chemistry, and pollutant types(Kataoka et al., 2019) investigating the adsorption of chemical pollutants by MPs from an urban river perspective is crucial in identifying hotspots of potential risks associated with MPs pollution(Birch et al., 2020; C. Wang et al., 2022). As stated earlier, urban rivers often face increased pollution loads, altered flow regimes, and reduced habitat diversity, creating complex interactions between MPs and pollutants for higher adsorption in specific areas (Jambeck et al., 2018; Verster & Bouwman, 2020).

Microplastic research on how urban river land-use specificities influence the interaction between MP-pollutants is critical for predicting the fate and transport of these contaminants in different environments and in developing targeted mitigation strategies and remediation approaches tailored to specific river systems (Birch et al., 2020). Furthermore, because of increased plastic waste generation in cities, studies of plastic pollution in urban watersheds, particularly those with large population densities and industrial activities, are critical in terms of source apportionment and transport of both MPs and emerging chemical contaminants of concern in aquatic ecosystems (Birch et al., 2020; Wang et al., 2022). As detailed in Chapter 3, land-use patterns in urban catchments are linked to different sources of MP accumulation and organic chemical pollutants. Spatial variability of MP contamination are critical factors in the emergence of MPs in urban runoffs (Xia et al., 2020; Wang et al., 2022).

4.2 Materials and Methods.

The materials, methodology and statistical data analysis followed in this chapter are described in Chapter 3 (deployment along the Bloukrans River; Section 3.2.1 to Section 3.2.8).

The deployment methodology followed the same experimental design described in section 3.2.4, chapter 3.Like in Bloukrans River, PP and PET MPs deployment in Swartkops River was done once in each of the two season’s i.e. summer on 21th, January 2022 and autumn on 8th, April 2022.

Microplastics retrieval

At periodic intervals of 7, 14 and 35 days calculated based from the day of deployment in each of the seasons (detailed experimental design similar to the of Bloukrans River Figure 3.2, section 3.2.4), a total of four mesh bags, two containing each polymer (PP or PET MP) of the respective size was retrieved per site. The retrieved mesh bags containing PP and PET separately were placed into cooler boxes and transported back to the water quality laboratory of the Institute for Water Research (IWR), Rhodes University. Samples were analysed within 24 – 48 hours after each retrieval.

Treatment of the PP and PET MPs after retrieval in each of the seasons, plus quantification of adsorbed concentration, and pollutants compound identification followed similar methodologies described in sections: 3.2.5, 3.2.6 and 3.2.7 respectively. (See chapter 3)

4.3 Statistical data Analysis

As described in Chapter 3, the data were subjected to univariate and multivariate statistical analyses to indicate statistical differences and elucidate relations and trends. The summary statistics were performed using the R Core Team (2022). Before analysis, the data were captured and prepared in Excel (Microsoft 2010 Office) and exported to R software packages Version R 4.3.0. The normality of the data was tested using the Shapiro-Wilk normality test. Non-parametric tests were chiefly used because most data violated the assumption of normality. The Kruskal-Willis test was used to compare the concentration of the same organic compound between study sites, followed by Tukey's honestly significant difference test (Tukey's HSD) for multiple comparisons. Statistical significance was set at $P < 0.05$ for all the tests.

4.3.1 Box and whisker plots

Box plots were used to represent the spatiotemporal variability of the concentration of adsorbed organic chemical pollutants based on:(i) land-use type as a factor (S1, S2, S3, and S4);(ii) MPs size range as a factor (Type1 (2mm<-≤5mm), and Type2 (0.5mm<-≤2mm)), and (iii) season as a factor (summer and autumn). The box plots display summary statistically significant differences.

4.3.2 The Kruskal-Willis test

Being a non-parametric univariant, the Kruskal-Willis multiple comparison tests was used to determine the significant differences of organic chemical pollutant concentrations adsorbed in terms of study sites (land-use type), microplastic size ranges (Type1 and Type2), and seasonal differences. R software package Version R 4.3.0 was used.

4.3.3 Two-factor analysis of variance (ANOVA)

As a parametric test, the analysis of variance the Two-way (ANOVA)was used to test for differences in the means of the measured concentration of the same organic chemical pollutants adsorbed on the MPs using both sites and days (period) as factors. This was followed by Tukey's honestly significant difference test (Tukey's HSD) for multiple comparisons to indicate land-use types that differed. Statistical significance was set at $P < 0.05$ for all the tests.

Before using Two-way ANOVA, the basic assumption of normality was investigated using the Shapiro-Wilk normality test. Data from Swartkops River greatly violated the assumption of normality, thus non-parametric tests were used. R software package Version R 4.3.0 was used.

4.4 Results

4.4.1 Spatial-temporal variability of the adsorbed organic chemical pollutants

Spatial differences in concentration adsorbed (based on land-use differences).

Three out of the four targeted pharmaceutical pollutants were detected from MP samples retrieved from the Swartkops River, that is, sulfamethoxazole, ciprofloxacin and 17β-estradiol. The 4-(2, 6-dimethyl-2-heptyl) Phenol was not detected on any of the MPs samples retrieved.

Spatial differences in the adsorbed concentration were observed, for instance: in the case of Sites S1 to S4, for PET Type2 MPs, the highest adsorbed concentrations of ciprofloxacin were at Site S3 (132.6525ngL^{-1}), an agriculture-impacted area, and the lowest at S1 (15.4381ngL^{-1}) a WWTW-impacted area. The highest adsorbed concentration of sulfamethoxazole was at Site S1 (176.0181ngL^{-1}) and lowest at S3 (86.6515ngL^{-1}). The highest concentration of 17β-estradiol, was detected at site S3 (11624.5611ngL^{-1}), followed by site S1 (498.9038ngL^{-1}). Similar to sites along the Bloukrans River, the control SiteS4, recorded the lowest mean concentrations of the targeted chemical pollutants, that is, (1.064e-4ngL^{-1}), ND, and (2.0e-4ngL^{-1}) for ciprofloxacin, sulfamethoxazole, and 17β-estradiol, respectively (Table S3: Appendix S).

For PET MPs Type1, the highest concentration of ciprofloxacin was recorded at Site S2 (264.8761ngL^{-1};Table S3: Appendix S). The mean highest concentrations of sulfamethoxazole, and 17β-EstradiolwereatS2, (92.2214ngL^{-1}), and S1 (178.3661ngL^{-1}).Lowest concentrations of sulfamethoxazole, ciprofloxacin, and 17β-estradiolwererecorded at S2 (59.0895ngL^{-1}), S1 (55.2396ngL^{-1}) and S3(116.2456ngL^{-1}), respectively.

For PP Type1, the highest mean adsorbed concentration for sulfamethoxazole was169.3613 ngL^{-1} at site S2, followed by (160.0227 ngL^{-1}) at S3and the lowest at S1 (101.0469 ngL^{-1}). The highest concentration of 17β-estradiolwas at S1 (54.0228 ngL^{-1}), followed by S2 (53.2831 ngL^{-1}) and the lowest at S3 (22.9989 ngL^{-1})(Table S4- appendix S). The mean highest concentration of Ciprofloxacin adsorbed was at S1 (70.3114ngL^{-1}), followed by S2 (55.1876 ngL^{-1}), and lowest at S3 (33.9281 ngL^{-1}). In the case of PP Type2 MPs, ciprofloxacin was highest at S2 (264.876 ngL^{-1}), followed by S1 (234.9993 ngL^{-1})and lowest at S3 (123.1972 ngL^{-1}).

For sulfamethoxazole, the highest concentration adsorbed was recorded from S3 (1762.1391 ngL^{-1}), followed by S2 (530.3466ngL^{-}) and lowest at S1 (214.0419 ngL^{-1}) (Table S4 – appendix S). In the case of 17β-estradiol, the highest adsorbed concentration recorded was from S2 (19613.2610 ngL^{-1}), the municipal waste discharge site, and the lowest at S3 (4913.3441 ngL^{-1}), a mainly agriculture area.

Generally, S1 (WWTW-impacted area) recorded higher concentrations of the three chemicals, sulfamethoxazole, ciprofloxacin, and 17β-estradiolin comparison to other sites for both PP and PET MPs (Figures4.1- 4.2), with the exception of 17β-Estradiol on PET Type2 MP (Figure 4.2C). Statistically, the mean concentrations recorded at S1, S2, and S3 differed significantly from those recorded at S4 ($P < 0.05$, Tukey HSD post-hoc test; Table U1, appendix U). In all cases, the recorded concentration from Site 4 differed significantly from S1, S2, and S3 (Figures4.3–4.6).

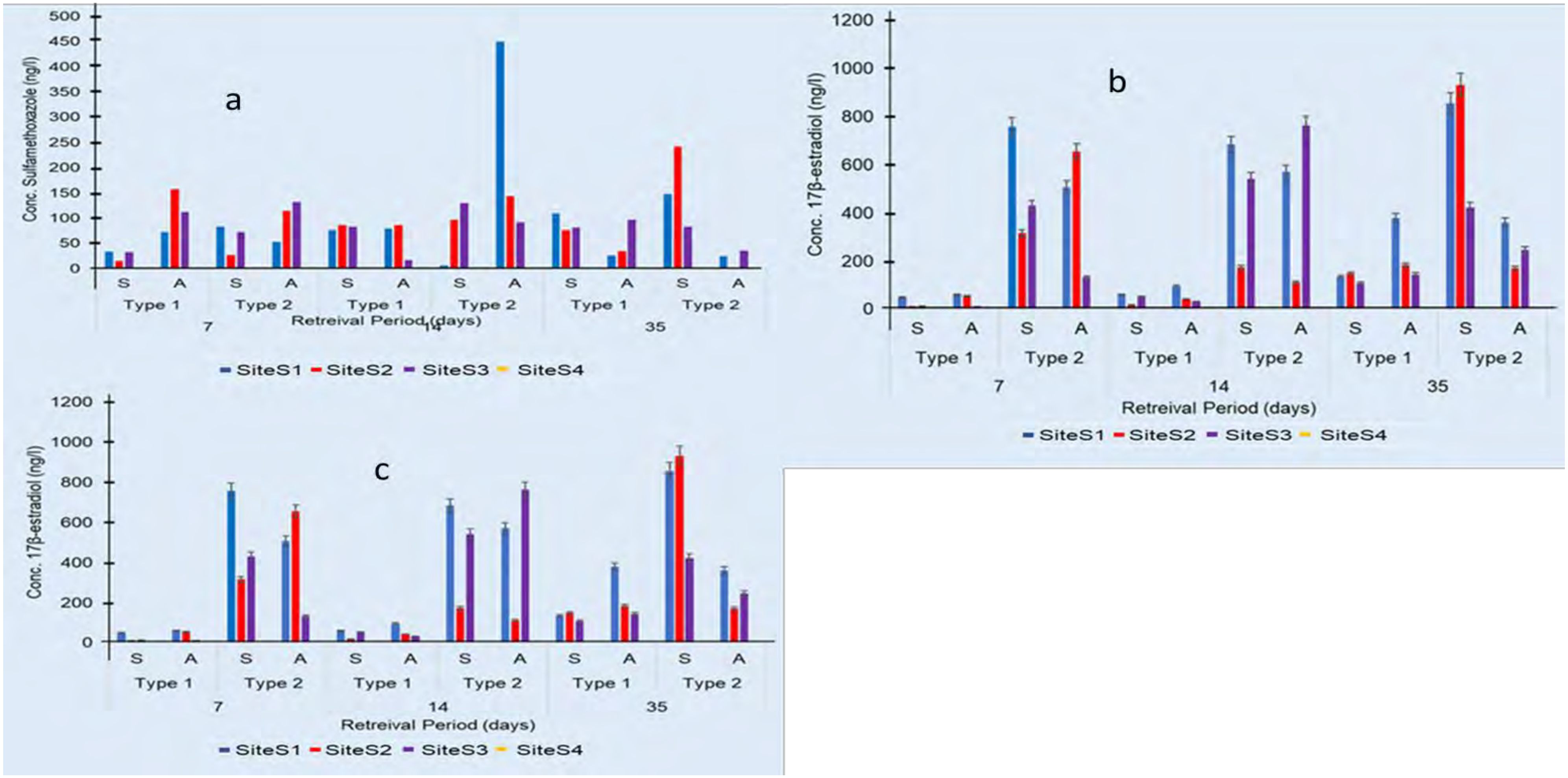


Figure 4.1: Spatial, temporal, and seasonal variability of adsorbed concentration of Sulfamethoxazole (a), Ciprofloxacin (b), and 17β-Estradiol (c) onto PET Type1 and Type2 MPs at the four study sites: S1, S2, S3 and S4 in the Swartkops River over two seasons, S = summer and A = autumn during the study period. Standard error (bars).

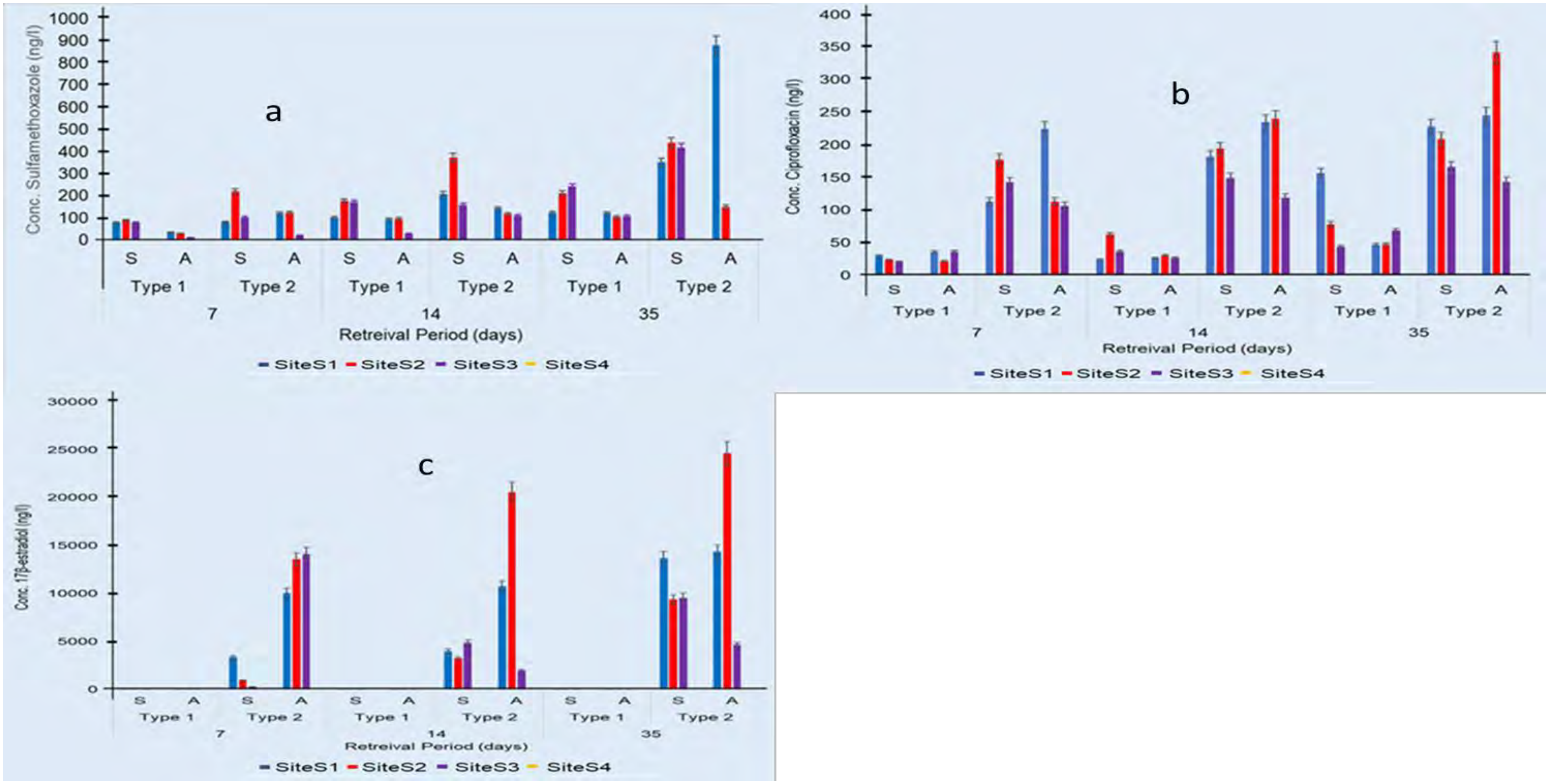


Figure 4.2: Spatial, temporal, and seasonal variability of adsorbed concentration of Sulfamethoxazole (a), Ciprofloxacin (b), and 17β-Estradiol (c) onto PP Type1 and Type2 MPs at the four study sites: S1, S2, S3 and S4 in the Swartkops River over two seasons, S = summer and A = autumn during the study period. Standard error (bars).

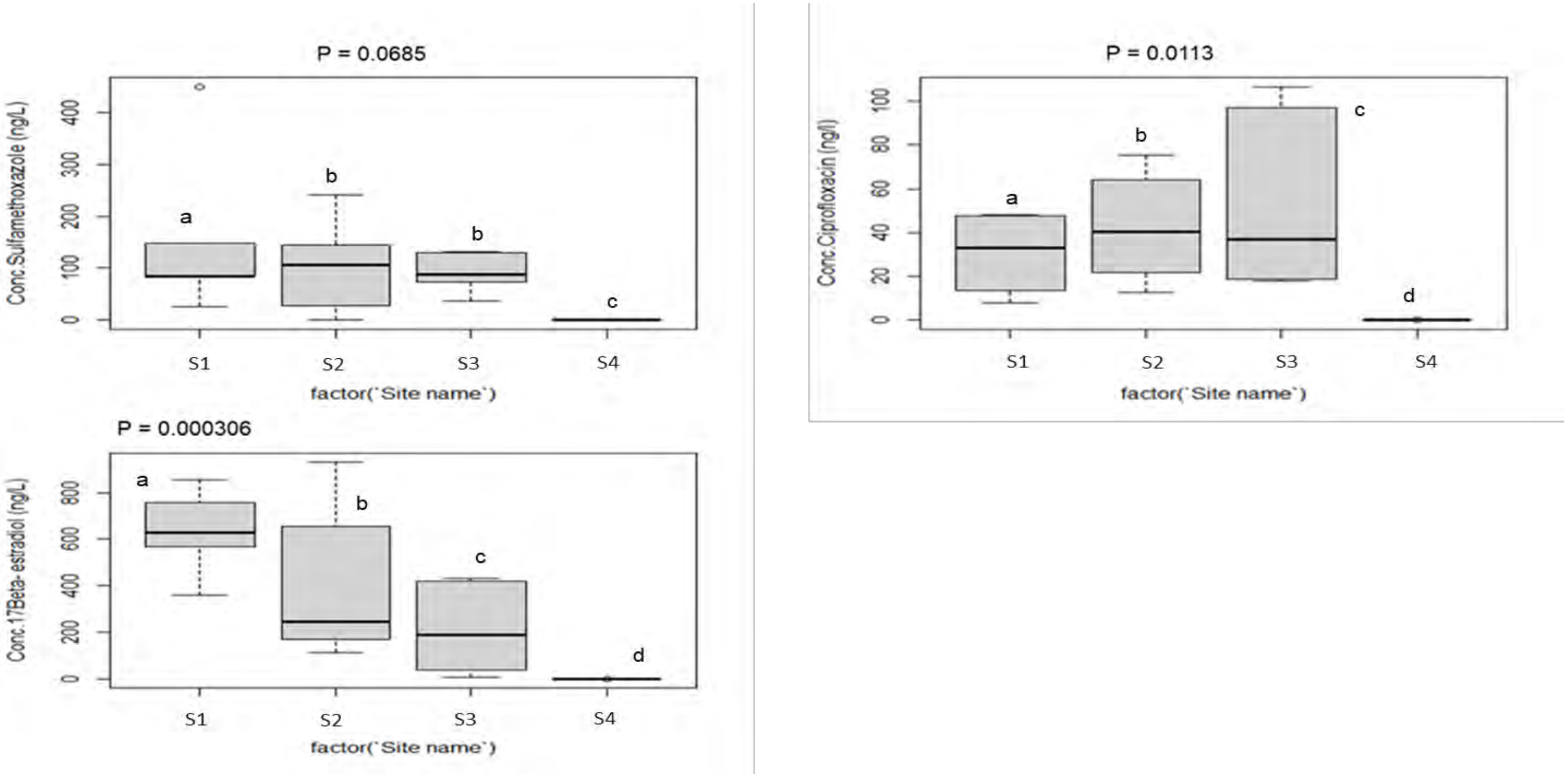


Figure 4.3: ANOVA (boxplots)-factor (sites) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol adsorbed onto PET Type-2 microplastics at the four study sites in the Swartkops Rivers (S1, S2, S3 and S4) during the study (January 2022 to May 2022 – summer to autumn). The same alphabet letter on the bars indicates no statistically significant difference ($P > 0.05$), whereas different alphabet letters show statistically significant difference ($P < 0.05$).

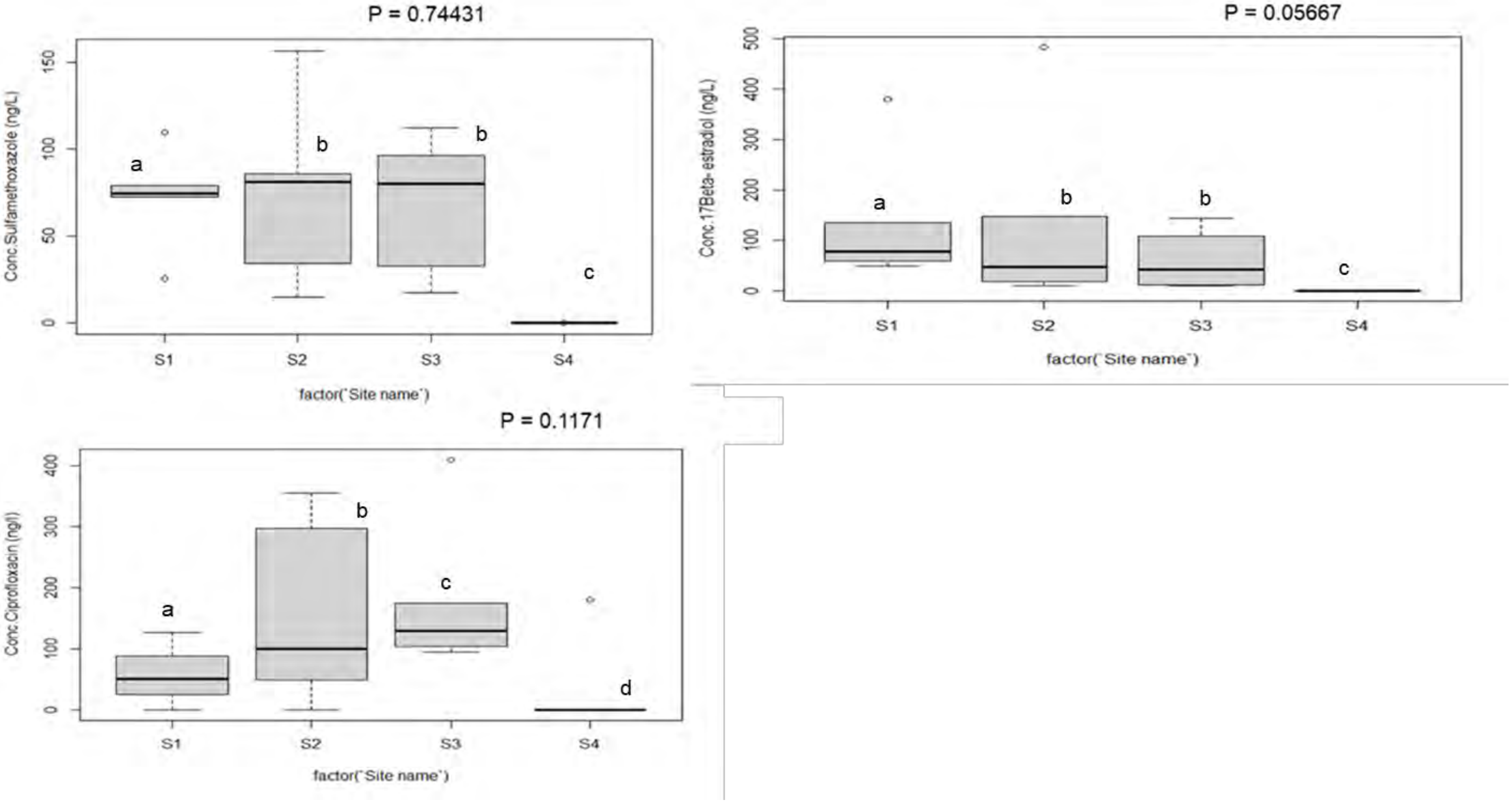


Figure 4.4: ANOVA (boxplots)-factor (sites) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol adsorbed onto PET Type1 microplastics at the four study sites in the Swartkops Rivers (S1, S2, S3 and S4) during the study (January 2022 to May 2022 – summer to autumn). The same alphabet letter on the bars indicates no statistically significant difference (P > 0.05), whereas different alphabet letters show statistically significant difference (P < 0.05).

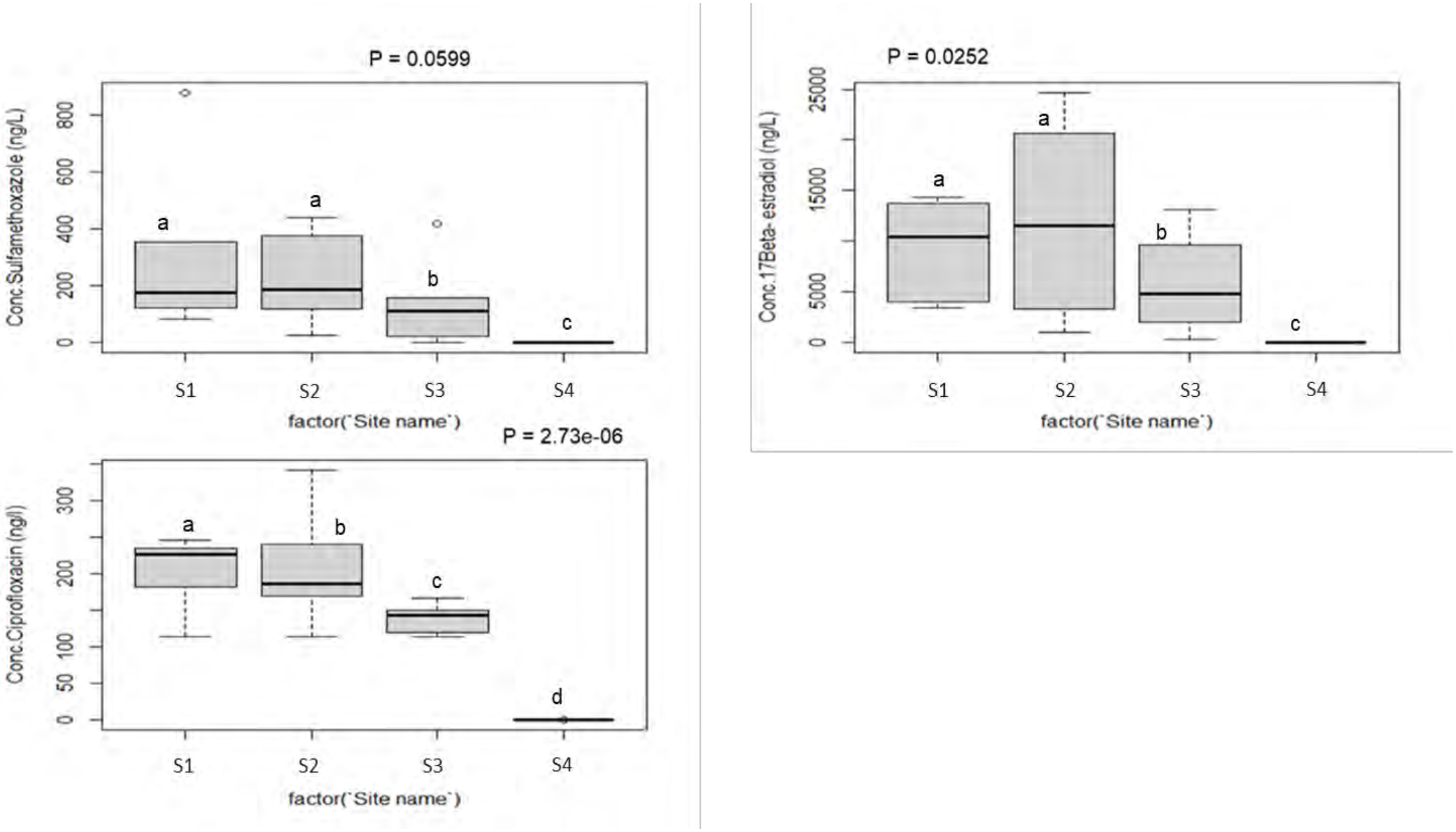


Figure 4.5:ANOVA (boxplots)-factor (sites) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol adsorbed onto PP Type2 microplastics at the four study sites in the Swartkops Rivers (S1, S2, S3 and S4) during the study (January 2022 to May 2022 – summer to autumn). The same alphabet letter on the bars indicates no statistically significant difference ($P > 0.05$), whereas different alphabet letters show statistically significant difference ($P < 0.05$).

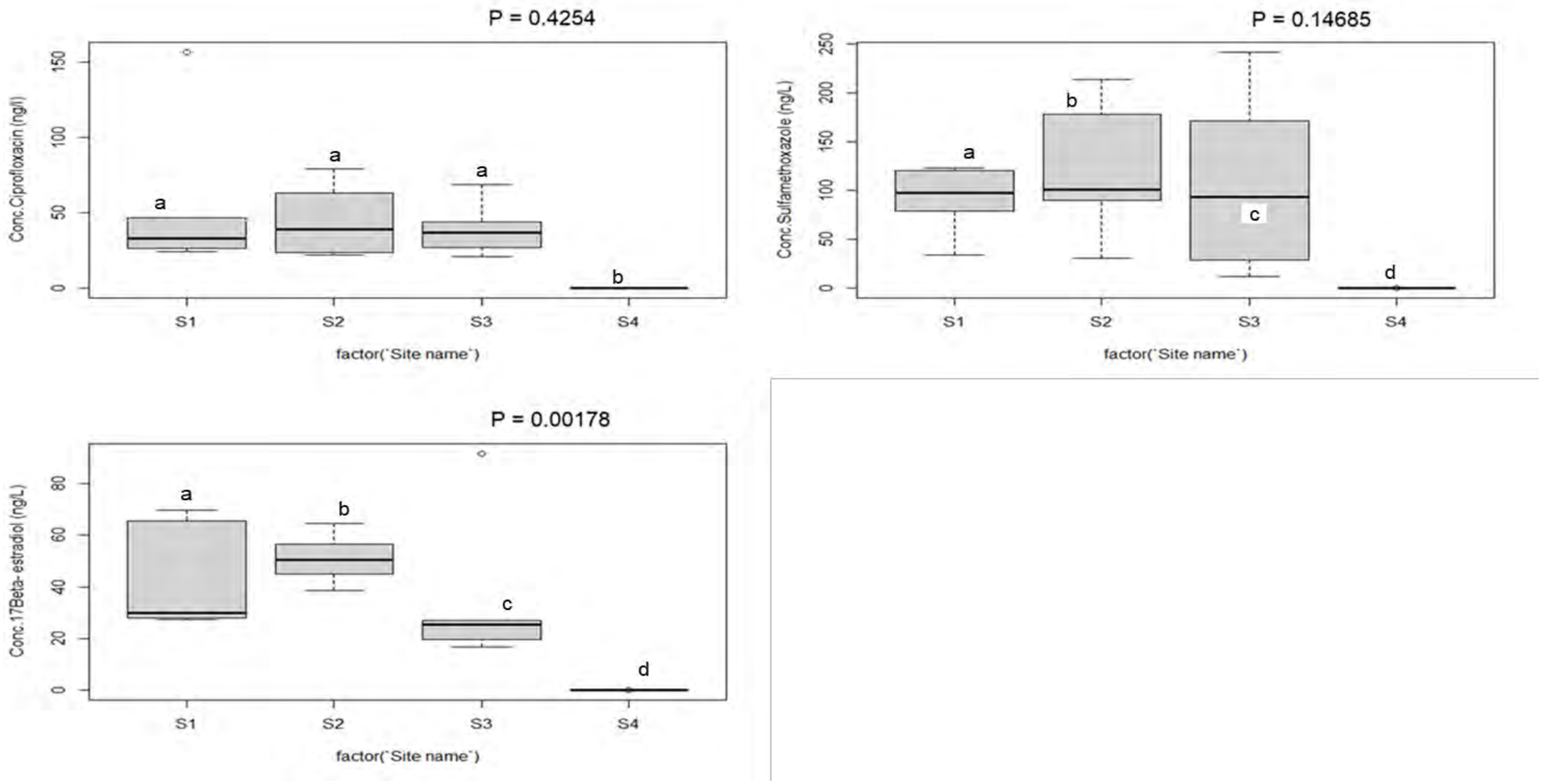


Figure 4.6: ANOVA (boxplots)-factor (sites) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol adsorbed onto PP Type1 microplastics at the four study sites in the Swartkops Rivers (S1, S2, S3 and S4) during the study (January 2022 to May 2022 – summer to autumn). The same alphabet letter on the bars indicates no statistically significant difference ($P > 0.05$), whereas different alphabet letters show statistically significant difference ($P < 0.05$).

Temporal differences in concentration adsorbed: Based on deployment period and seasonality.

The adsorbed concentrations on the MPs retrieved from the Swartkops River were subjected to temporal patterns; both in terms of period of deployment and seasonality to better understand the equilibrium interaction between the deployed MPs and the organic chemical pollutants.

For trend for the period of deployment is shown in Figures 4.7–4.9. Generally, the adsorbed concentration of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol at all the study sites (S1 to S4) was significant(ANOVA-test factor - Period of deployment; $P < 0.05$)and more pronounced after 35 days than other days, that is, a period of 7days and 14 days (Figures 4.7–4.9).

The Tukey multiple comparisons test (Tukey HSD post-hoc-test; (see Appendix T - Table T1)indicated that adsorbed concentrations of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiolweresignificant ($p< 0.05$) after 37days compared with adsorbed concentrations after a period of 7 days period at all study sites. Similarly, adsorbed concentrations between 35days and 14 days were also generally significant ($p< 0.05$). However, adsorbed concentrations after 14days and 7 days showed no significant differences, indicating that more than a month's deployment was needed to reach sorption equilibrium.

As with data from the Bloukrans River, the adsorbed concentration for all the targeted chemical pollutants was non-linear over the studied period of deployment (Figures 4.7–4.9) in all the instances that could be attributed to the temporal changing environmental factors, including ionic strength, pH and dissolved organic matter (DOM) that can facilitate adsorption and desorption processes of pollutants from the MPs to occur co-currently (Menéndez-Pedriza & Jaumot, 2020).

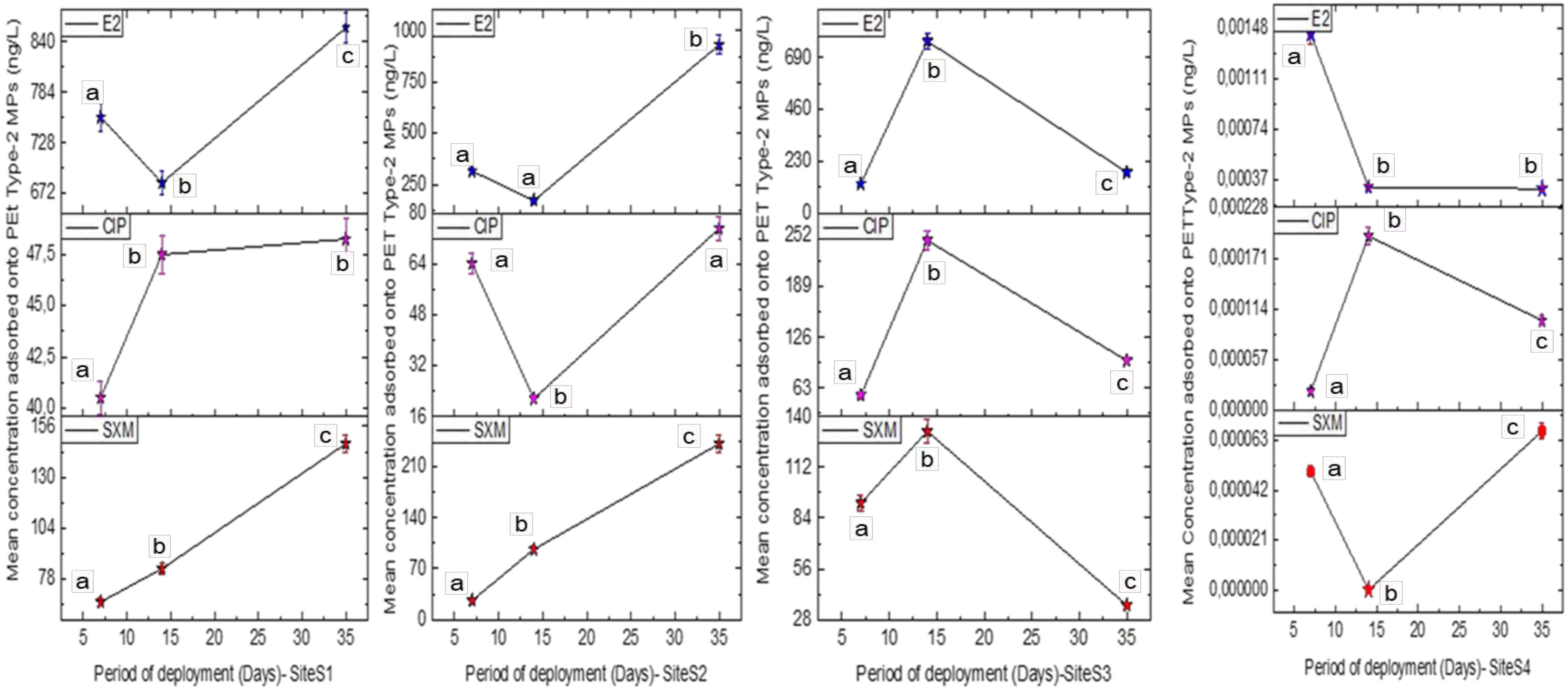


Figure 4.7: Time series (temporal) of mean (centre point) and standard deviation (bar) of adsorbed concentration trends of Sulfamethoxazole (SXM), Ciprofloxacin (CIP), and 17β-Estradiol (E2) onto PET Type2 MPs at the four sampling sites in the Swartkops River (S1, S2, S3 and S4) over a deployment period of 35 days during the study period (January 2022 to May 2022 – summer to autumn). Time series having completely different alphabet letters across the sites indicate statistically significant differences ($P < 0.05$), whereas series having the same alphabet letter in common across the sites indicate no statistically significant difference ($P < 0.05$).

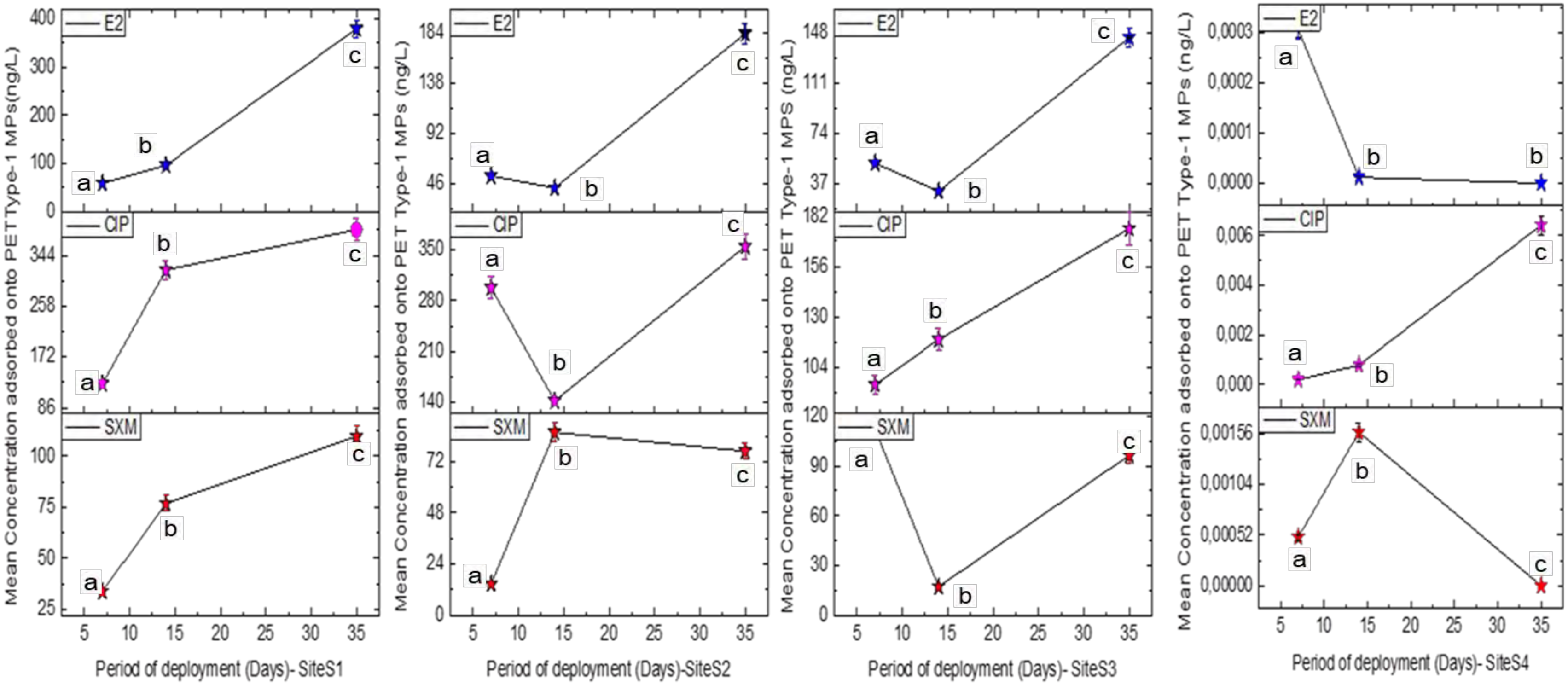


Figure 4.8: Time series (temporal) of mean (centre point) and standard deviation (bar) of adsorbed concentration trends of Sulfamethoxazole (SXM), Ciprofloxacin (CIP), and 17β-Estradiol (E2) onto PET Type1 MPs at the four sampling sites in the Swartkops River (S1, S2, S3 and S4) over a deployment period of 35 days during the study period (January 2022 to May 2022 – summer to autumn). Time series having completely different alphabet letters across the sites indicate statistically significant differences ($P < 0.05$), whereas series having the same alphabet letter in common across the sites indicate no statistically significant difference ($P < 0.05$).

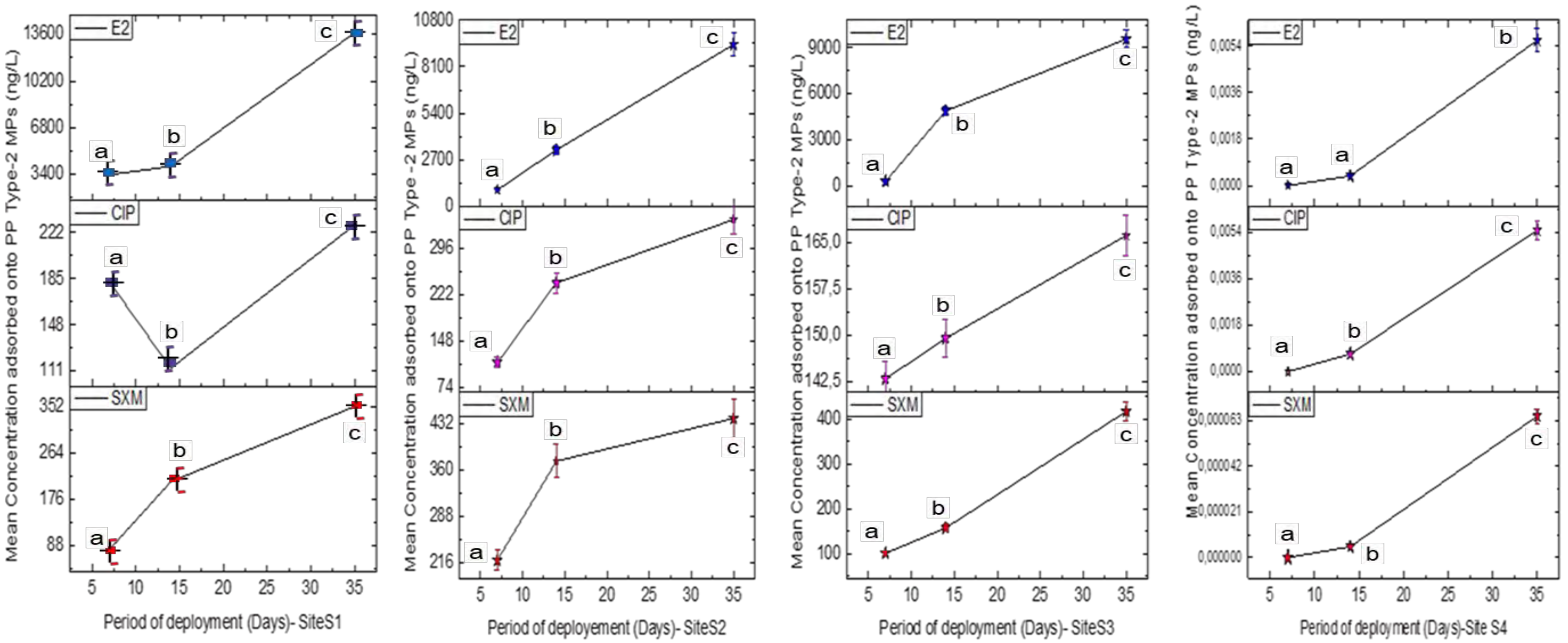


Figure 4.9: Time series (temporal) of mean (centre point) and standard deviation (bar) of adsorbed concentration trends of Sulfamethoxazole (SXM), Ciprofloxacin (CIP), and 17β-Estradiol (E2) onto PP Type2 MPs at the four sampling sites in the Swartkops River (S1, S2, S3 and S4) over a deployment period of 35 days during the study period (January 2022 to May 2022 – summer to autumn). Time series having completely different alphabet letters across the sites indicate statistically significant differences ($P < 0.05$), whereas series having the same alphabet letter in common across the sites indicate no statistically significant difference ($P < 0.05$).

Seasonal variations of the concentration adsorbed.

There were slight differences in adsorbed concentrations observed based on seasonal differences that is, summer and autumn. For instance, for PET Type2 MPs, at S1 (Tables D1and D2, Appendix D), the adsorbed concentration of Sulfamethoxazole was 99.04162 ngl^{-1} in summer compared to 176.0181 ngl^{-1} for the autumn season. Ciprofloxacin was 182.0338 ngl^{-1} during summer, and 234.9993 ngl^{-1}in autumn. For 17β-Estradiol, the summer concentration was 7028.2613 ngl^{-1} while for the autumn season, it was 11732.5000 ngl^{-1}. A one-fold increase in adsorbed concentration between summer and autumn was observedatS1. At S2, the summer concentration of Ciprofloxacin was 108.2164ngl^{-1} compared with the autumn concentration of 264.8761ngl^{-1}. The Sulfamethoxazole summer concentration was 121.5521ngl^{-1} compared with 128.9304ngl^{-1}for the autumn concentration. The summer season concentration of 17β-Estradiol was 312.4877 ngl^{-1} compared to 473.1463ngl^{-1}for the autumn concentration. The increase in concentrations as a factor of season between S1 and S2 was not even.

The summer concentration of Sulfamethoxazole for S3 was 95.1888 ngl^{-1} compared to 86.6575 ngl^{-1} in autumn. Ciprofloxacin measured 121.3471 ngl^{-1} during the summer season and 226.9621 during autumn. For 17β-Estradiol, the summer season was 116.2456 ngl^{-1}against 358.3556 ngl^{-1}in autumn. A similar trend observed: higher concentrations were adsorbed during the autumn than the summer.

Similar findings were observed for PP MP polymer type, Type2. At S1, Sulfamethoxazole measured 214.0419 ngl^{-1} during summer and 380.6985 during autumn. Ciprofloxacin concentrations were 182.0338 ngl^{-1} during summer and 234.9193 during autumn. Summer concentrations of 17β-Estradiol were7028.2613 ngl^{-1} during summer and11732.5000 ngl^{-1} for autumn. AtS2, Sulfamethoxazole was 344.2631 during summer and 530.3466 during autumn. Ciprofloxacin 193.6583 during summer compared with 231.9773 ngl^{-1} during autumn. 17β-Estradiol was 4536.5151 during summer compared with 19613.2610 ngl^{-1} for autumn. Similar trends of adsorbed concentration were observed at S3, with higher concentrations recorded during the autumn than the summer (TablesS3-S4; Appendix S).

For 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl) Phenol, the concentration differed irregularly between the two seasons. However, the Kruskal-Willis multiple comparison test and Tukey's honestly significant difference test (Tukey's HSD) for multiple comparisons that were used to indicate the difference between the sampling seasons in terms of the adsorbed concentration of the same pharmaceutical were only significant ($P < 0.05$) with 17β-Estradiol and 4-(2, 6-dimethyl-2-heptyl)Phenol ($P = 0.0482$ PET MPs Type1; Figure 4.10) and 17β-Estradiol ($P = 0.0423$, PP MPs Type2, Figure 4.11). Differences were generally non-significant in the adsorbed concentration of the organic chemicals onto the two MPs over the two seasons (Figure 4.10–4.13).

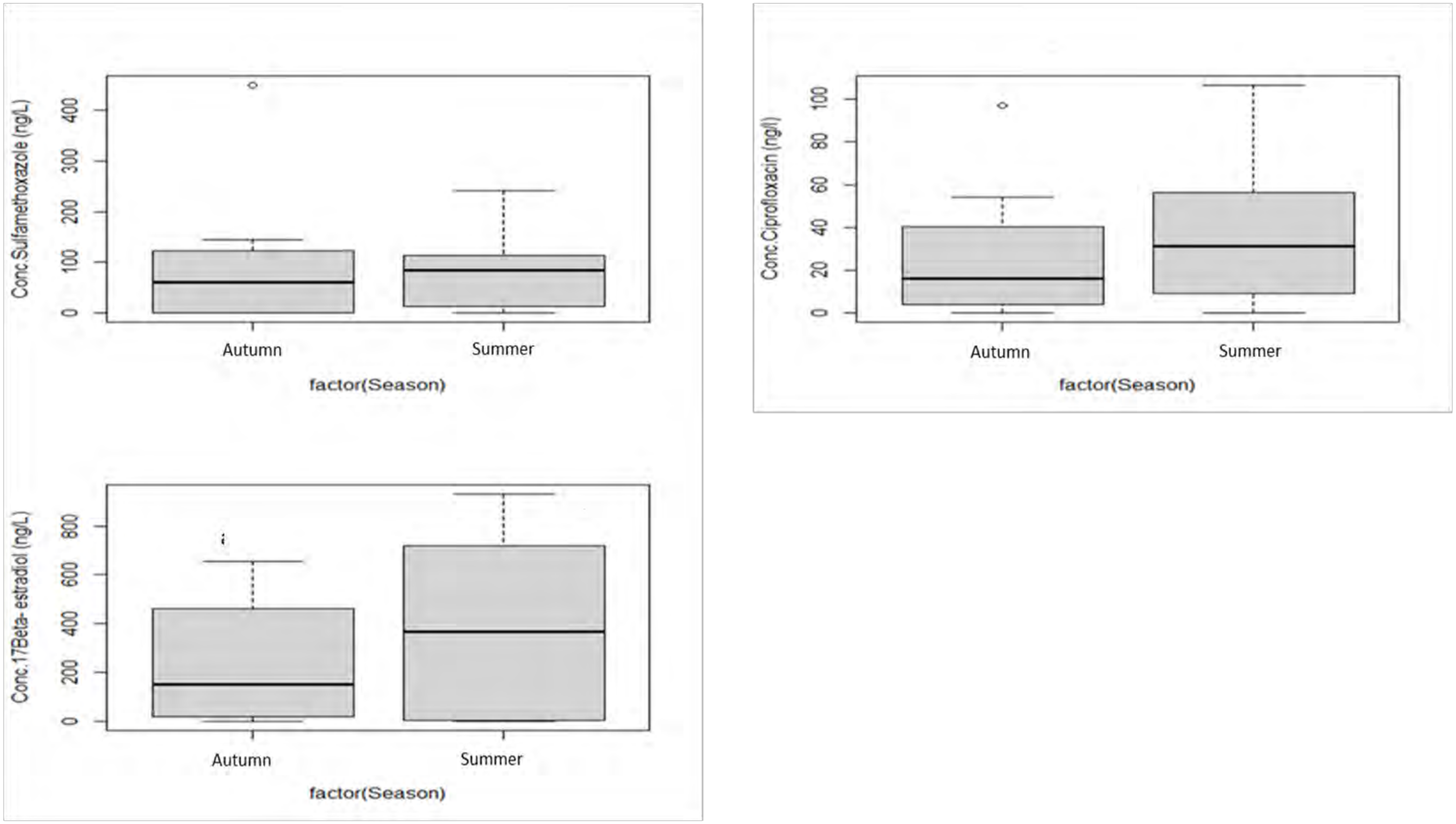


Figure 4.10:ANOVA (boxplots)-factor (seasons): Seasonal variability for the mean concentration of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol adsorbed onto PET Type2 microplastics in the Swartkops River (January 2022 to February 2022 – summer, March 2022 to May 2022 – autumn).

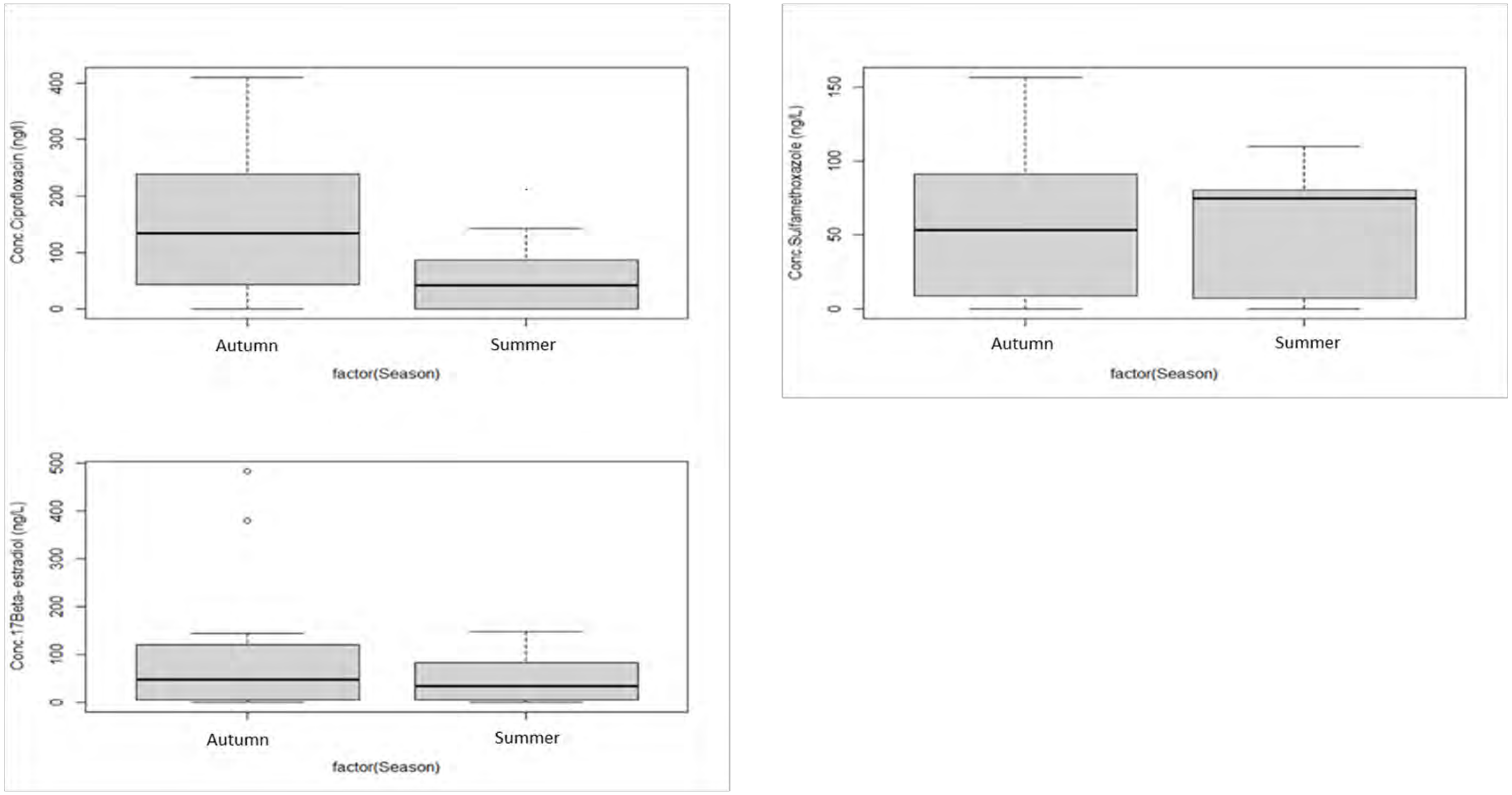


Figure 4.11: ANOVA (boxplots)-factor (seasons): Seasonal variability for the mean concentration of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol adsorbed onto PET Type1 microplastics in the Swartkops River (January 2022 to February 2022 – summer, March 2022 to May 2022 – autumn).

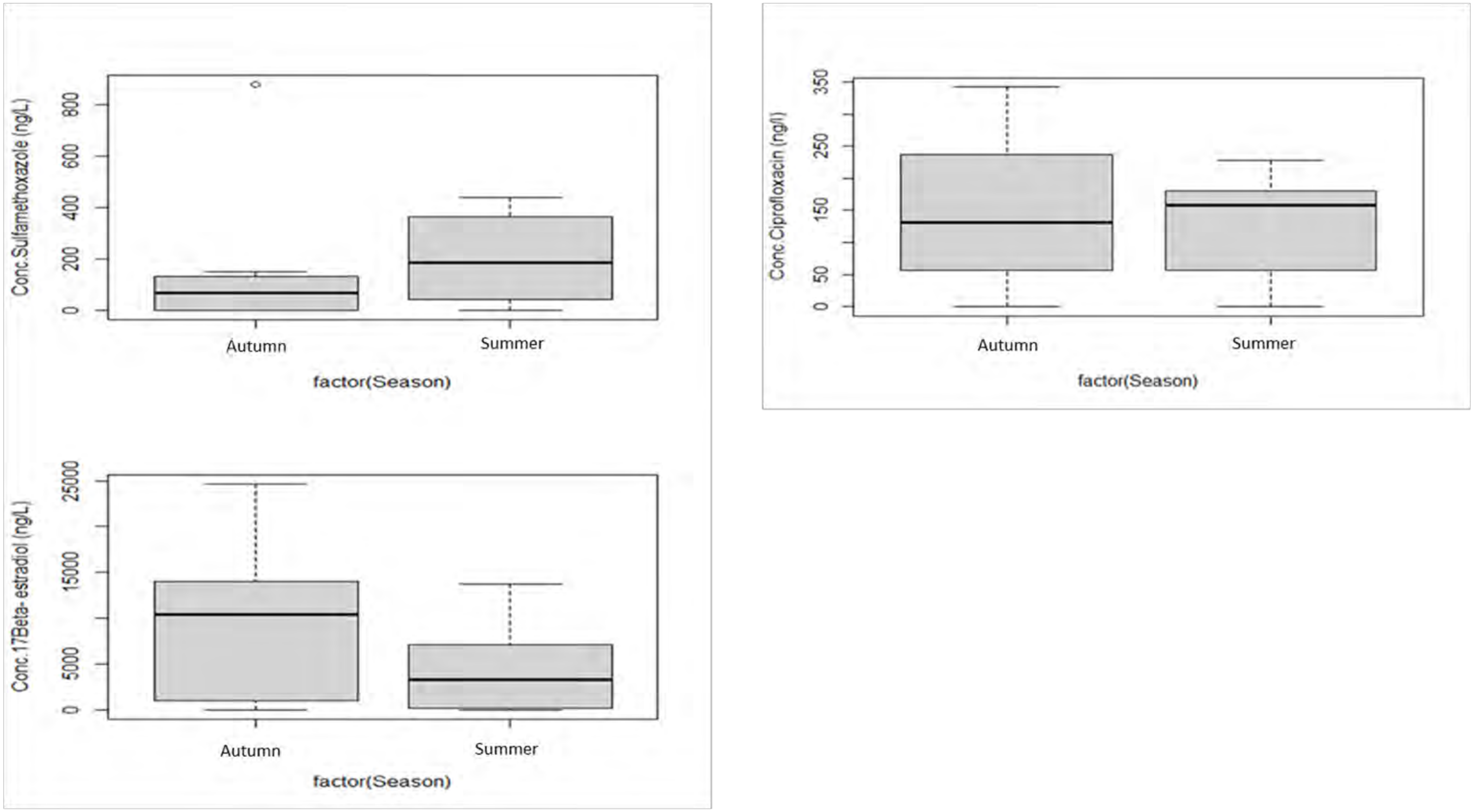


Figure 4.12: ANOVA (boxplots)-factor (seasons): Seasonal variability for the mean concentration of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol adsorbed onto PP Type2 microplastics in the Swartkops River (January 2022 to February 2022 – summer, March 2022 to May 2022 – autumn).).

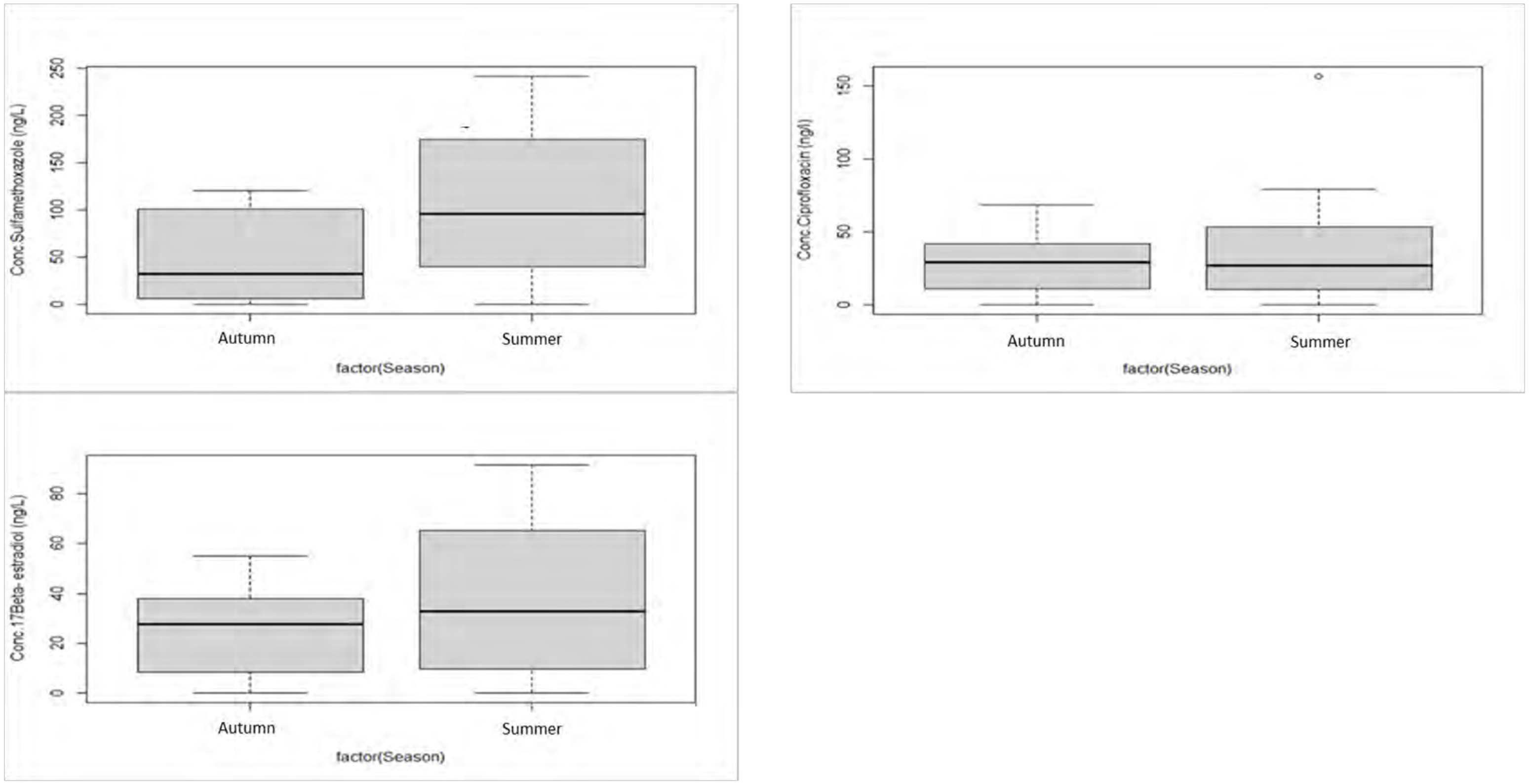


Figure 4.13: ANOVA (boxplots)-factor (seasons): Seasonal variability for the mean concentration of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol adsorbed onto PP Type1 microplastics in the Swartkops River (January 2022 to February 2022 – summer, March 2022 to May 2022 – autumn).

4.4.2 Relationship between microplastic particle size and concentration of adsorbed chemical pollutants

Differences between the MPs sizes, based on the adsorbed concentrations.

As explained in Chapter 3 (3.3.4), the mechanism of interaction between microplastics and contaminants is a complex process and prone to disruption by several factors, with MP size having a significant impact on the adsorption process (Ma et al., 2019). The influence of particle size of MPs on the adsorption of the organic chemical pollutants is shown in Figures4.14–4.17. It is evident that, for instance, the mean highest adsorbed concentration of Sulfamethoxazole (176.0181ngL^{-1} S3 Autumn) and 17βEstradiol (765.2759ngL^{-1} S1 Autumn) on PET MPs Type2 (0.5mm<-≤2mm) were approximately four and nine orders of magnitude higher, respectively, than the recorded concentration adsorbed of the same chemical pollutant on PET MPs Type1 (2mm<-≤5mm), Sulfamethoxazole (56.0460ngL^{-1}) and 17β-Estradiol (81.2188ngL^{-1}) (Table S3 – appendix S).

For PP MPs Type2, the highest adsorbed concentrations of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiolwere1762.1391 ngL^{-1} (S3 summer), 234.9993 ngL^{-1} (S1 autumn), 11732.5000 ngL^{-1} (S1 autumn), respectively, which were27, six, and 400 orders of magnitude higher than the concentration of the same pollutant on PP Type1 MPs, that is, 64.0227 ngL^{-1}, 36.5205 ngL^{-1}, 29.3831 ngL^{-1}, respectively.

In general, however, statistically at $P = 0.005$, there were no significant variations (differences) in the adsorbed concentrations of the chemical pollutant by either PET or PP MPs of the size ranges (in most cases, $P > 0.05$), with the exception of 17β-Estradiol (P=0.00443 PET MPs summer season, Figure4.15), Ciprofloxacin (P=0.00735 PP MPs summer season, Figure4.16), 17β-Estradiol (P= 0.00807 PP MPs summer season, Figure4.16), Ciprofloxacin (P=0.00223 PP MPs autumn season, Figure4.17), and 17β-Estradiol (P=0.000816 PP MPs autumn season, Figure4.17).

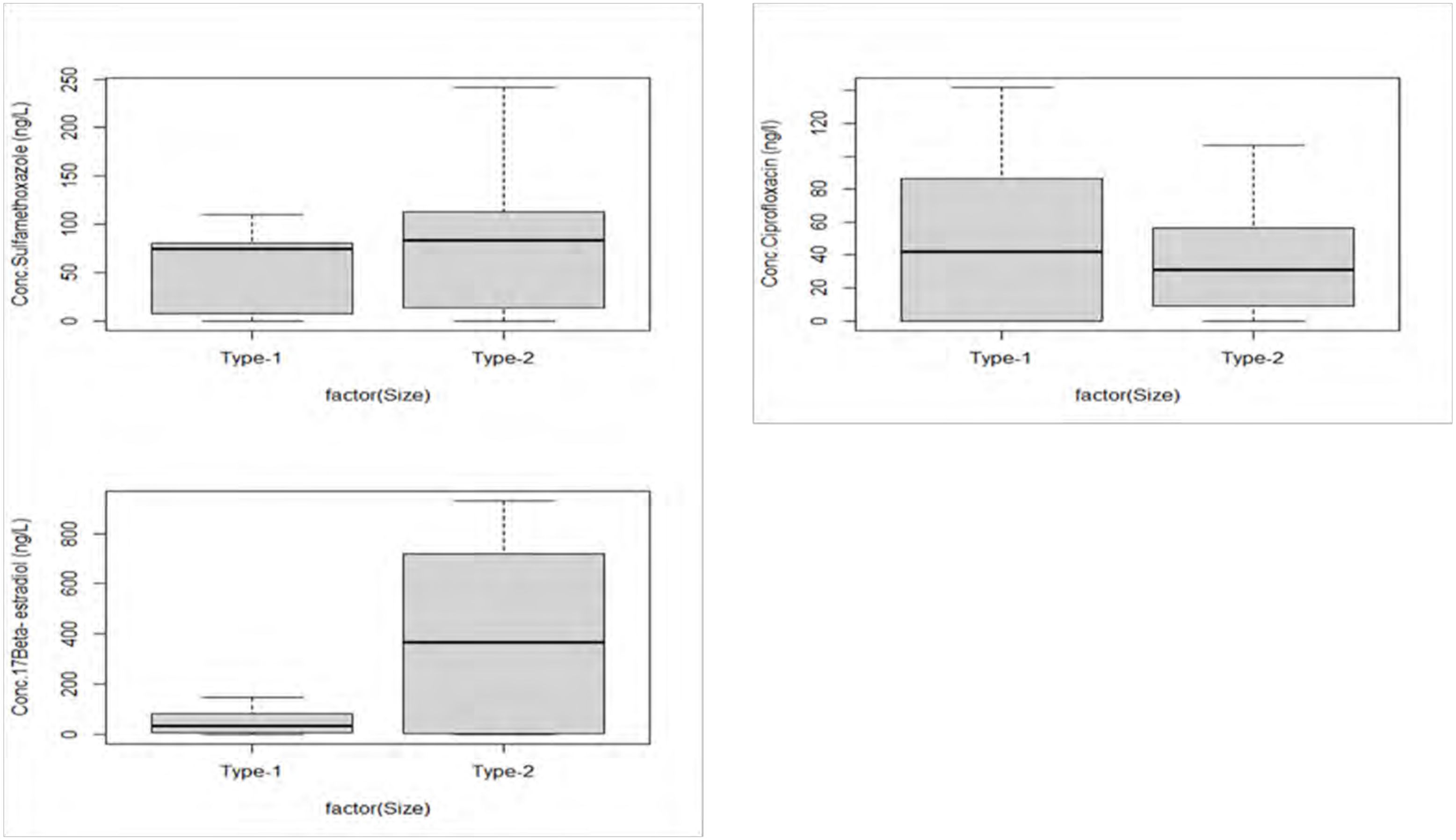


Figure 4.14: ANOVA (boxplots)-factor (MP – size range) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol adsorbed onto PET microplastics, Type1 (2mm<-≤5mm), and Type 2 (0.5mm<-≤2mm), during the summer season in the Swartkops River during the study (January 2022 to February 2022).

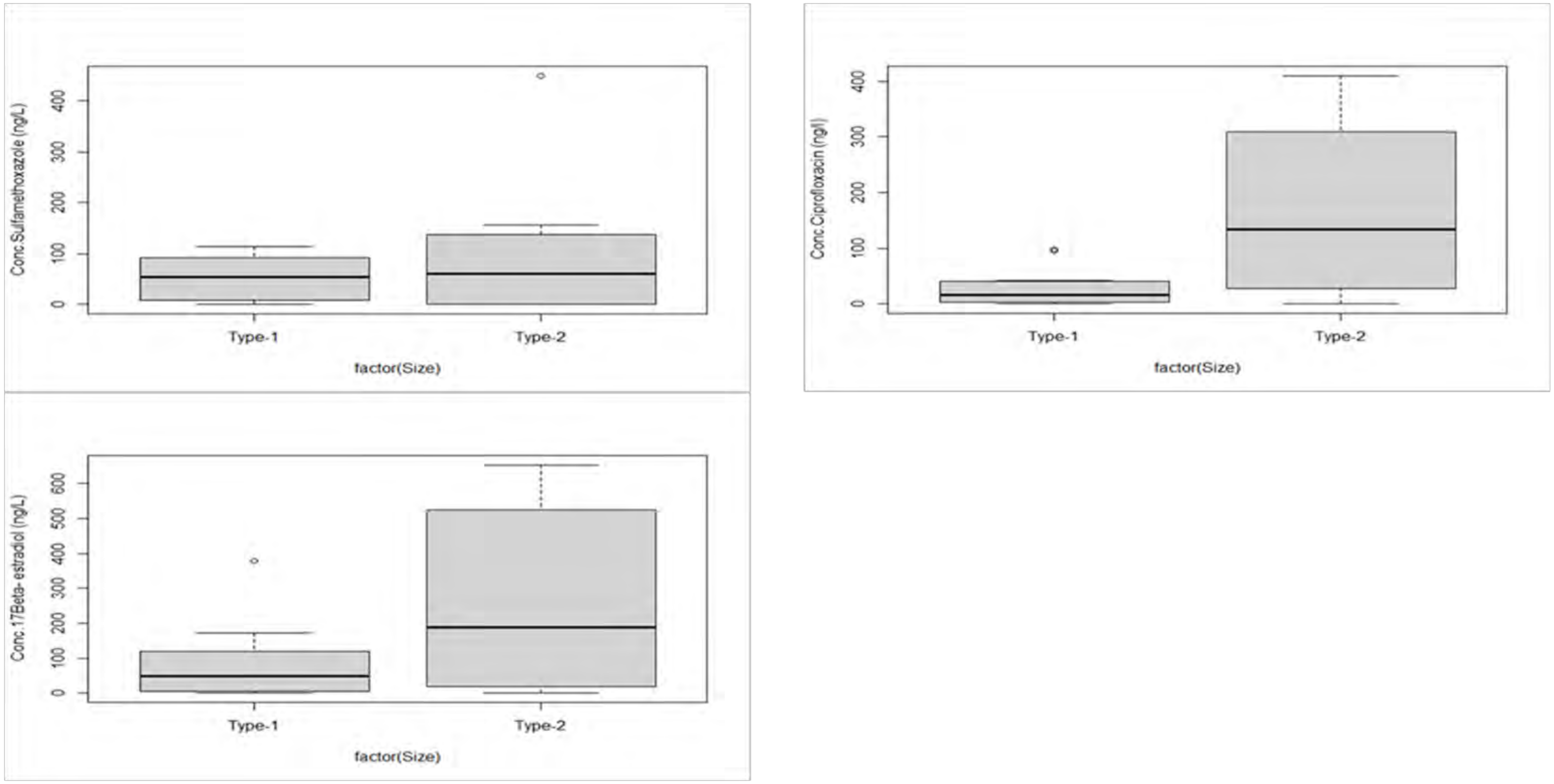


Figure 4.15: ANOVA (boxplots)-factor (MP – size range) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol adsorbed onto PET microplastics, Type1 (2mm<-≤5mm), and Type2 (0.5mm<-≤2mm), during the autumn season(March 2022 to May 2022) in the Swartkops River during the study.

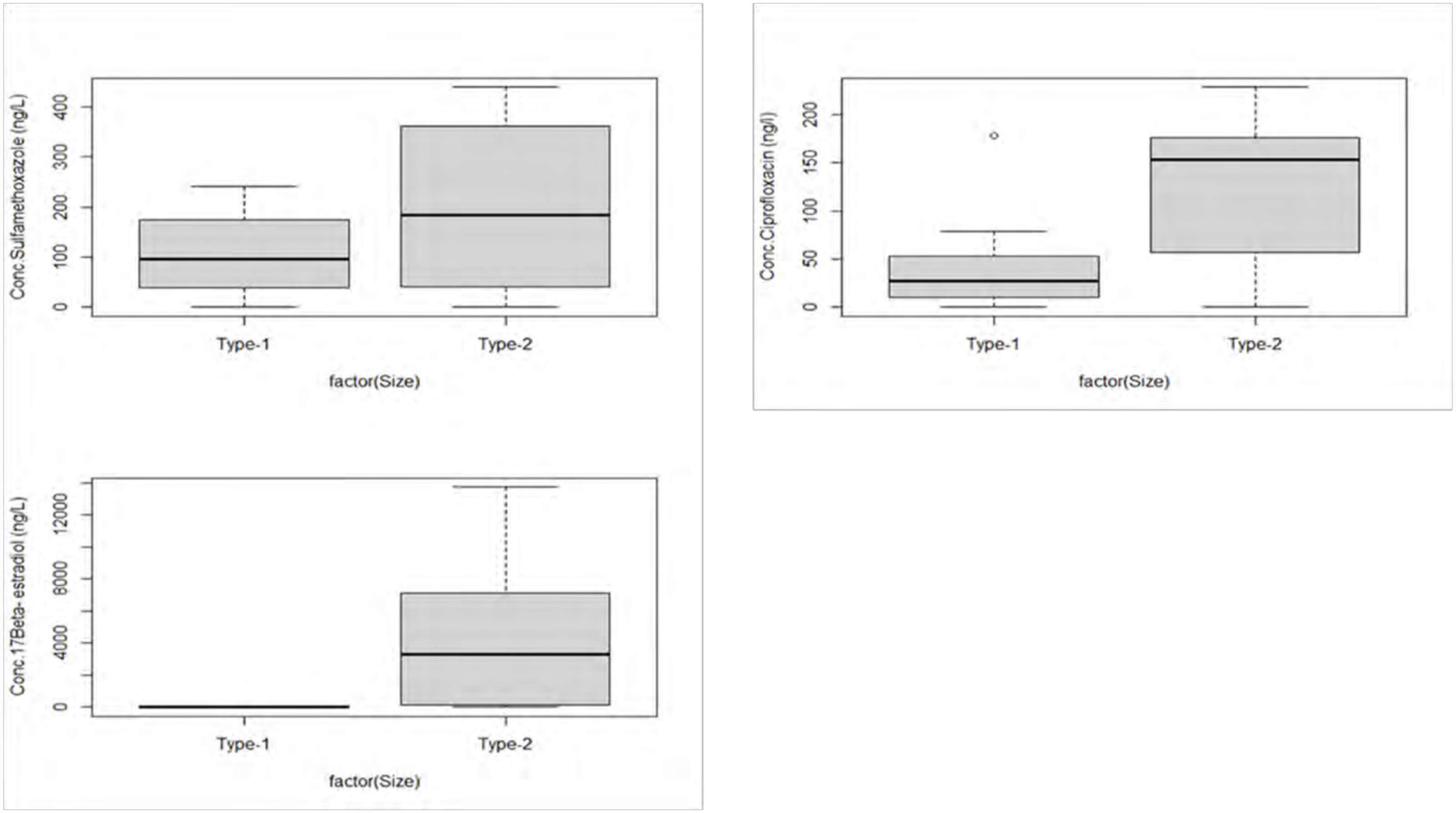


Figure 4.16: ANOVA (boxplots)-factor (MP – size range) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol onto PET microplastics PP microplastics, Type1 (2mm<-≤5mm), andType2 (0.5mm<-≤2mm), during the summer season (January 2022 to February 2022) in the Swartkops River.

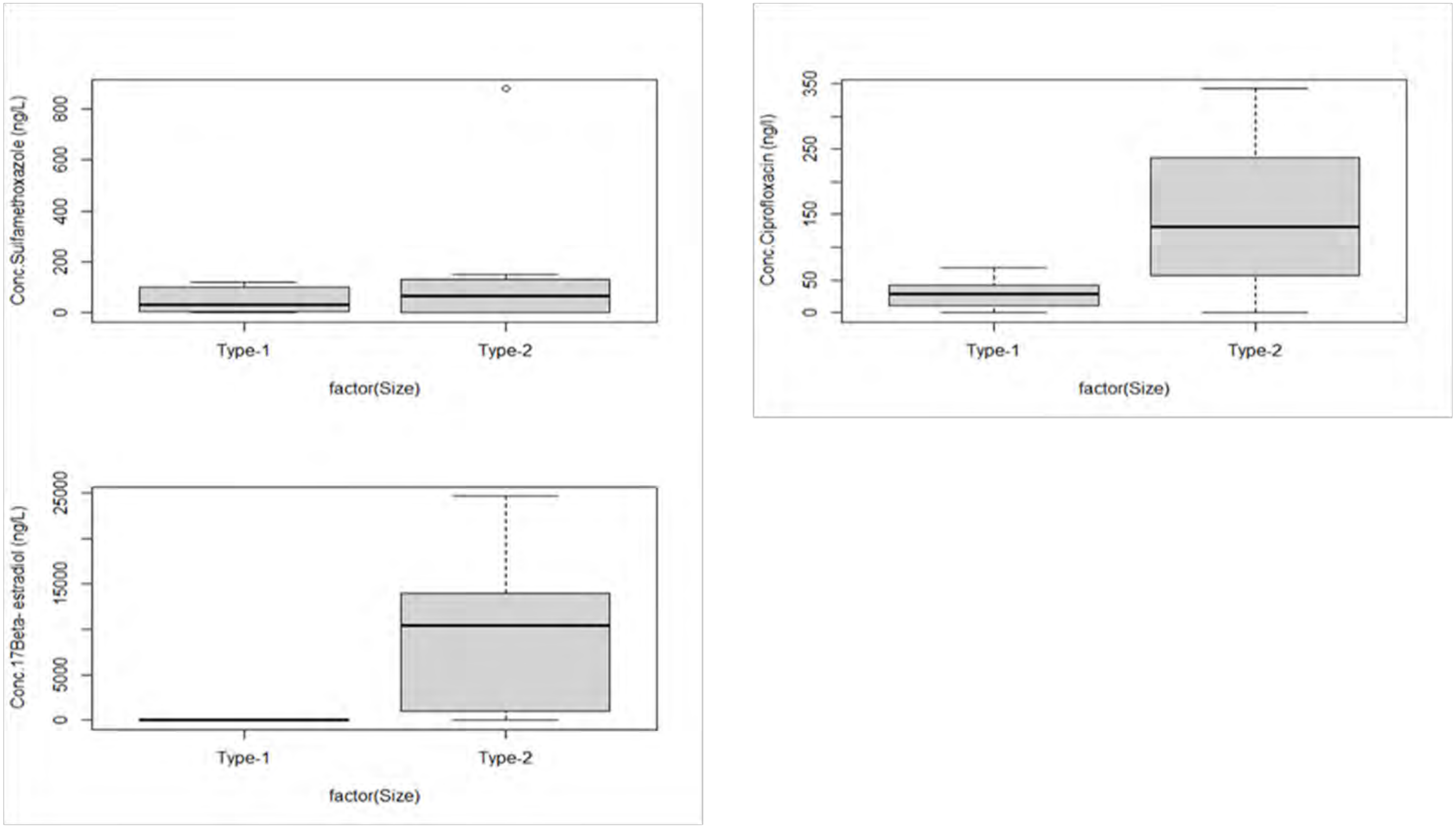


Figure 4.17: ANOVA (boxplots)-factor (MP – size range) for the mean concentration of Sulfamethoxazole, Ciprofloxacin, and 17β-Estradiol adsorbed onto PP microplastics, Type1 (2mm<-≤5mm), and Type 2 (0.5mm<-≤2mm), during the autumn season (March 2022 to May 2022) in the Swartkops River during the study.

4.5 Discussion

Three out of the four targeted organic chemical pollutants, sulfamethoxazole, ciprofloxacin and 17β-estradiol, were detected on the two deployed MP polymer types, PET and PP, at the four study sites in the Swartkops River during the two seasons, with 4-(2, 6-dimethyl-2-heptyl)Phenol not detected on any of the retrieved MPs samples. Similar to the Bloukrans River catchment results, differences in adsorbed concentration of individual chemical pollutants were associated with specific site characteristics, such as surrounding land use, a wastewater treatment plant (WWTWs), human settlements, animal husbandry and crop-farming activities. Unlike the Bloukrans River, the concentration of the adsorbed pharmaceuticals was significantly higher at B2 (WWTWs proximity) in the Swartkops River; the adsorbed concentration was considerably higher at S2 (Domestic-urban household refuse area) ($P<0.05$ Tukey multiple comparisons of means – see Appendix T-Table T2) for all the pharmaceutical concentrations recorded than at other study sites. This observation further emphasises the complexity of spatial factors affecting the adsorption process by MPs. Site 2 was in a more populated area of the Swartkops River watershed, near the industrial zone of Uitenhage.

17β-estradiol was present at greater concentrations than sulfamethoxazole and ciprofloxacin at all the sites (Figures 27–32). The highest concentration of 17β-estradiolrecorded on PP MPs was 11624ng/L at S2 during the summer and 19613ng/L during the autumn season. The highest concentration of sulfamethoxazole and ciprofloxacin was recorded on PP MPswas1762.1391ng/L (S3) and 234.9993ng/L (S1). The highest adsorbed concentration on PET MPs was 176.0181ng/L for sulfamethoxazole and 132.6525ng/L for ciprofloxacin. This difference in adsorbed concentration between the two polymer types, PP and PET, could be because the surfaces of most MPs, such as non-polar PP, are structurally homogeneous, inert, and non-wetting, leading to the accumulation of more organic chemical pollutants, including pharmaceuticals, even when they are hydrophilic. Polar PET with hydrophilic groups such as -CO and -COOH increase water accessibility on the material surface, decreasing the binding sites and reducing the enrichment of the organic chemical pollutants (Upadhyay et al., 2022). With chemical pollutant concentrations in water systems influenced by factors intrinsic to changing seasons (Kolpin et al., 2002; W. Xu et al., 2013), results of adsorbed concentrations of Sulfamethoxazole, Ciprofloxacin and 17β-Estradiol on MPs retrieved from the Swartkops River generally indicated no significant differences between summer sampling results and autumn results with few exceptions (Figures4.10 – 4.13).

Although MP particle size could play a more minor role than other physical parameters (Atugoda et al., 2021), it can affect the adsorption rate, rate of equilibrium establishment, and adsorption capacity (Hartmann et al., 2017). Like the results discussed in Chapter 3, the results from MPs retrieved from Swartkops River confirmed that the adsorption capacities of the two MP polymer particles deployed followed the size trend despite the dissimilarity in the polymer composition. This trend is depicted in Figures 4.14–4.17, with high adsorption of sulfamethoxazole, ciprofloxacin, and 17β-estradiol observed for the smaller particles (MP Type2). A significant correlation was observed ($P < 0.05$) between the adsorption capacity for sulfamethoxazole, ciprofloxacin, and 17β-estradiol and the microplastic deployment size. As discussed in Chapter 3, the adsorption capacity was in the order of MP-Type2 > MP-Type1. This suggests that as microplastic disintegrates into tiny particles in aquatic environments, the increased polymer surface area increases pollutant adsorption (Mo et al., 2021; X. Liu et al., 2022). The highest concentration of sulfamethoxazole, ciprofloxacin, and 17β-estradiolrecordedon PP Type2 was 1762.1391ngL^{-1}, 234.9993ngL^{-1}, 19613.2610ngL^{-1}, respectively, compared to PP Type1 MPs where the highest concentration was, 164.0227ngL^{-1}, 70.3114ngL^{-1}, 54.0228ngL^{-1}, respectively (Table S4, appendix S). These results provide further evidence for our hypothesis that the smaller the MP particle size, the larger the specific surface area (SSA) of the MPs, and the greater the number of adsorption sites and the amount of organic chemical pollutants that are adsorbed onto a variety of microplastics.

4.6 Conclusion

The Swartkops River is an urban river system compared to the Bloukrans River catchment, as it is densely populated (Hamandawana et al., 2020; Odume et al., 2022). Three of the four targeted chemical pollutants, sulfamethoxazole, ciprofloxacin and 17β-estradiol, were present on the retrieved MPs particles. 17β-Estradiol had the mean highest adsorbed concentration. The relatively intense concentration of adsorbed 17β-estradiol could be explained by the connection between an economic boom, a growing population and increasing amounts of estrogenic chemicals that are typically relatively abundant in municipal effluents of industrialised and urbanised river catchments (Roefer et al., 2000; Z. Wang et al., 2017). Additionally, the intrinsic hydrophobic properties and octanol-water partition coefficient (log_{oc} and $logK_{ow}$) of estrogenic compounds make them tend to adsorb strongly onto suspended particles in aquatic systems (Z. Wang et al., 2017).

The results for the effects of plastic properties and spatial-temporal factors presented in this chapter reveal further the significant relation between the adsorption of the three pharmaceuticals onto MPs with spatial scale (land-use types) where adsorption capacity between the different sampling sites was, in most cases, statistically significantly attributed to the differences observed in land use. The highest adsorbed concentration of the pharmaceuticals was in the more densely populated site (S2), indicating the human population's contribution to pollution in water systems. Temporal scale – sampling over several days and repeated seasonal sampling – was the other key factor influencing MP adsorptivity, although the MP adsorption capacity for the pharmaceuticals between summer and autumn was not statistically significant, as observed in the ANOVA analysis.

The MP property of size was more marked in determining the adsorbed concentration of the pollutants. MP Type2 (small size) exhibited high adsorption capacity, demonstrating further the significant role of particle size and surface area in the adsorption of organic chemical pollutants. The results given in Chapter 4 provide a further foundation for predicting the fate, transport, and risk of MPs adsorbed contaminants as an emerging concern in freshwater ecosystems.

CHAPTER 5: Relating Physico-Chemical Variables to the Adsorption Potential of Selected Pharmaceutical Pollutants and Endocrine-Disrupting Compounds on Microplastics

5.1 Introduction

Abiotic and biotic factors in a river system may impact the adoption of chemicals by microplastics(Lehmann et al., 2019; Santana-Viera et al., 2021; Cássio et al., 2022). Abiotic factors, such as pH, and biotic factors, such as microbial biofilms on MP surfaces, can modify the charges on the surfaces of MP and lead to increased chemical adsorption by MPs (Vieira et al., 2021; Avazzadeh et al., 2023). In a study by Liu et al. (2019a), the role of water chemistry in the adsorption process was explained as follows: (i) electrostatic repulsion will increase as solution pH increases, suppressing electrostatic interaction between differently charged sorbate and sorbent; (ii) an increase in pH may promote the dissociation of the hydrophobic neutral sorbate molecules into hydrophilic, negatively charged species, thereby resulting in reduced hydrophobic interaction; (iii) a high pH can increase the pi (π) donor ability of sorbate, thus enhancing (π-π) interactions (Liu et al., 2019a).

Studies have also shown that MPs tend to aggregate in aquatic environments where the pH ranges between 5 to 9 (Chowdhury et al., 2015; P. Wang et al., 2016); this aggregation has been attributed to the influence of pH on the surface charges of MPs (Vieira et al., 2021). In a study by Xu et al. (2018a), the highest adsorption of tetracycline on PE, PP, and polystyrene (PS)in freshwater systems was at pH 6, with a decreasing trend observed after that. The authors argued that the decreasing trend below pH of 6 was partly a result of electrostatic repulsion between anionic tetracycline and the negatively charged MP surfaces. Similarly, Zou et al. (2020) established that the adsorption affinity of Cu, Cd and Pd onto chlorinated polyethene (CPE), PE, and polyvinyl chloride (PVC) MPs increases as pH rises from 2 to 6.5. In other studies, pH did not influence the adsorption of several chemical compounds onto MP surfaces(Jiang et al., 2020; Liu et al., 2019a).

Salinity, dissolved compounds, especially soluble salts, and temperature have also been studied in the adsorption of chemical compounds by MPs (Vieira et al., 2021). For instance, Xu et al. (2018a) explored the effects of salinity on the adsorption of tetracycline on MPs. They concluded that enhanced salinity had no significant impact on the adsorption of tetracycline onto three MPs (PE, PP, and PS). In another study by Jiang et al.(2020) on the adsorption properties of tetrachlorobiphenyl (PCB77) by PP MPs in freshwater and simulated seawater (3.5wt% NaCl in ultrapure water), the results indicated that Kd and maximum adsorption capacity were approximately 2 and 1.8 times higher in the simulated seawater than in freshwater. These results suggest that salinity may also influence chemical adsorption by MPs.

Ionic strength, an indicator of the total concentration of ions in solution, has increased the adsorption affinity of hydrophobic organic compounds (Avazzadeh et al., 2023). This increase is attributed to a salting-out effect that facilitates increased adsorption affinity (Liu et al., 2019a). The same authors reported more significant Diethyl phthalate (DEP) adsorption and Dibutyl phthalate (DBP) onto MPs in freshwater with increased NaCl. However, other studies, for example, Zuo et al. (2019), reported that increased ions in solution do not influence the adsorption of Phenanthrene onto PE or PS in freshwater.

Temperature can also influence the rate and capacity of MPs' adsorption of chemical pollutants. A study by Zhan et al. (2016) found that the adsorption of PCB77 onto PP decreases with increasing temperature in freshwater systems. The authors explained that increasing temperature affects the surface tension, which hinders the adsorption process. However, this trend has yet to be observed for all polymer types. For instance, the adsorption of PAHs onto PS and PE MPs appeared to be endothermic, meaning that the adsorption capacity is lower in colder temperatures (Sørensen et al., 2020).

Most current existing data on the influence of physicochemical variables on the adsorption of chemical pollutants by MPs stem from studies that evaluate the influence of each variable separately (laboratory studies), yet the natural environment parameters exist in a complex and dynamic water matrix (Fred-Ahmadu et al., 2020; Talbot & Chang, 2022). Even though some parallels have been drawn and extrapolations made to situations in freshwater systems, many studies remain primarily centred on seawater systems (Avazzadeh et al., 2023). Besides, land-use types significantly influence water physicochemical variables by influencing erosion and sedimentation, surface water heat and material balance, which can alter water chemistry(Baker, 2005; Cheng et al., 2022). Few studies have used the river land-use approach in evaluating how water chemical variables affect the adsorption of pollutants by MPs (Fred-Ahmadu et al., 2020;

Atugoda et al., 2021). More importantly, understanding the relationship between water physicochemical variables and the adoption of pollutants by MPs is crucial for developing effective strategies to protect and manage water resources (G. Liu et al., 2019b; Fred-Ahmadu et al., 2020; Jiang et al., 2020).

This chapter addresses the second objective stated in Chapter 1: To determine the influence of selected physicochemical variables on the adsorption of targeted organic chemical pollutants on microplastics. For this purpose, this chapter provides field-based data from an *in situ* experiment that investigated how the adsorption of selected antibiotics and endocrine-disrupting chemicals is influenced by various physicochemical variables, including pH, temperature, conductivity, turbidity, and dissolved solids that vary between nearby land-use types.

5.2 Methodology

5.2.1 Water physico-chemical variables

Water physico-chemical variables were measured at the experimental sampling sites over the study period (beginning late summer, January 2022–February 2022; autumn, April 2022–May 2022) coinciding with the retrieval of deployed MPs. For each sampling event, the physico-chemical variables measured onsite included dissolved oxygen (DO), electrical conductivity (EC), temperature, and pH using the meter probe HACH SL1000. Turbidity was measured using the portable turbidity HANNA H19023 meter. Water depth and river width were measured using a tape measure and dip stick. Water flow rates were measured using a Marsh-McBirney Flo-Mate 2000 flow meter. For water physico-chemical variables requiring laboratory analysis, water samples were collected using 1L polyethene acid wash bottles which were then transported to the water quality laboratory of the Institute for Water Research, Rhodes University. In the laboratory, samples were preserved in a cold room at a temperature of 3 °C until analysis. Preservation of the samples was not longer than 24 hours, before they were processed for total dissolved solids (TDS), total suspended solids (TSS), alkalinity and total hardness.

In the laboratory, TSS and TDS were analysed following the method described in Abubakar et al. (2020). Using the Whatman filter paper 47 mm nominal pore size and vacuum pump (Gravimetric Method), 100 cm^3 of the sampled water was measured using a measuring cylinder, and subsequently filtered using the vacuum filtering apparatus. The residues remained on the filter paper and, to remove any remaining traces of water, the filter paper and the residue were dried in an oven at 103 °C and subsequently taken back to the desiccator. The mass of TSS in grams was taken by a weighing balance. The filtrate was collected in a dried conical flask which, together with its contents, was placed on a hot plate and heat dried at 104 °C to complete dryness. The mass of TDS in grams was taken using a weighing balance.

TSS present = TSS (mg/l) $= \frac{(TSS)g}{100}\ x\ 10^6$

TDS present = TDS (mg/l) $= \frac{(TDS)g}{100}\ x\ 10^6$.

TSS and TDS results for all the water samples are given in Table B1 and B2,Appendix B.

Alkalinity and hardness were estimated chemically through the titrimetric method, and the EDTA titration method, respectively, for fresh samples immediately after sample collection using a method described in Islam et al. (2018). To measure alkalinity, three drops of methylene indicator were added to 100 cm^3 of the sampled water and titrated against 0.02N H_2SO_4 acid from the burette until the solution turned from pink to yellow. Alkalinity of the solution was calculated using the formula = $\left(\frac{V1x0.02x50x100}{100\ (volume\ sample\ used)}\right)\ mg/l$

V1 = is the used average volume used of the acid. Alkalinity results for all the water samples are indicated in TablesQ1 and Q2 in Appendix Q. In all titrations the phenolphthalein indicator end point was zero. Therefore, the alkalinity of the water samples was only due to the presence of bicarbonate ions HCO_3^{2-}.

For hardness, to 100 cm^3 of the sampled water, 5 ml of buffer solution pH =10.3 was added, followed by addition of 2 ml of Eriochrome black T. The resultant solution titrated against a standard solution of 0.01M EDTA solution from the burette. The equation of the reaction between the calcium ion and or magnesium ions and EDTA is given by:

$[Mg/Ca \rightarrow EriochromeBalck\ T] + EDTA\ \rightarrow [Mg/Ca - EDTA] + Eriochrome\ Black\ T$

EDTA forms 1:1 complex with Ca^{2+} and Mg^{2+}ions.

1000 ml of 1 M EDTA = 1 Mole of $CaCO_3$ = 100 g of $CaCO_3$

1 ml of 0.05M EDTA = 100 $x\ \frac{1}{1000}\ x\ \frac{1}{100}\ g\ CaCO_3\quad = 1mg\ of\ CaCO_3$

Vml of 0.01 EDTA used (Volume from the burette readings) = 1 x V mg of $CaCO_3$.

100 ml of the sampled water will contain (1 x V) mg of $CaCO_3$.

1000 ml of the sampled water contain = $\frac{1\,x\,V}{100}\,x\,1000\,mg/l\;of\;CaCO3$. This is the total hardness of the sampled water. The total hardness results for all the water samples are indicated in Tables B1 and B2, Appendix B.

5.3 Statistical data Analysis

The summary statistics were performed using the R programming environment (2022). Before analysis, the data were captured and prepared in Excel (Microsoft 2010 Office) and then exported to R software package Version R 4.3.0. The data were subjected to relevant univariate and multivariate statistical analyses to indicate statistical differences and elucidate relations and trends of the water physico-chemical variables between different sampling sites.

5.3.1 Box and whisker plots

Box plots were used to visualise the variations of the different water quality variables of the study sites based on the land-use and season. The box plots in this chapter display summary statistically significant differences of the water quality based on the land use, and seasonality.

5.3.2 Two-factor analysis of variance (ANOVA)

ANOVA was used to investigate whether the study sites' physico-chemical variables differed between the control site and impacted sites. Before using ANOVA, the basic assumption of normality was investigated using the Shapiro-Wilk normality test. The water physico-chemical data were logarithmically transformed and normalised if the assumption still needed to be met. ANOVA was conducted using R software package Version R 4.3.0.

5.3.3 Linear regression analysis

Linear regressions were done to elucidate the relationship between the water quality variables and the concentration of the same pharmaceuticals adsorbed and the water physico-chemical variables. Water parameters included temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), turbidity, alkalinity, hardness, total dissolved solids (TDS), and total suspended solids (TSS). The linear regression was undertaken using R software package Version R 4.3.0.

5.4 Results

5.4.1 Spatial-temporal variation in the concentration of the measured physico-chemical variables in the Bloukrans and Swartkops River.

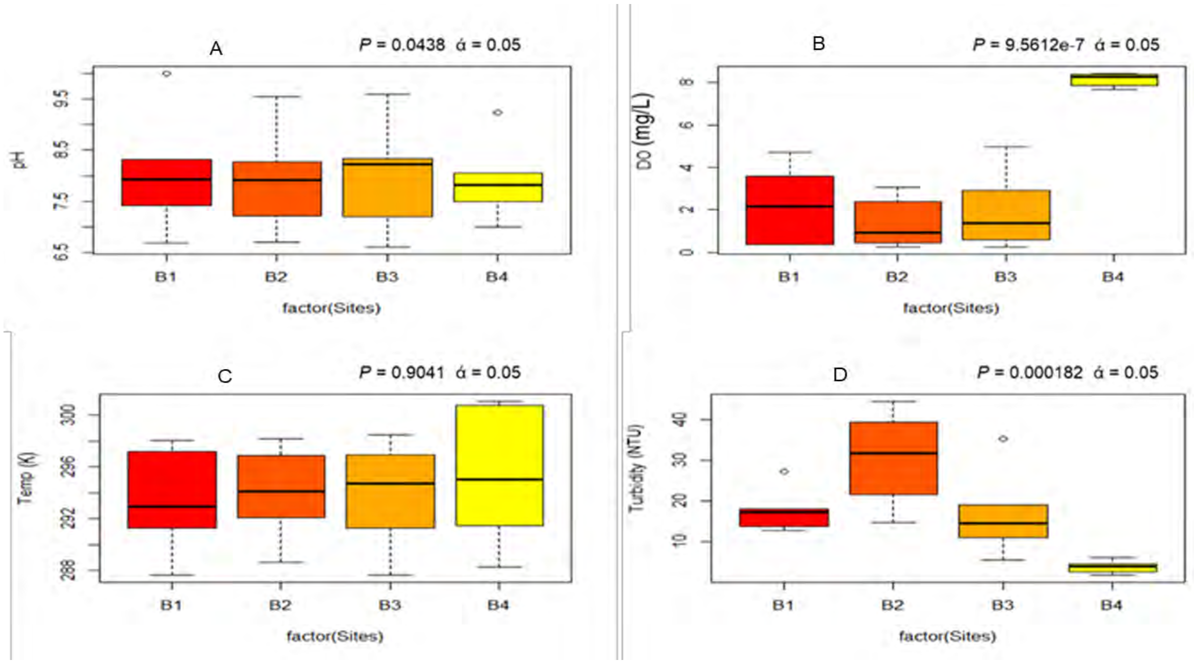
There were significant differences (Kruskal-Willis; $P < 0.05$) for water quality variables data between different land-use types in both the river systems (Figures5.1 and 5.2).

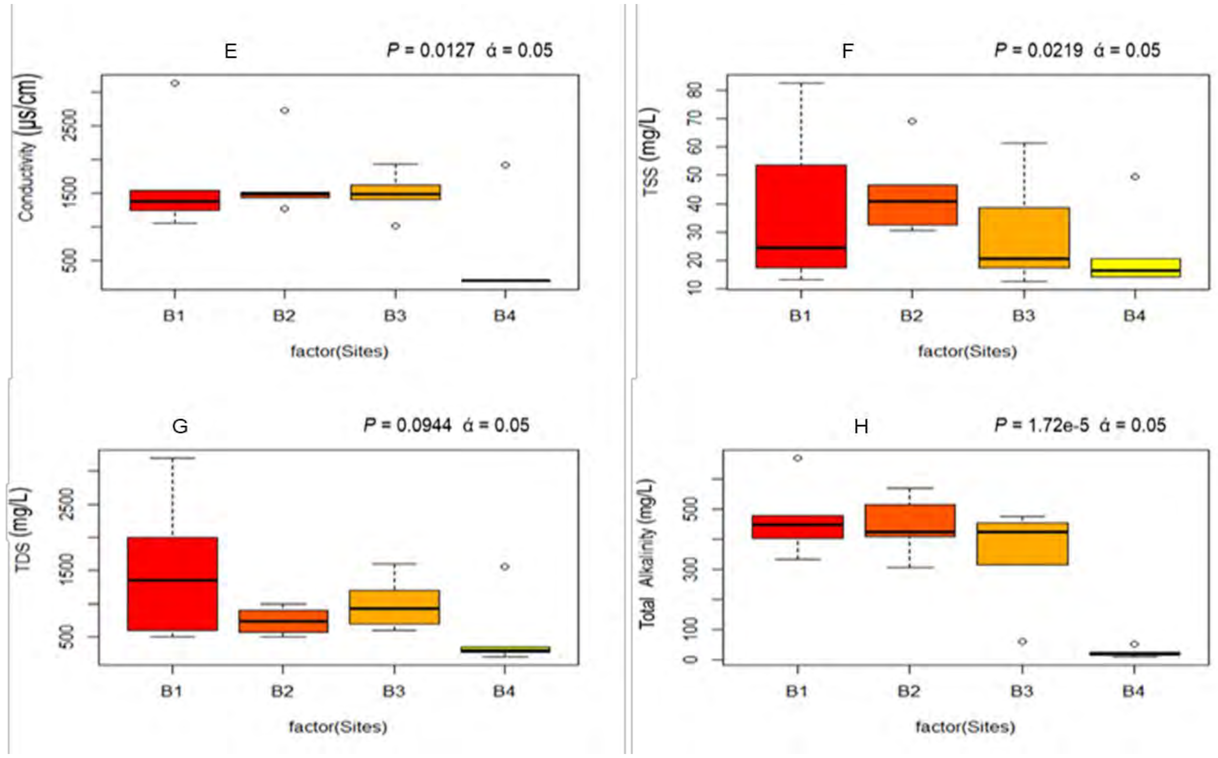
For the Bloukrans River, the mean highest recorded values of water quality variables included EC (3477 µs/cm) at B2, TDS (1916.675 mg/L) at B1, Alkalinity (478.35 mg/L) at B1, pH (7.9075 mg/L) at B2, TSS (5.3.667 mg/L) at B2 and Hardness (382.05 mg/L) at B1 (Appendix A,Table B1)

The following values were recorded for the Swartkops River: EC (2138.75 µs/cm)at S2, TDS (1083.3325 mg/L) at S2, Alkalinity (336.375 mg/L)at S2, pH (7.9775 mg/L)at S1, TSS (82.3875 mg/L) at S1, and Hardness (336.375 mg/L) at S2(Appendix Q, Table Q2)

The highest mean DO concentration was recorded in the least impacted sites for both river systems: 8.07 mg/L at B4 and 8.1275 mg/L at S4 (Appendix Q, TablesQ1, and Q2). The Shapiro-Wilk test indicated non-normality and Kruskal-Wallis was consequently used to compare the variations in environmental variables across the different sites.

Pairwise comparisons using the Kruskal-Wallis multiple comparison *p* values for water quality variables between the multiple study sites across the two river systems was carried out to assess the significant differences of the physico-chemical variables between different study sites (Figures 5.1 and 5.2). Similarly, Kruskal-Wallis multiple comparison *p* values for water quality variables between the two seasons (summer and autumn; Figures 5.3 and 5.4) for the two river systems was carried out to assess the significant differences of the physico-chemical variables. In all cases, with the exception of temperature, there were no significant differences ($P > 0.05$) in the physico-chemical variables across the two seasons.


A
P = 0.0438 ά = 0.05
pH
6.5
7.5
8.5
9.5
B1
B2
B3
B4
factor(Sites)
B
P = 9.5612e-7 ά = 0.05
DO (mg/L)
0
2
4
6
8
B1
B2
B3
B4
factor(Sites)
C
P = 0.9041 ά = 0.05
Temp (K)
288
292
296
300
B1
B2
B3
B4
factor(Sites)
D
P = 0.000182 ά = 0.05
Turbidity (NTU)
10
20
30
40
B1
B2
B3
B4
factor(Sites)


E
P = 0.0127 ά = 0.05
Conductivity (µs/cm)
500
1500
2500
B1
B2
B3
B4
factor(Sites)
F
P = 0.0219 ά = 0.05
TSS (mg/L)
10
20
30
40
50
60
70
80
B1
B2
B3
B4
factor(Sites)
G
P = 0.0944 ά = 0.05
TDS (mg/L)
500
1500
2500
B1
B2
B3
B4
factor(Sites)
H
P = 1.72e-5 ά = 0.05
Total Alkalinity (mg/L)
0
100
300
500
B1
B2
B3
B4
factor(Sites)

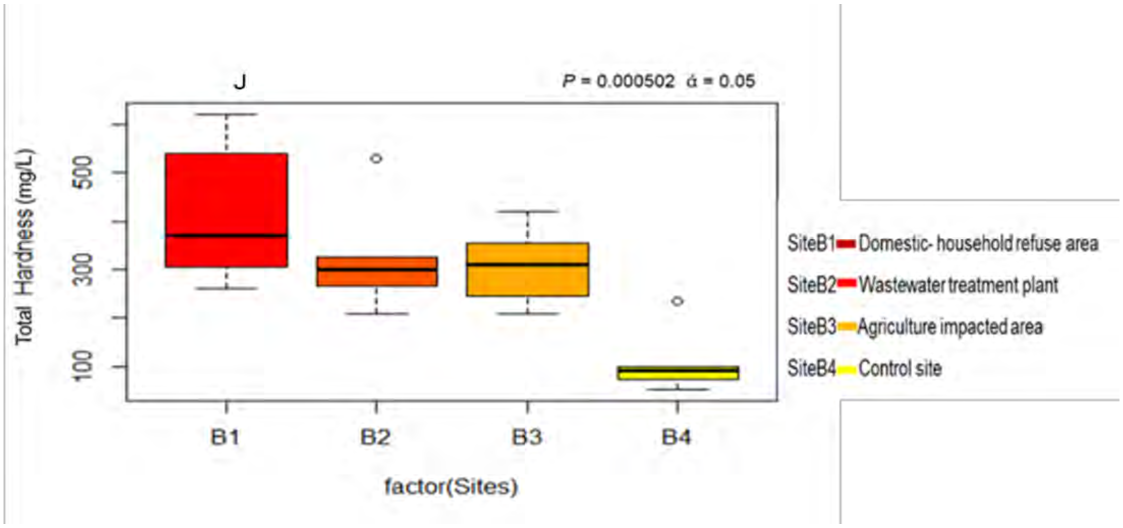
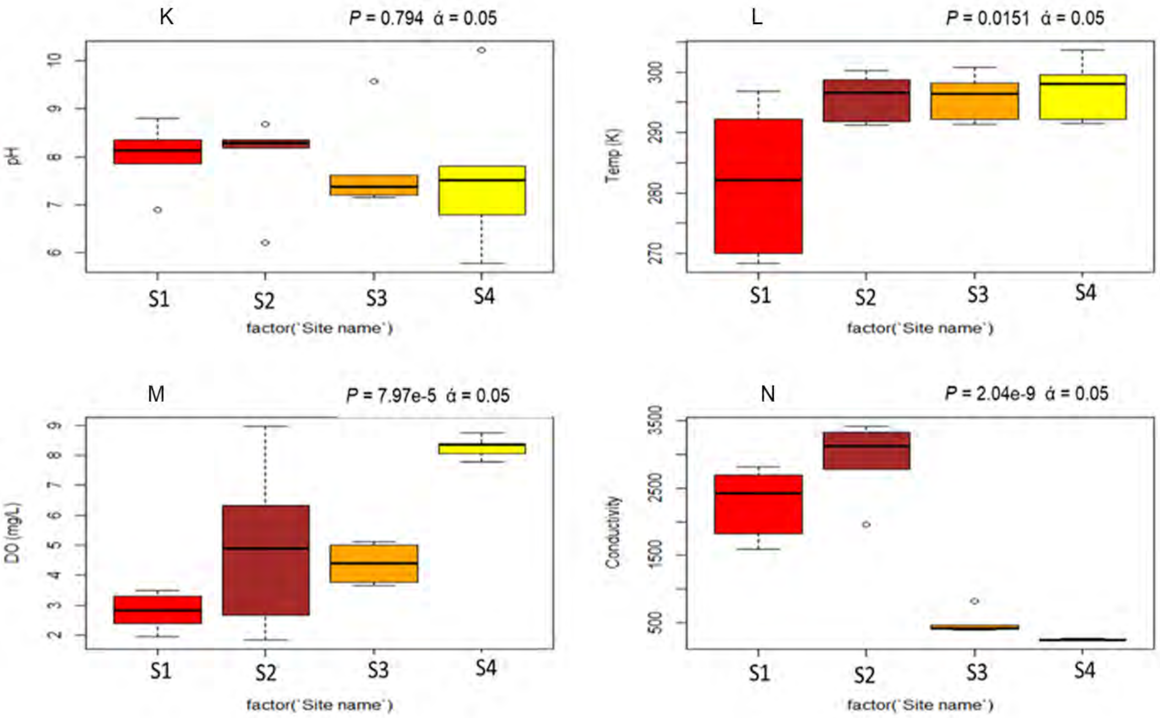


Figure 5. 1:Spatial variability of the mean concentration of pH (A), dissolved oxygen (DO) (B), temperature (C), turbidity (D), electrical conductivity (EC) (E), total suspended solids (TSS) (F), total dissolved solids (TDS) (G), total alkalinity (H) and total hardness (J) between the four sampling sites along the Bloukrans River (B1, B2, B3) and Palmiet river (B4) during the study period (January 2022 to May 2022 – summer to autumn).



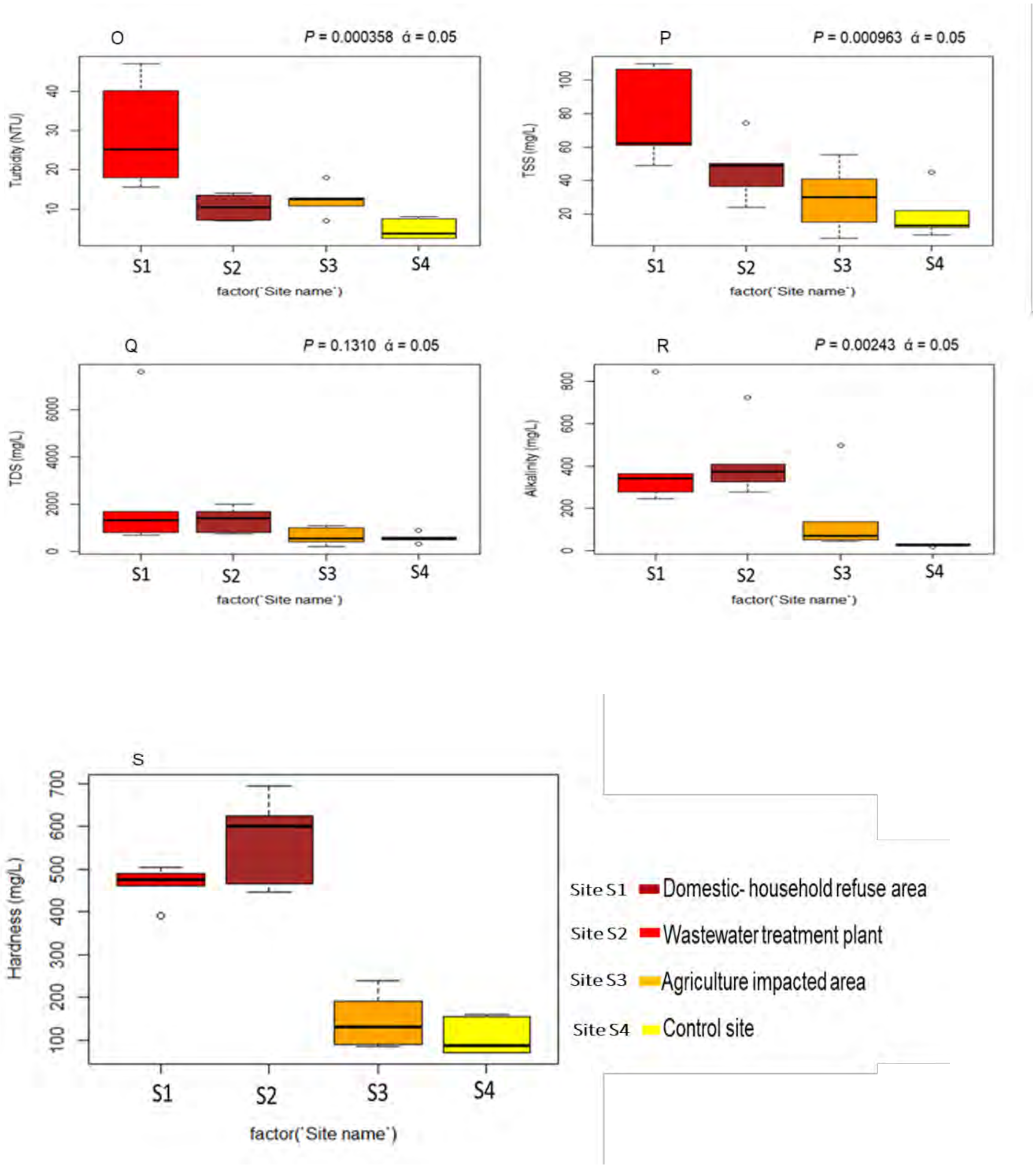
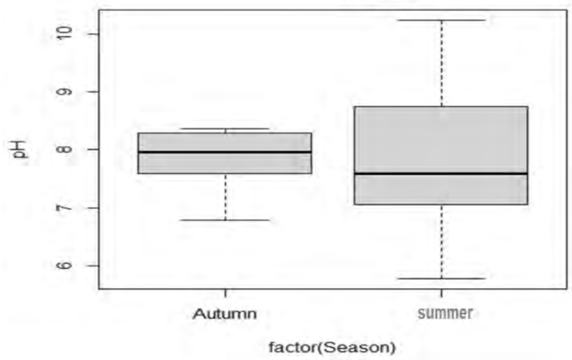
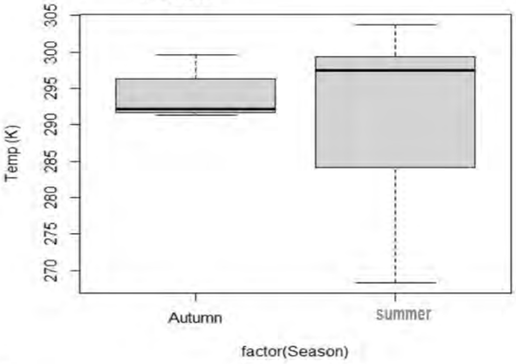


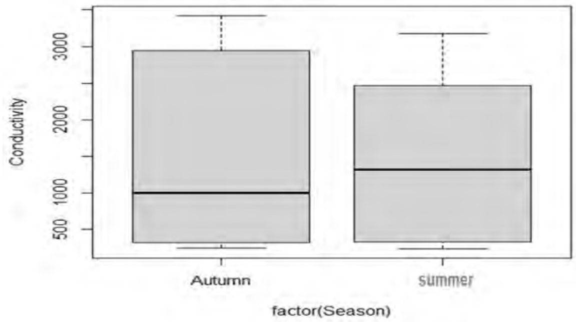
Figure 5.2: Spatial variability of the mean concentration of pH (K), dissolved oxygen (DO) (M), temperature (L), turbidity (O), electrical conductivity (EC) (N), total suspended solids (TSS) (P), total dissolved solids (TDS) (Q), total alkalinity (R) and total hardness (S) between the four sampling sites in Swartkops River (S1, S2, S3 and S4) during the study period (January 2022 to May 2022 – summer to autumn)



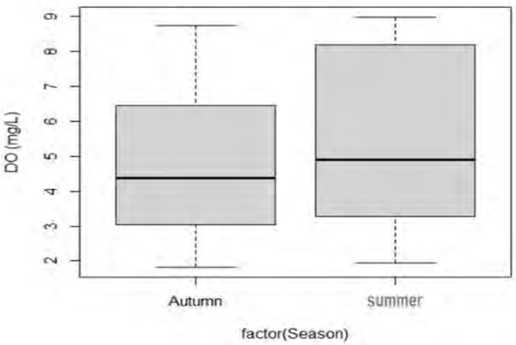
***P*= 0.1036**



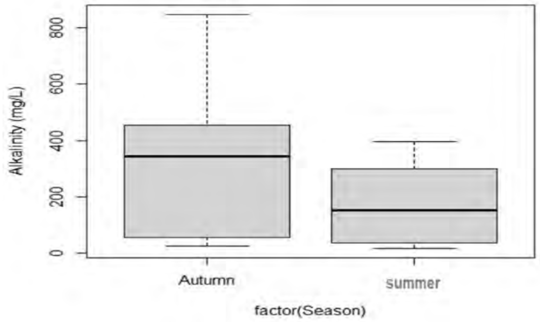
***P*= 0.6591**



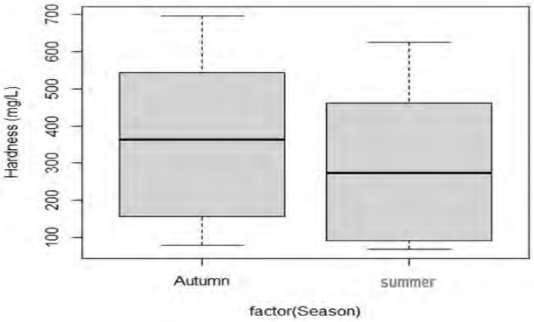
***P*= 0.9421**



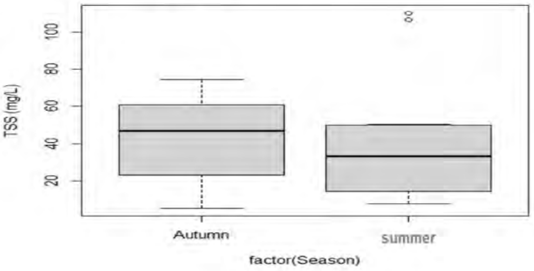
***P*= 0.6012**



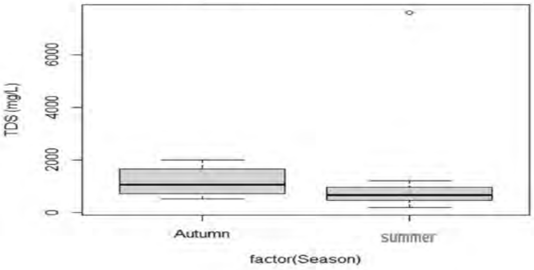
***P*= 0.7665**



***P*= 0.9524**



***P*= 0.7685**



***P*= 0.5392**

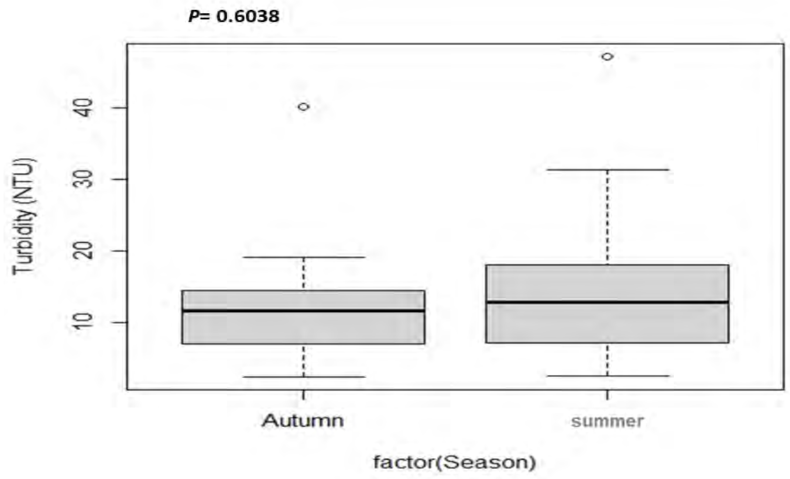
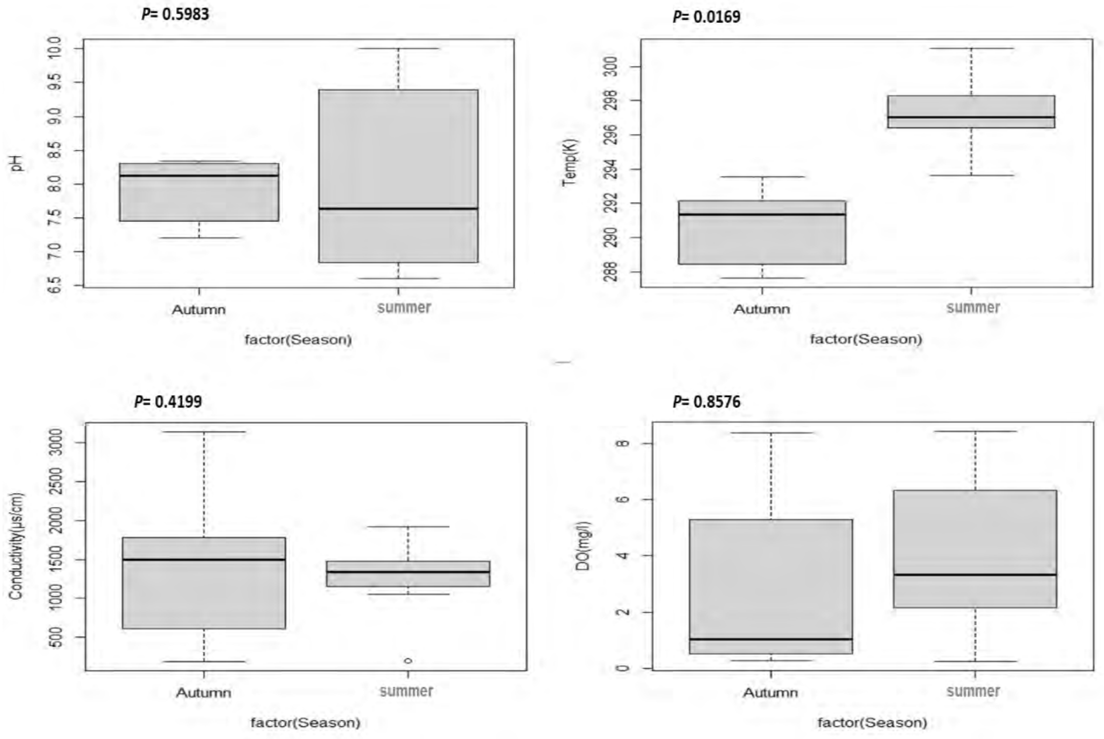


Figure 5.3: Seasonal variability of the mean concentration of pH, Temperature(K), dissolved oxygen (DO), electrical conductivity (EC), total suspended solids (TSS), total dissolved solids (TDS), total alkalinity, total hardness and turbidity between the two sampling seasons (summer and autumn) in the Bloukrans River.



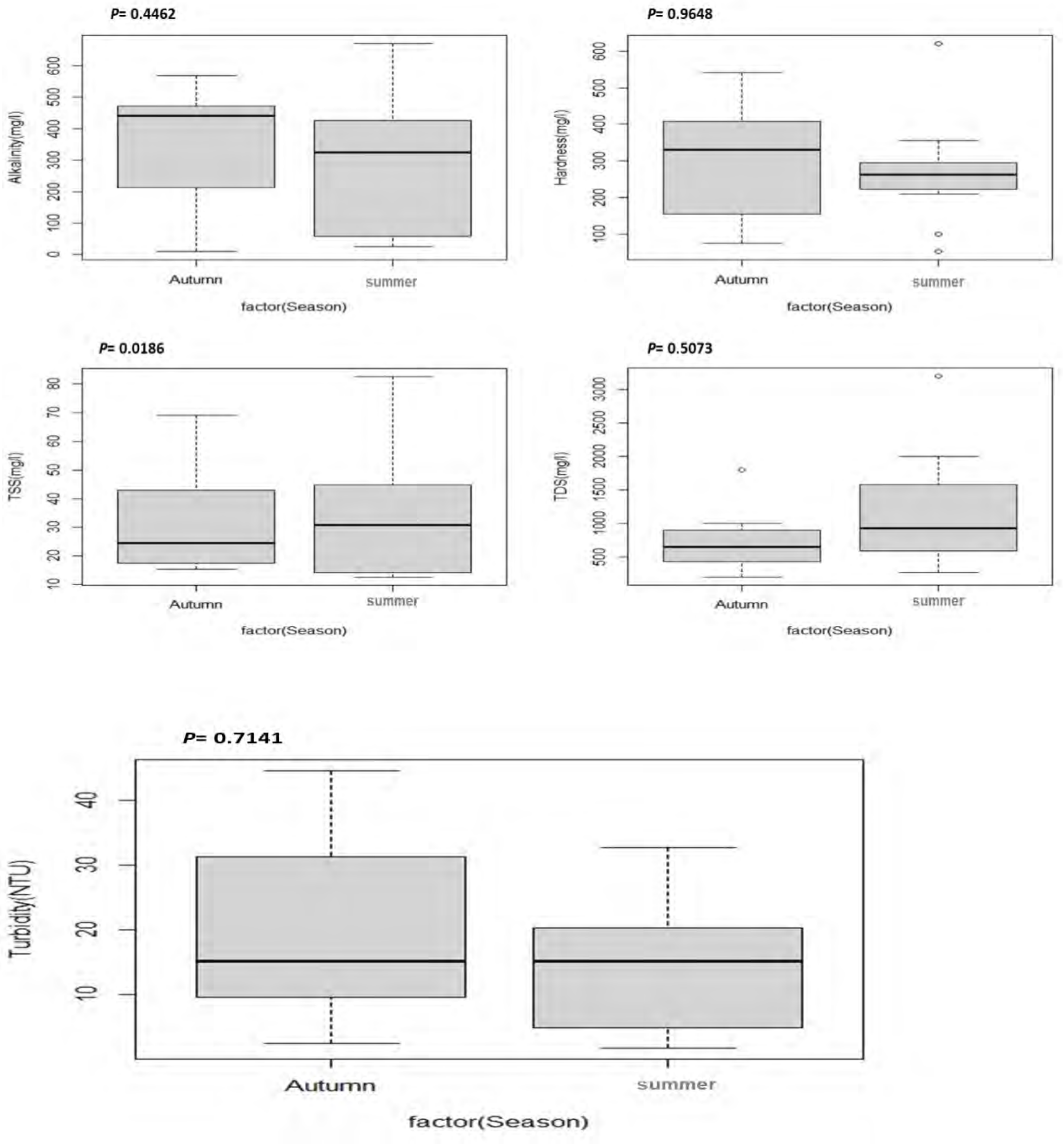


Figure 5.4: Seasonal variability of the mean concentration of pH, Temperature(K), dissolved oxygen (DO), electrical conductivity (EC), total suspended solids (TSS), total dissolved solids (TDS), total alkalinity, total hardness and turbidity between the two sampling seasons, summer and autumn, in the Swartkops River.

5.4.2 Relating the measured physico-chemical variables to the concentrations of adsorbed organic chemical pollutants by MPs across the sites.

In order to establish a relationship between the recorded physicochemical properties and adsorbed concentration of the targeted chemical pollutants onto the MPs, linear regression analysis was conducted. The regression coefficient and regression coefficient of determination (r and R^2 values) were used to reveal the extent of influence on river physicochemical properties on the adsorption chemical pollutants onto MPs

Regression analysis results (Table 5.1 – 5.4) and regression graphs (Figure 5.5 – 5.12) for the relationship between water physicochemical variables and the adsorbed concentration of the targeted pollutants on deployed PET and PP MPs showed that, in most instances, adsorbed concentrations of the targeted pollutants on the MPs were significantly positively associated ($P <$ 0.05 and (r) = positive) with changes in measured water physicochemical variables, with the exception of temperature and DO a significant negative regression ($p < 0.05$ and (r) = negative values Table 5.1 – 5.4 and Figure 5.7 and Figure 5.8 respectively). The slopes of the regression graphs and correlation coefficient in the case of regression analysis indicated a positive or negative association between the analytes and the water physicochemical variables.

For instance for measured DO and temperature , the correlation coefficient (regression analysis-Table 5.1 – 5.4) and the slopes of the regression graphs (Figure5.7 and Figure 5.8 respectively) were negative ((r) = negative values), indicating that the greater the Temperature or dissolved oxygen, the lower the determined concentration of the targeted chemical pollutants onto the MPs. Indeed higher dissolved oxygen (DO) values were recorded at control sites with the lowest concentration adsorbed onto MPs recorded i.e. B4 (on Palmiet River in the Bloukrans catchment) and S4 (on Swartkops River) (the least impacted site, the control site) (Tables 6 and 7).

However, for the rest of the water physicochemical variables (alkalinity, turbidity, conductivity, total suspended solids, and pH), slopes were positive, indicating a positive correlation between the measured physicochemical values and concentration adsorbed of the targeted chemical pollutants onto MPs. The mean water depth and water flow were similar (Kruskal-Wallis $p >$ 0.05) across two catchment zones (Tables5.1 – 5.4).Indeed, generally, higher values of conductivity, turbidity, total hardness, and total alkalinity were recorded at the impacted sites where high concentration of the targeted pollutants was recorded i.e. Bloukrans River (B1, B2 and B3) and Swartkops River (S1, S2 and S3) an indicator that poor water quality are typically associated with pollution that leads to higher chemical adsorption onto MPs.

The higher concentration of the adsorbed organic chemical pollutants was associated with the low DO, Temp and TDs and high conductivity, Alkalinity, pH, Hardness, TSS (Figure 5.5 – 5.12) physicochemical variables that indicate poor water quality. This signifies that water pollution as result of the different land-use activities was the main contributor to higher concentration ad(r) = negative adsorbed. Organic chemical pollutants concentration adsorbed on PET Type 2 and PP Type 2 was used to test the linear regression analysis between physico-chemical variables and adsorbed concentration since the concentration trends adsorbed were same for the two sizes of MPs used.

Table 5.1: Results of a linear regression analysis for a relationship between water quality variables and the concentration of the adsorbed organic pollutants on the retrieved microplastics from Bloukrans River (PET Type 2 MP). Double asterisk (**) means the P value is significant at 0.01 confidence level and a single asterisk (*) means P value is significant at 0.05 level.

Targeted chemical pollutants pollutants /Water quality variable		pH	Temperature	Alkalinity	Turbidity	Conductivity	Hardness	DO	TSS	TDS
Sulfamethoxazole	Regression coefficient (r)	0.5136	- 0.2134	0.4987	0.5110	0.3231	0.5492	-0.6659	0.3473	- 0.1750
	Regression coefficient of determination(R^2)	0.2638	0.0468	0.2487	0.2612	0.1044	0.4117	0.4434	0.1206	0.0306
	P-value	0.0876	0.0109**	0.0494*	0.0891	0.0305	0.0443	0.0104**	0.0268*	0.0586
Ciprofloxacin	Regression coefficient (r)	0.1005	- 0.1440	0.5434	0.3567	0.40316	0.3924	-0.6901	0.0802	- 0.1015
	Regression coefficient of determination(R^2)	0.1272	0.0205	0.4307	0.1272	0.1625	0.154	0.4762	0.0812	0.0103
	P-value	0.1551	0.6552	0.0249*	0.1551	0.0103**	0.0207*	0.0123**	0.3722	0.0753
17β-Estradiol	Regression coefficient (r)	0.1585	- 0.2885	0.6318	0.4136	0.3725	0.5027	-0.5792	0.0051	- 0.2314
	Regression coefficient of determination(R^2)	0.2569	0.0823	0.4667	0.1710	0.0944	0.2527	0.3356	0.2681	0.0683
	P-value	0.06233	0.0361*	0.01433**	0.0331**	0.0104**	0.0195*	0.0484*	0.0374*	0.0504*
4-(2, 6-dimethyl-2-heptyl)Phenol	Regression coefficient (r)	0.17518	-0.2097	0.30237	0.30237	0.57196	0.5374	-0.506	0.3882	0.4103
	Regression coefficient of determination(R^2)	0.02704	0.6097	0.3475	0.08259	0.4612	0.4755	0.3944	0.1562	0.0223
	P-value	0.4429	0.6665	0.00236**	0.1733	2.643e-4**	1.934e-4**	1.018e-3**	0.0509*	0.4856

Table 5.2: Result of a linear regression analysis for a relationship between water quality variables and the concentration of the adsorbed organic pollutants on the retrieved microplastics from Bloukrans River (PP Type 2 MP). Double asterisk (**) means P value is significant at 0.01 confidence level and a single asterisk (*) means P value is significant at 0.05 level.

Pharmaceutical pollutants /Water quality variable		pH	Temperature	Alkalinity	Turbidity	Conductivity	Hardness	DO	TSS	TDS
Sulfamethoxazole	Regression coefficient (r)	0.6513	- 0.1316	0.5874	0.6150	0.2313	0.5673	-0.7660	0.4132	- 0.2651
	Regression coefficient of determination(R^2)	0.3386	0.0381	0.2814	0.1723	0.0552	0.3556	0.4514	0.2171	0.0154
	P-value	0.0768	0.0121**	0.0391*	0.07162	0.0413*	0.03142*	0.0133**	0.0187**	0.0664
Ciprofloxacin	Regression coefficient (r)	0.2111	- 0.4301	0.5431	0.2071	0.1941	0.2836	-0.8025	0.0507	- 0.3123
	Regression coefficient of determination(R^2)	0.1711	0.0153	0.3110	0.2361	0.1233	0.1432	0.3312	0.0711	0.0112
	P-value	0.3200	0.5713	0.0146*	0.2450	0.0141*	0.0212*	0.0100**	0.2282	0.0125*
17β-Estradiol	Regression coefficient (r)	0.1815	- 0.3041	0.7329	0.3161	0.2205	0.6219	-0.4393	0.0016	- 0.3425
	Regression coefficient of determination(R^2)	0.3601	0.0721	0.3602	0.1103	0.0466	0.1209	0.4512	0.0268	0.0652
	P-value	0.0611	0.0235*	0.0127*	0.0216*	0.0403*	0.0177*	0.0448*	0.0771	0.0543
4-(2, 6-dimethyl-2-heptyl)Phenol	Regression coefficient (r)	0.1183	-0.1071	0.2017	0.2817	0.7961	0.7182	-0.5451	0.3217	0.4370
	Regression coefficient of determination(R^2)	0.0202	0.0517	0.3475	0.0753	0.4612	0.5451	0.3944	0.1602	0.0264
	P-value	0.2442	0.7771	0.00243**	0.1380	1.714e-4**	2.3004e-4**	1.103e-3**	0.0569	0.4516*

Table 5.3: Result of a linear regression analysis for a relationship between water quality criteria parameters and concentration of the targeted adsorbed pharmaceutical pollutants on the retrieved from Swartkops River (PET Type 2 MP). Double asterisk (**) means P value is significant at 0.01 confidence level and a single asterisk (*) means P value is significant at 0.05 level.

Pharmaceutical pollutants /Water quality variable		pH	Temperature	Alkalinity	Turbidity	Conductivity	Hardness	DO	TSS	TDS
Sulfamethoxazole	Regression coefficient (r)	0.2181	-0.2295	0.3781	0.2401	0.4929	0.3615	-0.2805	0.2401	0.2924
	Regression coefficient of determination(R^2)	0.0561	0.0351	0.1692	0.3277	0.2224	0.1595	0.1327	0.0759	0.0193
	P-value	0.7281	0.5886	0.0458*	0.0034**	0.0228*	0.0532	0.0801	0.1926	0.5173
Ciprofloxacin	Regression coefficient (r)	0.0471	- 0.1090	0.1415	0.2536	0.3333	0.29091	-0.2608	0.3023	0.1617
	Regression coefficient of determination(R^2)	5.641e-4	0.0210	5.048e-5	0.0264	0.0298	0.03492	0.1411	0.0257	0.0066
	P-value	0.9123	0.4991	0.9737	0.4474	0.4197	0.3819	0.0704	0.4537	0.7047
17β-Estradiol	Regression coefficient (r)	0.21818	-0.2295	0.4102	0.5771	0.4682	0.4699	-0.5408	0.5255	0.2983
	Regression coefficient of determination(R^2)	0.05189	0.2875	0.3965	0.4855	0.3738	0.3941	0.4242	0.4841	0.1327
	P-value	0.2844	0.0069**	9.762e-4**	1.552e-4**	0.0015**	0.0010**	5.665e-4**	1.552e-4**	0.0801

Table 5.4: Result of a linear regression analysis for a relationship between water quality criteria parameters and concentration of the targeted adsorbed pharmaceutical pollutants on the retrieved from Swartkops River (PP Type 2 MP). Double asterisk (**) means P value is significant at 0.01 confidence level and a single asterisk (*) means P value is significant at 0.05 level.

Pharmaceutical pollutants /Water quality variable		pH	Temperature	Alkalinity	Turbidity	Conductivity	Hardness	DO	TSS	TDS
Sulfamethoxazole	Regression coefficient (r)	0.1508	-0.4172	0.1234	0.0129	0.3992	0.5331	-0.3456	0.3124	0.2441
	Regression coefficient of determination(R^2)	0.6431	0.0121	0.2003	0.3657	0.1453	0.0906	0.2134	0.0978	0.0124
	P-value	0.8175	0.8716	0.0876	0.00416**	0.0131*	0.0421*	0.0125*	0.1628	0.3004
Ciprofloxacin	Regression coefficient (r)	0.0187	- 0.1009	0.2121	0.2036	0.1236	0.2011	-0.3108	0.3061	0.2134
	Regression coefficient of determination(R^2)	4.431e-4	0.0103	6.1430e-5	0.0289	0.0312	0.0423	0.1534	0.0123	0.4321
	P-value	0.6213	0.4991	0.7172	0.3142	0.3489	0.7512	0.0855	0.3447	0.6543
17β-Estradiol	Regression coefficient (r)	0.8181	-0.4312	0.5123	0.7213	0.3217	0.5094	-0.6543	0.6754	0.3421
	Regression coefficient of determination(R^2)	0.0231	0.2134	0.7312	0.9124	0.7812	0.4123	0.3234	0.4176	0.3421
	P-value	0.3123	0.00981**	3.9012e-4**	2.1345e-4**	0.00231**	0.00654**	3.6541e-4**	1.8712e-4**	0.0975

5.4.3 Regression graphs for the relationship between concentration adsorbed of SXM, CIP, E2, $C_{18}H_{24}O$) onto PP and PET MPs and the physicochemical variables

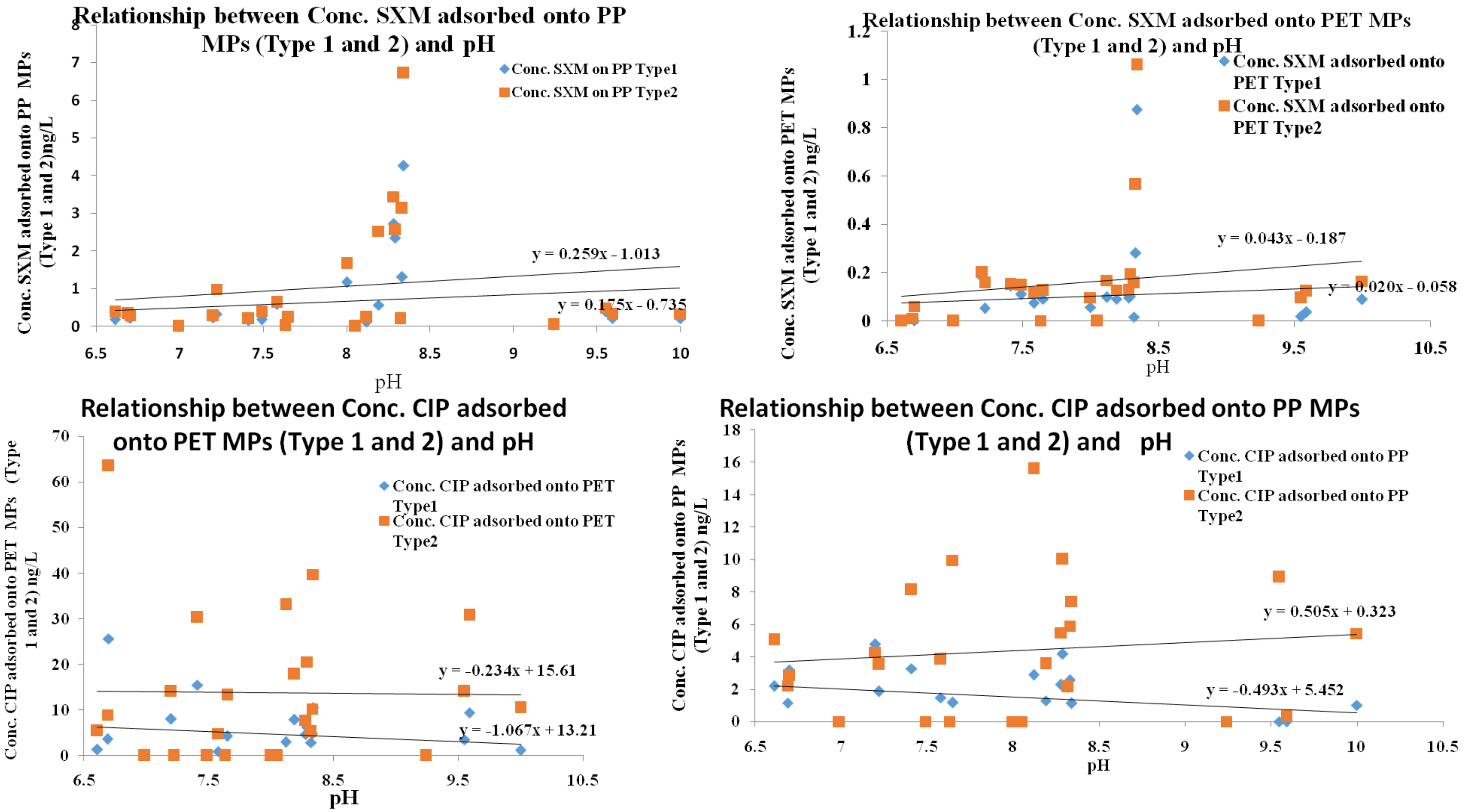


Figure 5. 5: Regression graphs for the relationship between concentrations adsorbed of SXM, and CIP onto PP (Type 1 and 2) and PET (Type 1 and 2) MPs and pH indicating the regression lines (y).

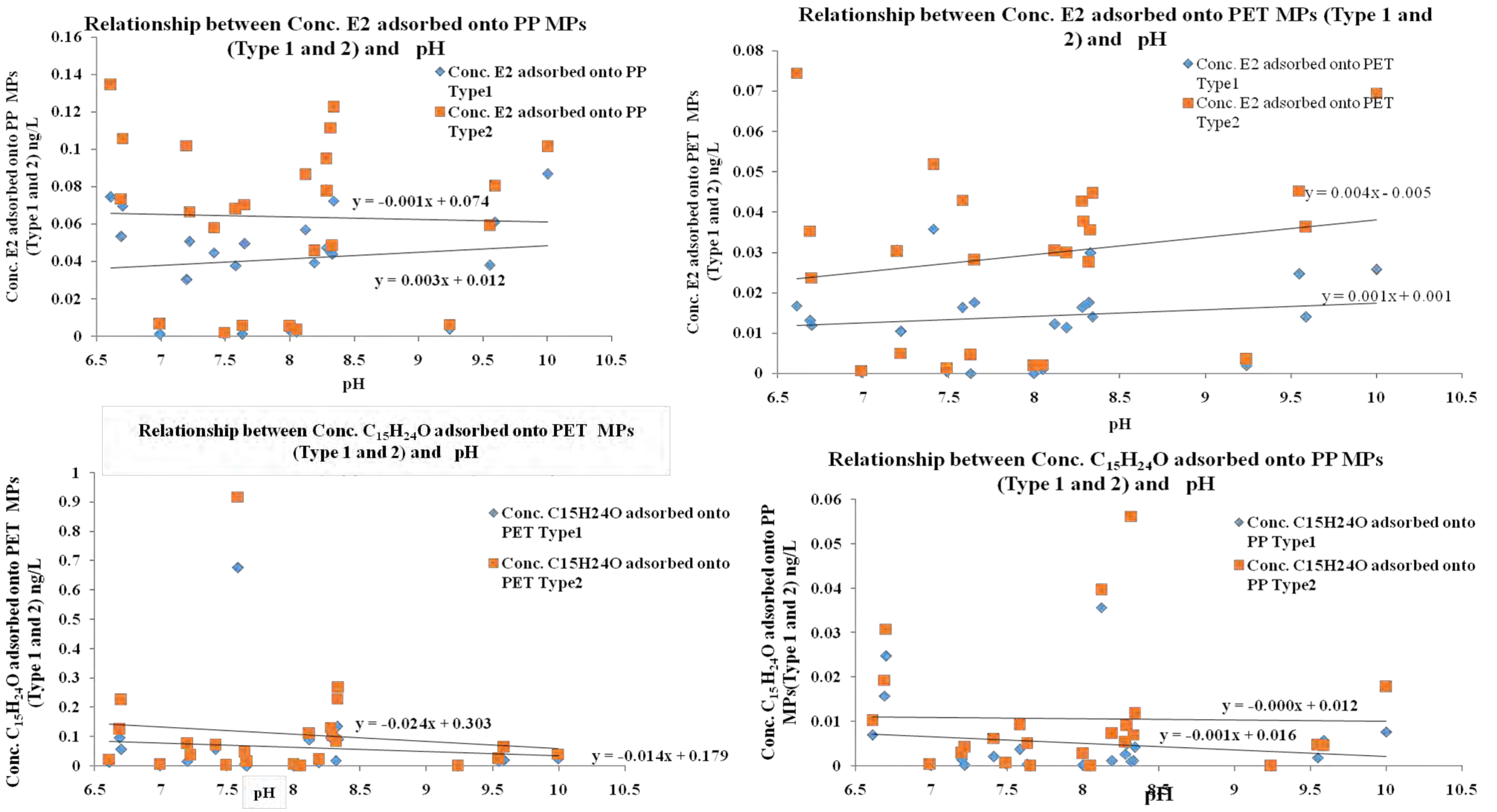


Figure 5. 6: Regression graphs for the relationship between concentrations adsorbed of E2, and $C_{18}H_{24}O$ onto PP (Type 1 and 2) and PET (Type 1 and 2) MPs and pH indicating the regression lines (y).

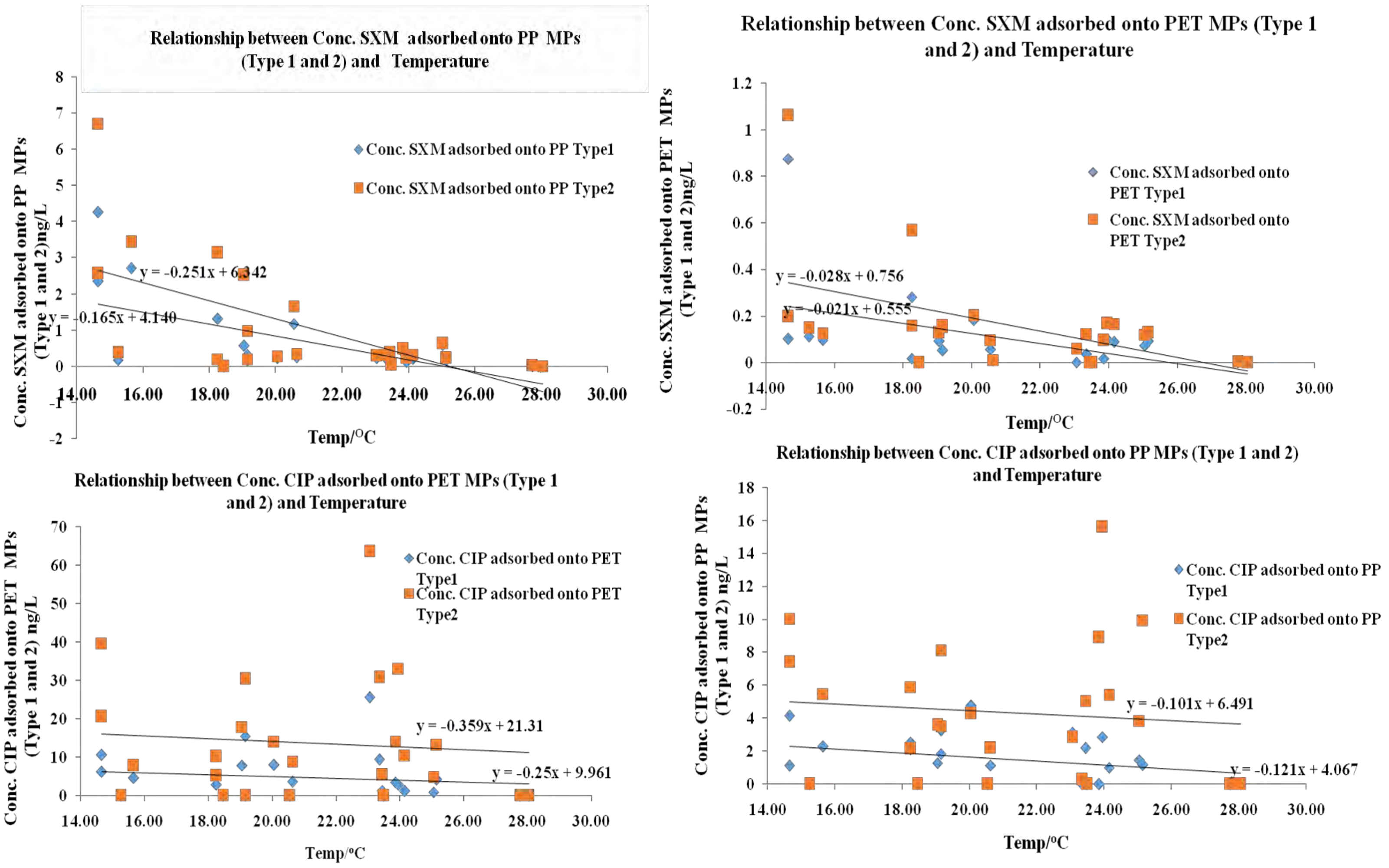


Figure 5. 7: Regression graphs for the relationship between concentrations adsorbed of SXM, and CIP onto PP (Type 1 and 2) and PET (Type 1 and 2) MPs and Temperature indicating the regression lines (y).

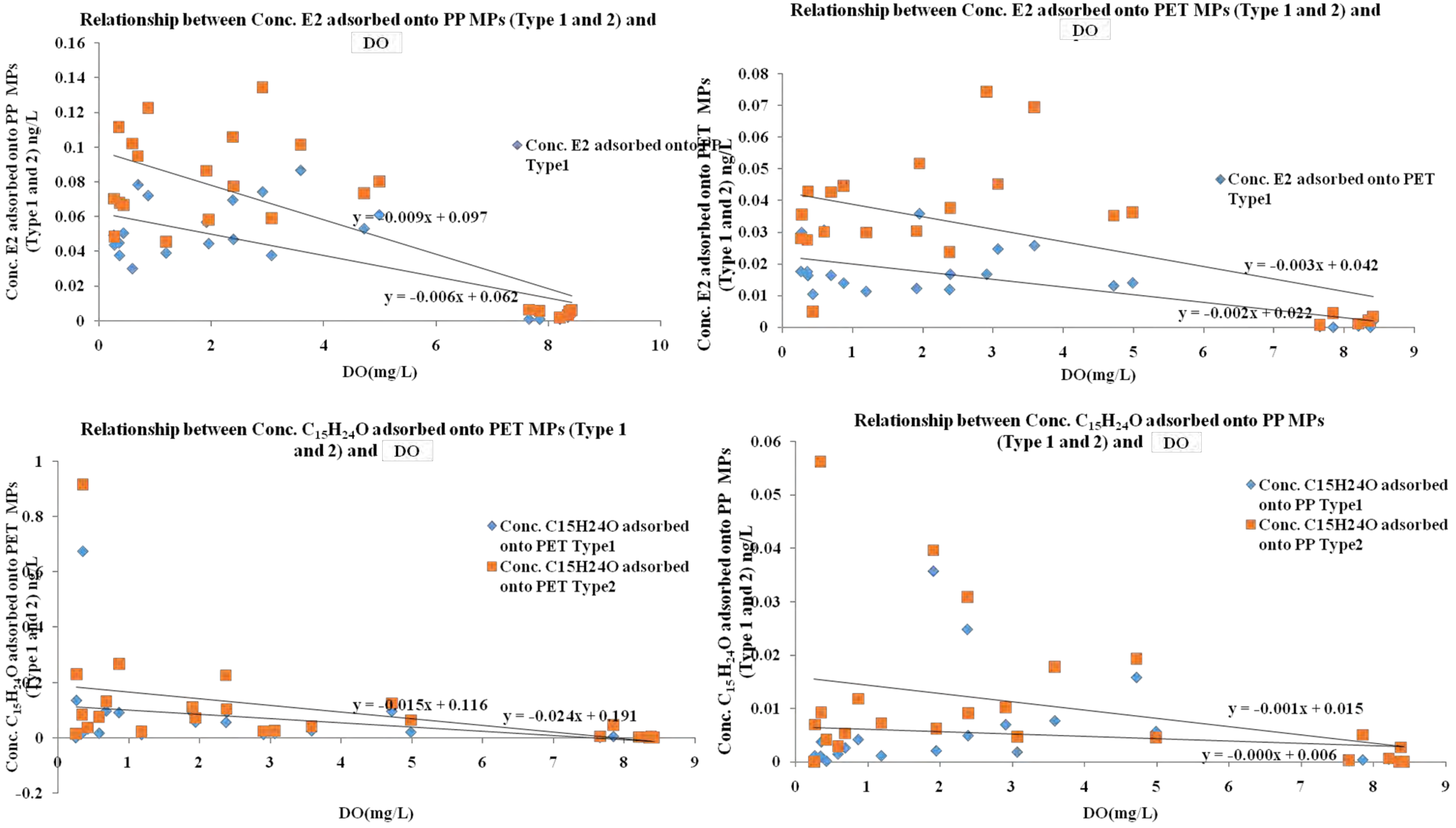


Figure 5. 8: Regression graphs for the relationship between concentrations adsorbed of E2, and $C_{18}H_{24}O$ onto PP (Type 1 and 2) and PET (Type 1 and 2) MPs and DO indicating the regression lines (y).

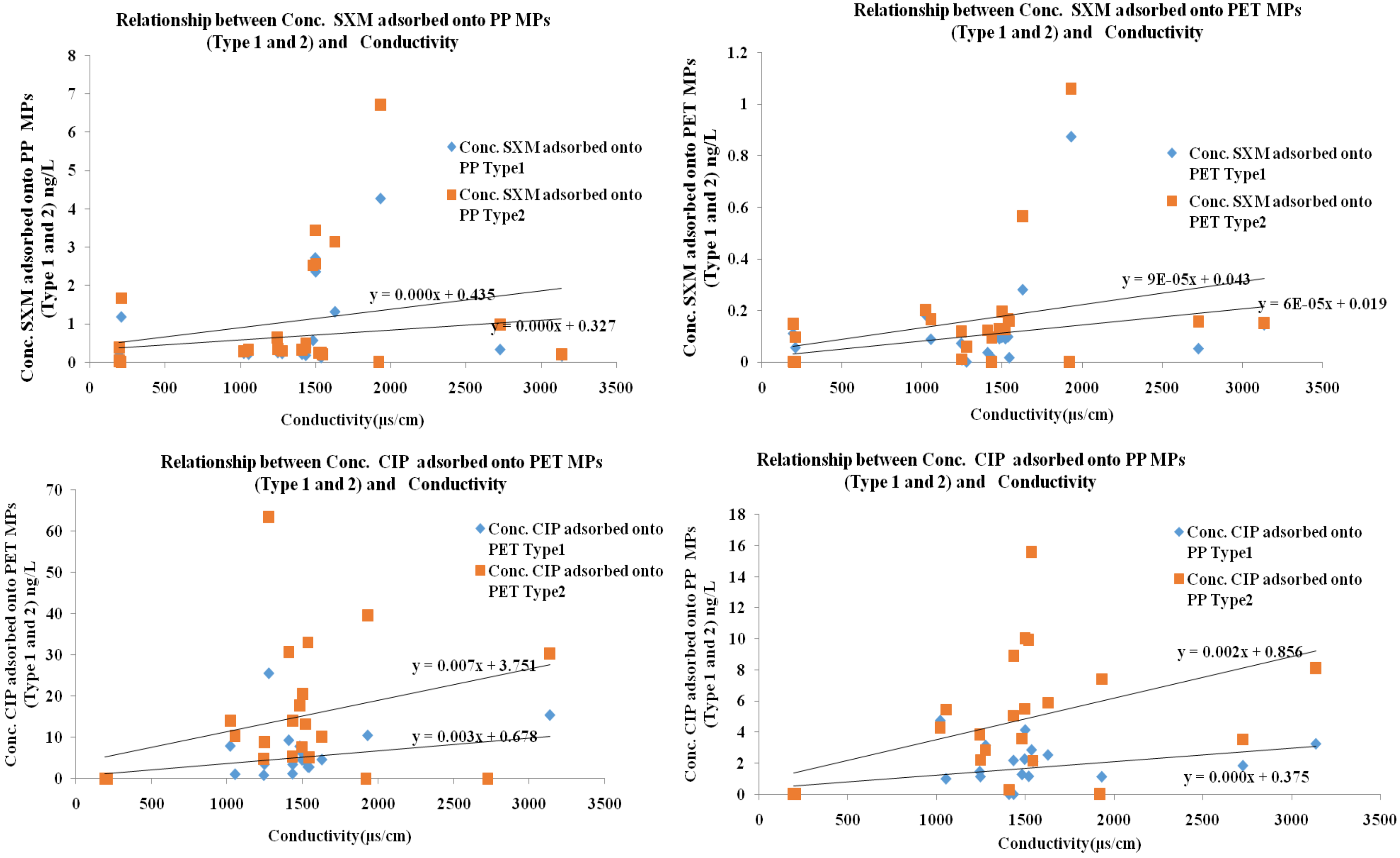


Figure 5. 9: Regression graphs for the relationship between concentrations adsorbed of SXM, and CIP onto PP (Type 1 and 2) and PET (Type 1 and 2) MPs and Conductivity indicating the regression lines (y).

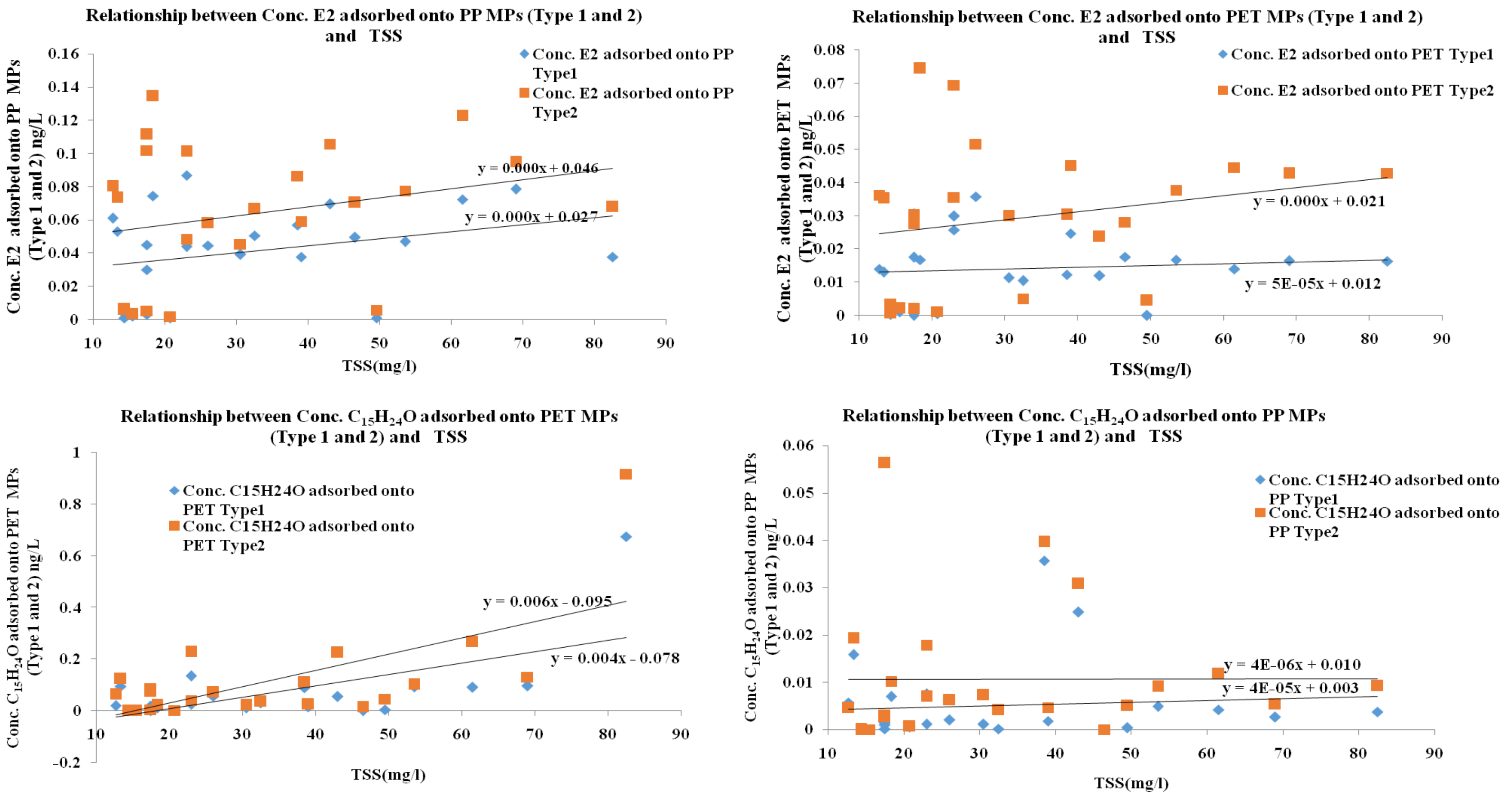


Figure 5. 10: Regression graphs for the relationship between concentrations adsorbed of E2, and $C_{18}H_{24}O$ onto PP (Type 1 and 2) and PET (Type 1 and 2) MPs and TSS indicating the regression lines (y).

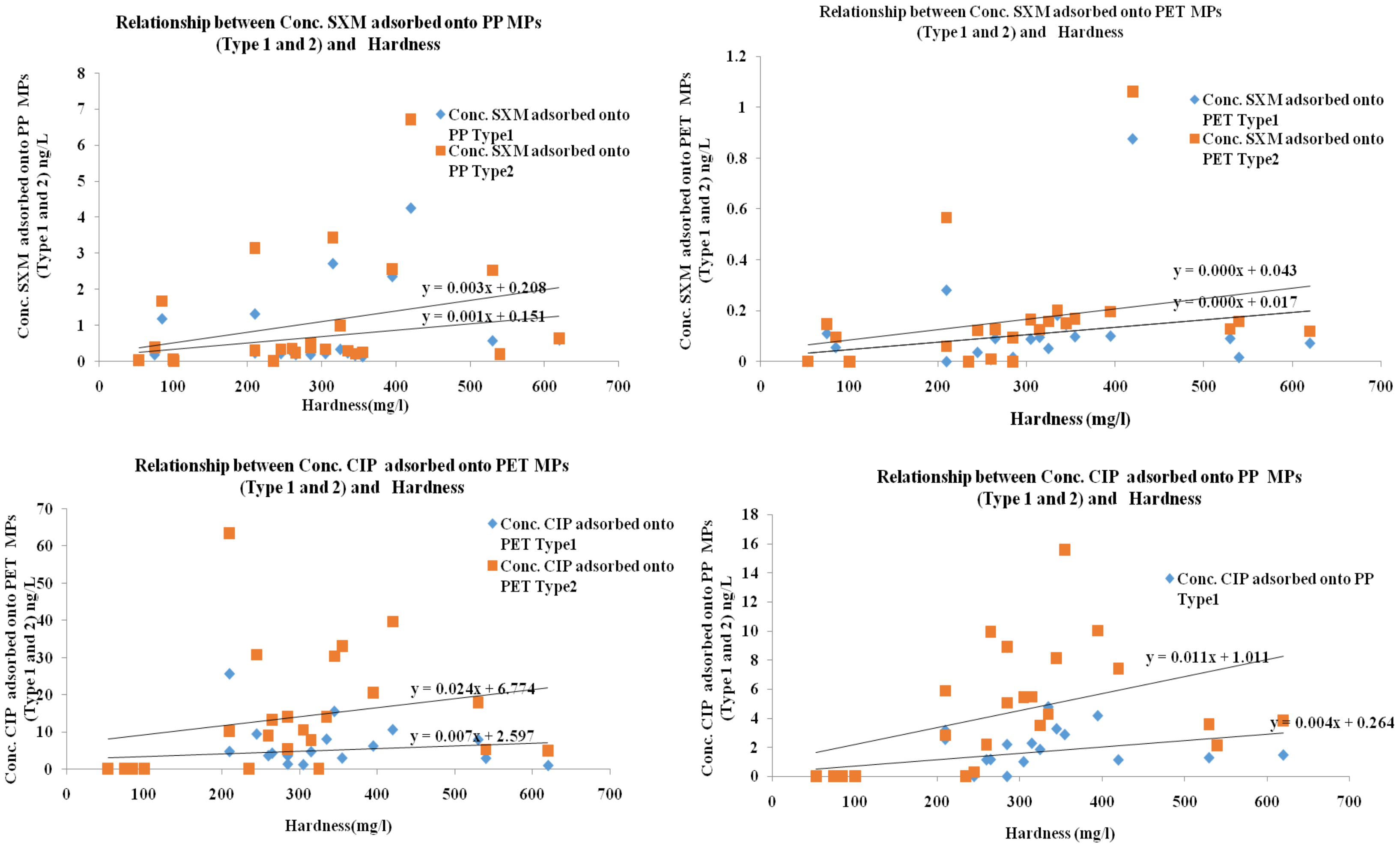


Figure 5. 11: Regression graphs for the relationship between concentrations adsorbed of SXM, and CIP onto PP (Type 1 and 2) and PET (Type 1 and 2) MPs and Hardness indicating the regression lines (y).

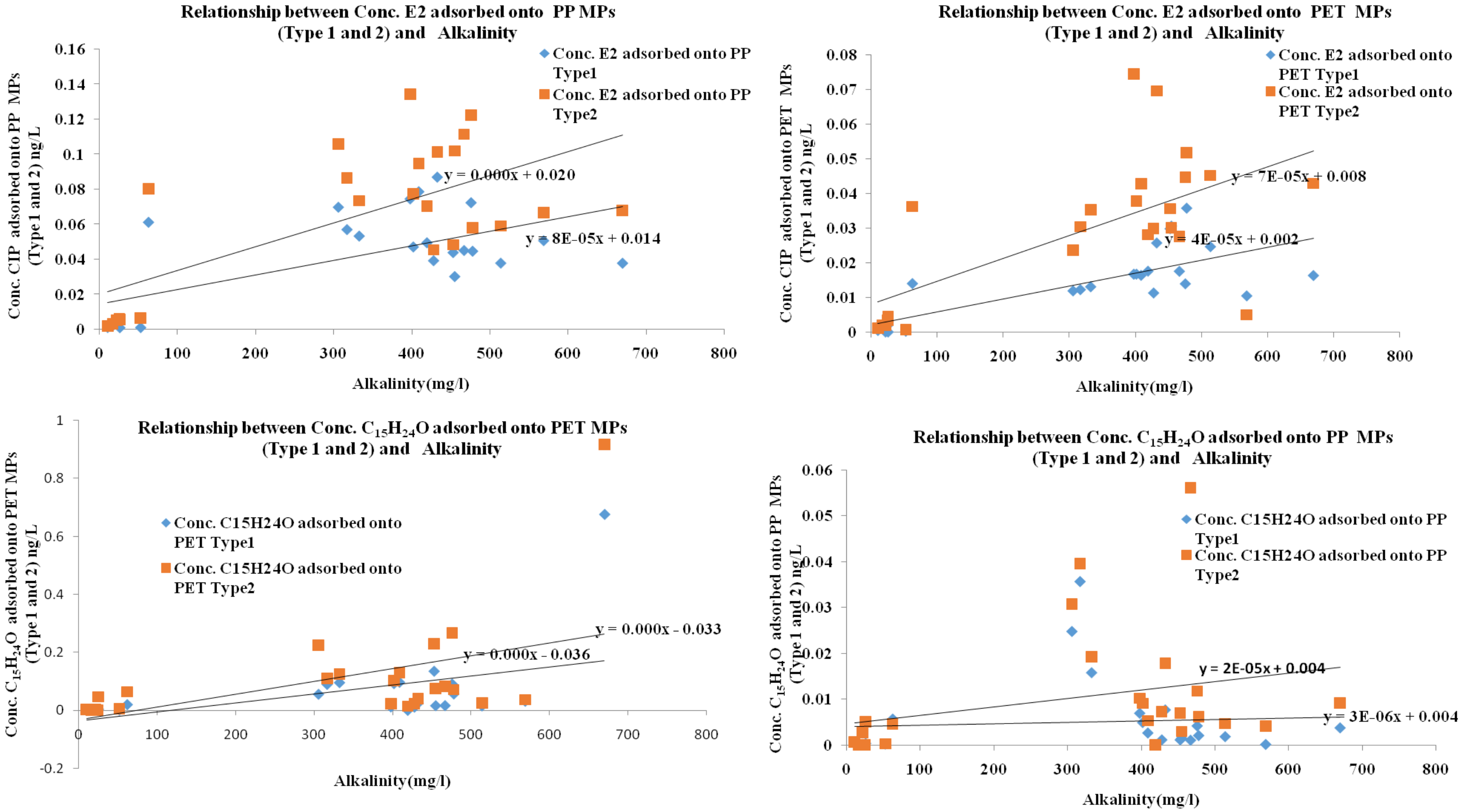


Figure 5. 12: Regression graphs for the relationship between concentrations adsorbed of E2, and $C_{18}H_{24}O$ onto PP (Type 1 and 2) and PET (Type 1 and 2) MPs and pH indicating the regression lines (y).

5.5 Discussion

Physico-chemical water variables differed between the different sampling sites in the two river systems, with sites B1, B2, B3 and B4 across the Bloukrans River and S1, S2, S3 and S4 across the Swartkops River (Appendix B: Table Q1 and Q2). According to Cheng et al. (2022), the land use-water quality relationship is complex, with any correlation observed in one catchment likely to be site- or region-specific. However, some water quality determinants and land covers show reproducible trends(Baker, 2005). For this research, the temperature, a crucial factor for chemical and biological reactions in water, ranged from 21^{o}C at B4 to the highest 23^{o}C in Bloukrans River. In contrast, for Swartkops Rivers, the temperature ranged from 19 ^{o}C to 28^{o}C. Since an adsorption reaction is usually an exothermic reaction (Hua, 2017; Melland et al., 2018; Kondraju & Rajan, 2019), the extent of adsorbed concentration of the chemical pollutants onto the MPs generally increased with decreasing temperature changes, and regression coefficient was negative (Tables 5.1 – 5.4 and Figure 5.7). These temperature changes indicate that land use types disproportionately influence water quality variables. Indeed, variation in temperature values tended to alter the concentration adsorbed to a significant extent ($P< 0.05$) (Table 5.1 – 5.4)

The pH of water can affect the surface charge of both MPs and pollutants, influencing the interaction processes (Avazzadeh et al., 2023). The pH ranged from 7.99 at B2 to 8.34 at B3 for the Bloukrans River and from 6.21 at S1 to 10.23 at S4 for the Swartkops River. This changing pH value indicates a relationship between land use and water chemistry parameters, probably due to the strong contrast between catchment land-use practices (Abu Bakar et al., 2020). It was observed that sites with higher pH values (B4 - and S4 -) had low concentrations adsorbed by the targeted chemical pollutants. This could be attributed to the fact that other sites (B1 – B3) and (S1 – S4), which were impacted sites, were more polluted, and therefore, other substances such as mineral salts and suspended solids could reduce pH values and enhance the adsorbability of the chemical pollutants targeted (Avazzadeh et al., 2023).

The electrical conductivity (EC) ranged from the highest at B2 (1690 µs/cm) to the lowest at B1 (1499 µs/cm) for the Bloukrans River and 254 µs/cm at S4 to 3330 µs/cm at S2 for the Swartkops River. The less impacted sites (control sites) associated with low concentrations of targeted chemical pollutants adsorbed recorded the lowest conductivity values. Indeed, the regression coefficient (Table 5.1 – 5.4) and regression graphs (Figure 5.9) had a positive coefficient of regression implied sites that recorded greater conductivity values and had higher concentrations of organic chemical compound adsorbed. This indicates that sites with poor-quality water properties were strongly associated with higher concentrations adsorbed in both river systems. The targeted compounds are polar, and only a few studies have been conducted on the influence of the partitioning of polar compounds (Verla et al., 2020; Ohoro et al., 2022). The EC values are suitable for giving a screening of the level of pollution. However, when they are associated with organic contaminant concentration, they can provide definite information about anthropogenic sources of contaminant discharge (Ohoro et al., 2022). Indeed, a significant high relationship ($P < 0.05$) between the concentrations adsorbed of E2 and electrical conductivity values was recorded (table 5.1 – 5.4). High electrical conductivity is brought about by increased salt content and high hydrophilic organic concentration, which subsequently can lead to higher adsorption on plastic particles (Ohoro et al., 2022).

The turbidity level of water which depends on the quantity of solid matter present in the suspended state (Liu et al., 2019); in this research, it ranged from 2.53 NTU at B4 (control site) to the highest at site B2 with 34.60 NTU. In the Swartkops River catchment turbidity ranged from 2.54 NTU at S4 to the highest value of 15.58 NTU at S1. Turbidity is generally a result of the dispersion of suspended particles. From the regression analysis data for turbidity values and concentration adsorbed onto the MPs (Table 5.1 – 5.4), it was observed that there was a positive relationship between high turbidity values and higher concentration adsorbed onto the MPs that were statistically significant ($P < 0.05$, Table 5.1 – 5.4).

Dissolved oxygen (DO), an index of physical and biological processes in water, is one of the most important parameters for assessing water quality (Kale, 2016). In this research, average DO ranged from the lowest 1.233mg/l at B2, the WWTW impacted site, to the highest value of 8.38mg/l at the B4 control site. For the Swartkops River, DO values ranged from 2.52mg/l at the S1 WWTW-impacted site to 8.47mg/l at the S4 control site, an indicator of land-use practices impacting DO depletion. DO was observed to have a negative correlation with the concentration adsorbed of the targeted chemical pollutants onto the MPs, indicating that sites with greater values of DO had the concentration of the organic pollutants, leading to lower adsorbed concentration onto MPs. However, the correlation was weak, indicated by the r values less than

0.5 but statistically ($P < 0.05$). This could be a result of biodegrading increases under aerobic conditions (Ohoro et al., 2022). Similar other research studies have observed low organic compound concentration that has a relationship with low concentration adsorbed with DO concentration (negative correlation) similar trends (de Souse et al., 2014; You et al., 2015).
Total dissolved solids (TDS) measure the organic and inorganic substances in water's molecular, ionised, or suspended forms (Abu Bakar et al., 2020). The substances dissolved in the water in this research were between 1250 and 3610 mg/L for the Bloukrans River samples and 600 mg/l to 7600 mg/l for the Swartkops River samples.

The physicochemical water variable values between seasons were not statistically significant, as indicated in Figure 5.4; however, some studies have indicated that land-use characteristics usually explain water quality variations in some seasons (Deng et al., 2020; Yu et al., 2020). The ANOVA of the water quality variables between the different sites (Figures 5.1 and 5.2) indicated a clear relationship between agricultural land-use impacted sites and TDS, alkalinity (Sites B3 and S3), urban settlements and EC and turbidity (sites B1 and S2), an indicator of increased animal and human waste-runoff into the river systems. It should be noted that metal cations, alkalinity, and pH are usually correlated with adding lime to leached soils to improve agricultural productivity (Melland et al., 2018). Elsewhere, research has indicated significant linear associations between water quality variables and the concentration of organic chemical compounds in water systems (Yu et al., 2020; J. Zhang et al., 2019; Cheng et al., 2022).

Together with a higher concentration adsorbed of the organic chemical pollutants targeted at the experimental study sites (B1, B2, and B3 for Bloukrans and S1, S2 and S3 for Swartkops), a strong association of concentrations adsorbed with decreasing water quality properties (Table 5.1to Table 5.4 and regression graphs, Figure: 5.7 – 5.12 strongly suggest that pollution via both the Bloukrans River and Swartkops River systems is responsible for a more significant part of the higher organic chemical pollutants in the two river systems. Sites B4 and S4 (control sites) may not receive pollution directly from this source, as their relative composition differs, and generally, a low concentration of adsorbed targeted organic pollutants was detected. Similar relations were observed in other studies (Shen et al., 2018; Chen et al., 2019; Mejías et al., 2023) that have attributed high pollutant loading in aquatic ecosystems to water chemistry differences.

5.6 Conclusion

The result of this chapter has shown that the physicochemical properties of river water from Swartkops and Bloukrans River systems with different land-use characterisation under ambient natural/field conditions influence the concentration of sulfamethoxazole, ciprofloxacin, 17β-estradiol, and 4-(2, 6-dimethyl-2-heptyl) phenol adsorbed onto PP and PET MPs. However, other factors like climate change and biodegradability of the organic chemical pollutants may influence the impact of physicochemical parameters on the pollutant's concentration that affects their adsorption, thus making it quite challenging to quantify the adsorption of organic pollutants in aquatic environments (Verla et al., 2020; Ohoro et al., 2022)

The results of this chapter further revealed that the Bloukrans and Swartkops River waters were alkaline and that the values of TDS, total hardness, and total alkalinity of these areas were within World Health Organisation (WHO) limits. Data also showed that turbidity, electrical conductivity, and neutral pH are likely to increase the level of concentration of Sulfamethoxazole, Ciprofloxacin, 17β- Estradiol, and 4-(2, 6-dimethyl-2-heptyl) phenol adsorbed onto PP and PET MPs, while decreased temperature and dissolved oxygen will possibly give decreased concentration of sulfamethoxazole, ciprofloxacin, 17β- estradiol, and 4-(2, 6-dimethyl-2-heptyl) phenol adsorbed onto PP and PET MPs.

Generally, the correlation observed in the river catchment studied earlier was site-specific. However, some water quality determinants and land use showed reproducible trends. Sites with human influence and land-use practices often determine the concentration of organic chemical pollutants that influence their adsorption (for example, see Herlihy et al., (1998). However, because the environmental data are usually complex, nonlinear and cluttered, as in this study, it was sometimes quite challenging to clarify explicitly how the concentration adsorbed onto MPs and the measured water physicochemical parameters interact with each other.

This study established that the water physicochemical parameters across the two river systems are clearly influenced by the surrounding anthropogenic land-use practices, mainly agricultural and urban settlement land uses, which influence the concentration of organic pollutants in surrounding water systems, thus affecting their adsorption. However, land use is forever changing, and future land cover changes will be driven by the interplay between population growth, climate change, and policymakers, which will vary in different parts of the world.

Understanding human land use and natural vegetation adaptations to water quality and river response is essential. However, all factors studied in this chapter merit attention in managing organic chemical polluted waters, and they also inform the mitigation process.

CHAPTER 6: Exploring the Kinetic Processes between Selected Pharmaceutical Pollutants on Microplastics

6.1 Introduction

The adsorption kinetics between microplastics and adsorbed chemical pollutants is an essential factor worthy of consideration to understand better the so-called vector effect of MPs in aquatic environments (Atugoda et al., 2020, 2021; Karapanagioti & Werner, 2019). Adsorption kinetics govern several vital processes, such as the interaction between the chemical pollutant and microplastics, the movement of the chemical pollutant within the MPs, and whether the chemical is adsorbed onto the surface of the MP or otherwise (Karapanagioti & Werner, 2019). Empirical evidence suggests that adsorption onto the surfaces of MP is usually fast and governed primarily by physical processes, whereas diffusion within the micropores is usually slower(Zhan et al., 2016; Xu et al., 2018b). These processes have significant human health and ecological implications about the significance of the so-called vector effects of MPs. For example, the bio-availability of chemicals adsorbed onto the surface of MPs through physical processes may differ from those governed through chemical processes within the micropores (Karapanagioti & Werner, 2019).

Atugoda et al. (2020) investigated the adsorption behaviour of Ciprofloxacin (CPX) antibiotic on polyethene (PE) microplastics under various environmental conditions. They established that adsorption kinetics data were compatible with the parabolic diffusion model and the Elovich model (R^2 of 0.90) in the presence of humic acid, suggesting that the adsorption was assisted through diffusion-controlled processes. The isotherm equilibrium data fitted well for the Hill (Hill cooperativity coefficient, $n > 1$) and Dubinin–Radushkevich models, implying multilayer adsorption through physical adsorption processes on the heterogeneous PE microplastic surface. Hence, they concluded that PE microplastics could be a potential vector to transport CPX in a natural aquatic environment where the adsorption mechanism is influenced primarily by several environmental conditions. In another study, Xu et al. (2018b) investigated the adsorption kinetics and isotherms of Sulfamethoxazole on polyethene (PE) microplastics. They observed that the data closely fitted a pseudo-second-order model (PSOM) ($R^2 = 0.98$) and linear model ($R^2 = 0.99$), respectively, indicating that the adsorption process was a partition-dominant interaction. They concluded that the primary binding mechanism was possibly the van der Waals interaction for hydrophilic Sulfamethoxazole onto hydrophobic PE microplastics and that PE microplastics may serve as a vector for Sulfamethoxazole in the aquatic environment. However, they recommended that the kinetics of hydrophilic Sulfamethoxazole with other microplastic polymers, besides PE types, be further explored.

Atugoda et al. (2020) and Zhan et al. (2016) described the adsorption kinetics using the pseudo-first-order-rate reaction, emphasising the importance of time and pollutant concentration. Authors such as Karapanagioti and Werner (2019) and Xu et al. (2018b) have previously used the pseudo-second-order kinetic model to describe adsorption processes between chemical pollutants and microplastics. Irrespective of the model adopted, several essential factors impact the adsorption processes. They include the region of the research study, the plastic particle size and its crystallinity, the diffusion behaviour of the pollutant, the size of the plastic material, the segmental mobility of the pollutant, the time of interaction between the pollutant and the MP, as well as the concentration of the pollutant in the solution to which the MP is exposed(Chen et al., 2019; Ma et al., 2019).

For instance, Chen et al. (2019) investigated the size effect of plastic particles on pollutants associated with open ocean plastics giving PAH concentrations nd-6.2, nd- 6298.8, and nd-8.3 µg/kg ($p = 0.323$) and PCB concentrations nd-210.1, nd-412.4, nd-694.0 µg/kg ($p = 0.4193$) that did not significantly differ among small, medium, and large plastic particle sizes, respectively. The results suggesting that pollutant concentrations versus particle size did not occur explained the likelihood of partition sorption mechanisms of PAHs and PCBs onto plastics. However, it was concluded that the size effect might need to be further verified by decreasing the size range as their unpublished data had found that polyethene plastic (µm-mm) adsorption ability for antibiotics had increased with decreasing plastic size.

In the same study, Chen et al. (2019) examined the influence of the polymer type on HOC concentrations in plastics. Concentrations of organic pollutants on Styrofoam (were much more significant than those in un-aged and aged pellets. A similar phenomenon was observed by Rochman et al. (2013), who found that PS behaved as a sink for parent PAHs, with 8–200 times greater sorption of PAH concentrations on PS than on polyethene terephthalate (PET), polyvinyl chloride (PVC), PE and PP. More significant adsorption on PS may be attributed to the different degrees of crystallinity of these polymers, which usually contain crystalline. The crystal region within the polymers has regularly arranged atoms in positions and motions.

Pollutants do not favourably adsorb to crystalline regions because high energy is required to disrupt tightly associated polymeric chains. However, to strengthen our understanding of the interaction between microplastics and environmental pollutants and related environmental risks and to help us deal with the comprehensive environmental risks caused by microplastics and pollutants, there is still a need to investigate the adsorption of other ionisable organic pollutants on various polymer types of microplastics, especially on environmentally weathered ones with more hydrophilic groups (Wang & Wang, 2018b; Xu et al., 2018b, 2018a; Atugoda et al., 2020).

Most current studies have tended to investigate the kinetic processes using one or two of the commonly used adsorption models to elucidate the kinetic processes, with the possibility of other potential mechanisms involved in the kinetic processes primarily limited in scope(Atugoda et al., 2020; Karapanagioti & Werner, 2019). This chapter aimed to explore the adsorption kinetic processes between the selected organic chemical pollutants (i.e., sulfamethoxazole and 17β-estradiol) and microplastics (i.e., PP and PET). The chapter investigates in detail the kinetic processes of the interaction between PP and PET of two size ranges (Type 1 (2mm<-≤5mm), and Type 2 (0.5mm<-≤2mm) and sulfamethoxazole and 17β-estradiol. The chapter provides further comparative analysis of the different adsorption kinetic models.

6.2 Materials and Methods

Microplastic samples (PP and PET polymer types) of two size ranges, their sourcing and characterisation was described in Chapter 3, Sections 3.2.1 and 3.2.2. Two model organic chemicals, Sulfamethoxazole and 17β-Estradiol were used. Analytical grades of sodium azide (NaN_3), anhydrous potassium chloride (KCl), calcium dihydrate ($CaCl_2.2H_2O$), sodium hydrogen carbonate ($NaHCO_3$), and magnesium sulphateheptahydrate ($MgSO_2$.7H2O) were purchased from Sigma Aldrich, Gauteng, South Africa. These chemicals were used to prepare the background test solution following the method described in AkkanenandKukkonen, (2003). The initial pH was adjusted to 7 by addition of either 0.1M HNO_3 or 0.1M NaOH.

6.2.1 Experimental design

The adsorption experiments were carried out in 250 ml polyethene acid glass wash bottles; 10 mg of microplastics samples were added into each bottle containing 100 ml of AFW, and 25mg/l of NaN_3 as a bio-inhibitor. The glass bottles containing the mixture were then conditioned for 24 hrs in the dark at 21 ^{o}C with continuous horizontal rotary agitation at 300 rpm in a laboratory shaker. Each bottle was subsequently spiked with the methanol solution containing a predetermined amount of the respective chemical, that is, Sulfamethoxazole or17β-Estradiol. The volume of methanol (spiking solvent) for all the samples was the same and less than 0.5% (v/v). The bottles were subsequently sealed using Teflon caps and horizontally shaken on a labcon orbital shaking incubator (3082UBruker, Germany) in the dark, temperature-controlled at 25 °C and rotated at 20 rpm. In total, test experiments and three Blanks were used (Figure 6.1).

After periods of 24 hrs, 48 hrs, 72 hrs, 96 hrs, and 5 days, 1ml aliquot of solution was collected from the bottles using a 0.2μm syringe filter to remove microplastic particles; the solution was transferred into HPLC vials. Subsequently the concentrations of Sulfamethoxazole and 17β-Estradiol in the aliquots were analysed using LC-MS/MS, following a methodology described in Sections 3.2.6 and 3.2.7 of Chapter 3.

Blank and control experiments were performed in parallel with the adsorption experiments. The blank test samples contained the same amount of AFW, NaN_3 and MPs particles as the samples, but without the sorbent chemicals and were subjected to conditions similar described in the test experiment described above. Control tests were carried out in 20 ml of AFW solution containing a same gradient Sulfamethoxazole and 17β-Estradiol concentration as the samples, but without the MP particles, to evaluate the amount of sorbent adsorbed; that is, the amount (C_t) of Sulfamethoxazole and 17β-Estradioladsorbedonto the MPs was calculated as the difference between the concentration of the same chemical between actual experiment and control experiment that included the organic chemical only. This was done to assess the concentration of the organic chemical that could have been adsorbed by the glass walls.

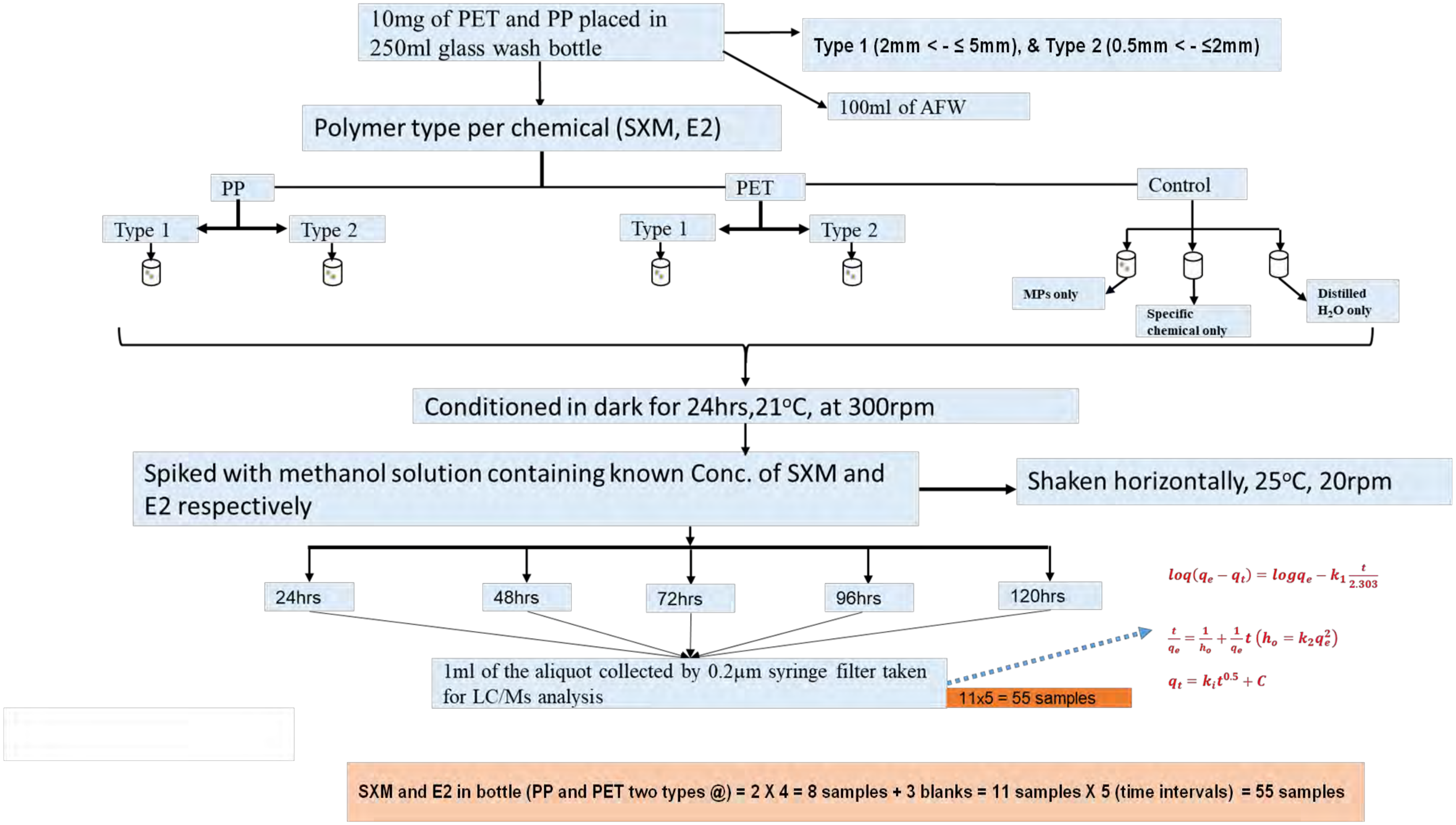


Figure 6.1: Summary of the experimental design setup employed for kinetic process experiments.

6.3 Statistical analysis

The concentrations of sulfamethoxazole and 17β-estradiol adsorbed per unit mass of the microplastic at equilibrium q_e (mg/g) and after a period (t-hrs.) q_t (mg/g) was estimated by the mass balance equation as follows.

$$\mathrm{q_e} = \left(\frac{\mathrm{c_o} - \mathrm{c}_e}{\mathrm{m}}\right)\mathrm{V}\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \qquad 6.1$$

$$\mathrm{q_t} = \left(\frac{\mathrm{c_o} - \mathrm{c_t}}{\mathrm{m}}\right)\mathrm{V}\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \qquad 6.2$$

Where $\mathrm{c_o}$, and $\mathrm{c_t}$representthe concentration of Sulfamethoxazole and 17β-Estradiol in (µg/L) in the control experiment (bottles without MPs) and the test experiment solution (adsorbed concentration at time t),c_e is the equilibrium concentration, V is the volume of solution (L), and m is the mass of the MP samples used in (mg). The parameters for the adsorption kinetics were determined by linear least-squares method using Microsoft Excel 2010. The fitted curves were performed with OriginLab environment. Three empirical kinetic models of pseudo-first-order (PFOM), pseudo-second-order (PSOM) and intraparticle diffusion model (Table 6.1) were adopted to depict the adsorption kinetics processes. The suitability of the different models was tested using the correlation coefficients (R^2).

Table 6.1: List of kinetic models used in this study.

Kinetic model	**Equation**	**Linear form**	**Plot**
PFOM	$\frac{dq_t}{dt} = k_1(q_e - q_t)$	$log(q_e - q_t) = logq_t - \frac{k_1}{2.303}t$	$log(q_e - q_t) vs\ t$
PSOM	$\frac{dq_t}{dt} = k_2(q_e - q_t)^2$	$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}t$	${}^{t}/_{q_t}\ vs\ t$
Intraparticle diffusion	$q_t = k_i t^{0.5} + C$	-	$q_t vs\ t^{0.5}$

For the pseudo-first-order, qt is the adsorbed organic chemical concentration at time (t); $\mathrm{q_e}$ is adsorbed organic chemical concentration at equilibrium, and $\mathrm{k_1}$ is the first order rate constant.

For the pseudo-second-order, K_2 in this case is the second order rate constant and$(h_o = k_2 q_e^2)$.For the diffusion kinetics described using the intraparticle diffusion model, k_i is the concentration fraction of pollutants adsorbed, t contact time (hrs.).

6.4 Results

6.4.1 Adsorption kinetics

The adsorption trends of Sulfamethoxazole and 17β-Estradiol on both PP and PET microplastics is presented in Figure 6.2. For Sulfamethoxazole, the maximum adsorption capacity was approximately 727.8551µg/g (PP Type1), 920.9270 µg/g (PP Type2), 688.2395 µg/g (PET Type1), and 798.7106 µg/g (PET Type2), at an initial Sulfamethoxazole concentration of 1 mg/L, and the adsorption equilibrium was achieved at 96 h (Figure 6.2a).

For 17β-Estradiol, the maximum adsorption capacity was approximately 798.77035 µg/g (PP Type1), 958.4474 µg/g (PP Type2), 761.4401 µg/g (PET Type1), 890.0671 µg/g (PET Type2) at an initial 17β-Estradiol concentration of 1 mg/L, and the adsorption equilibrium was achieved at 80 h (Figure 6.2b).

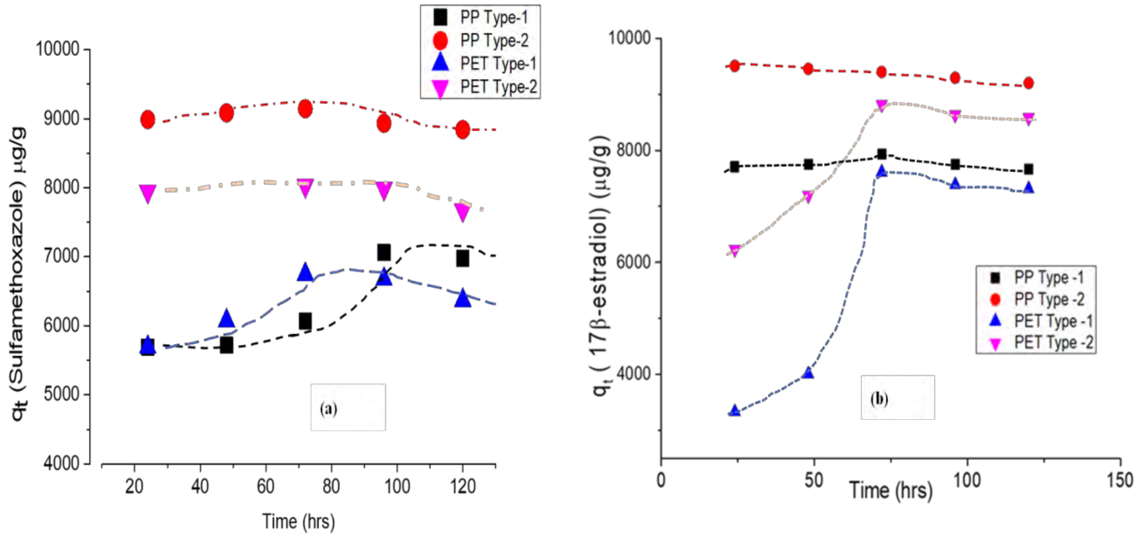


Figure 6. 2: Influence of contact time on the adsorption of (a)Sulfamethoxazole and (b) 17β-Estradiol on PET and PP microplastics of two size ranges, Type1 (2 mm<-≤5 mm), and Type2 (0.5mm<-≤ 2mm)

To understand the adsorption kinetics of Sulfamethoxazole and 17β-Estradiol onto the MPs, the experimental data were fitted onto a plot of $log(q_e - q_t)$ versus $t(hrs)$ for pseudo-first-order model. The rate constant $k_1(hrs^{-1})$ and the calculated equilibrium adsorption capacity $q_e(\mu g/g)$were obtained from the slope and intercept of the generated line, respectively (Figures 6.3, 6.4; Table6.2).

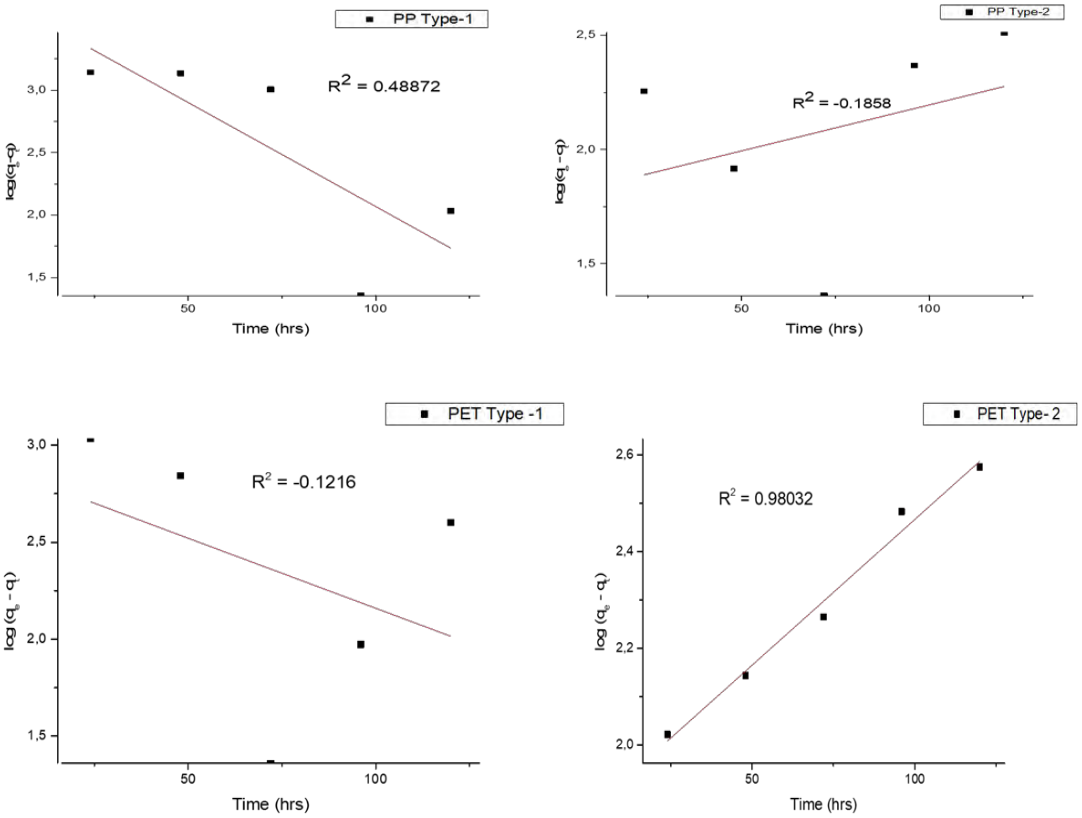


Figure 6.3: Linear plots for adsorption kinetics of Sulfamethoxazole on PET and PP microplastics particles of two size ranges, Type1 (2mm<-≤5mm), and Type2 (0.5mm<-≤2mm based on pseudo-first-order model.

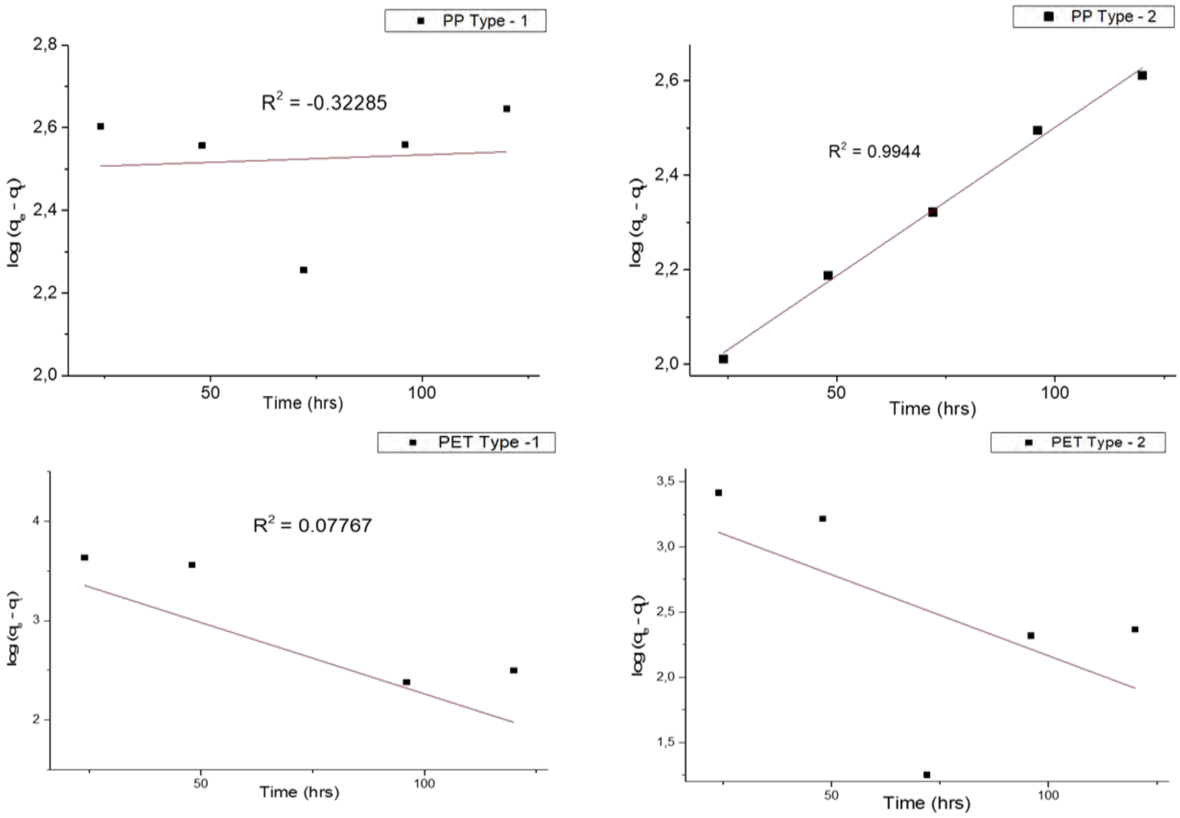


Figure 6.4: Linear plots for adsorption kinetics of 17β-Estradiol on PET and PP microplastics particles of two size ranges, Type1 (2mm<-≤5mm), and Type2 (0.5mm<-≤2mm based on pseudo-first-order model.

Table 6.2: Parameters calculated with different kinetic models for the adsorption of Sulfamethoxazole and 17β-Estradiolonto PET and PP particle of two size ranges.

Kinetic model	**Solid phase**	**Parameters**		
Pseudo-first-order Sulfamethoxazole		$k_2(h^{-1})$	$q_e(\mu g/g)$	R^2
	PP Type-1	0.0167	73.0550	0.4887
	PP Type-2	0.009281	761.9737	0.1859
	PET Type-1	0.016628	61.9755	0.1216
	PET Type-2	0.013887	542.2380	0.9803
Pseudo-second-order Sulfamethoxazole		$k_2(g/\mu\ h)$	$h(\mu g/g\ h)$	$q_e(\mu g/g)$

	PP Type-1	0.009916	5882.353	770.2021	0.9789
	PP Type-2	0.008649	6702.4129	880.2816	0.9993
	PET Type-1	0.004546	2052.966	672.0250	0.9919
	PET Type-2	**0.008106**	**4689.5517**	**760.6035**	**0.9997**
Pseudo-first-order 17β-Estradiol		$k_2(h^{-1})$		$q_e(\mu g/g)$	R^2
	PP Type-1	0.00832		498.4252	0.3229
	PP Type-2	0.01443		257.7508	0.9944
	PET Type-1	0.03300		74.8359	0.0776
	PET Type-2	0.02869		314.9271	0.0702
Pseudo-second-order 17β-Estradiol		$k_2(g/\mu\ h)$	$h(\mu g/g\ h)$	$q_e(\mu g/g)$	
	PP Type-1	0.002087	1730.1038	910.4980	0.9789
	PP Type-2	0.007911	7462.686	971.2509	0.9993
	PET Type-1	0.00177	1043.8413	767.5180	0.9719
	PET Type-2	**0.003277**	**2211.900**	**821.4902**	**0.9997**

Similarly data was also fitted onto Plot of ${}^{t}/_{q_t}$ versus $t(hrs)$ for pseudo-second-order kinetic model. The rate constant $k_2(g/\mu gh)$ and the calculated equilibrium adsorption capacity $q_e(\mu g/g)$ were obtained respectively from the intercept and slope of the generated line respectively (Figure6.5). The calculated q_e, k_2,R^2 values are listed in Table: 6.2 (see Page – 167).

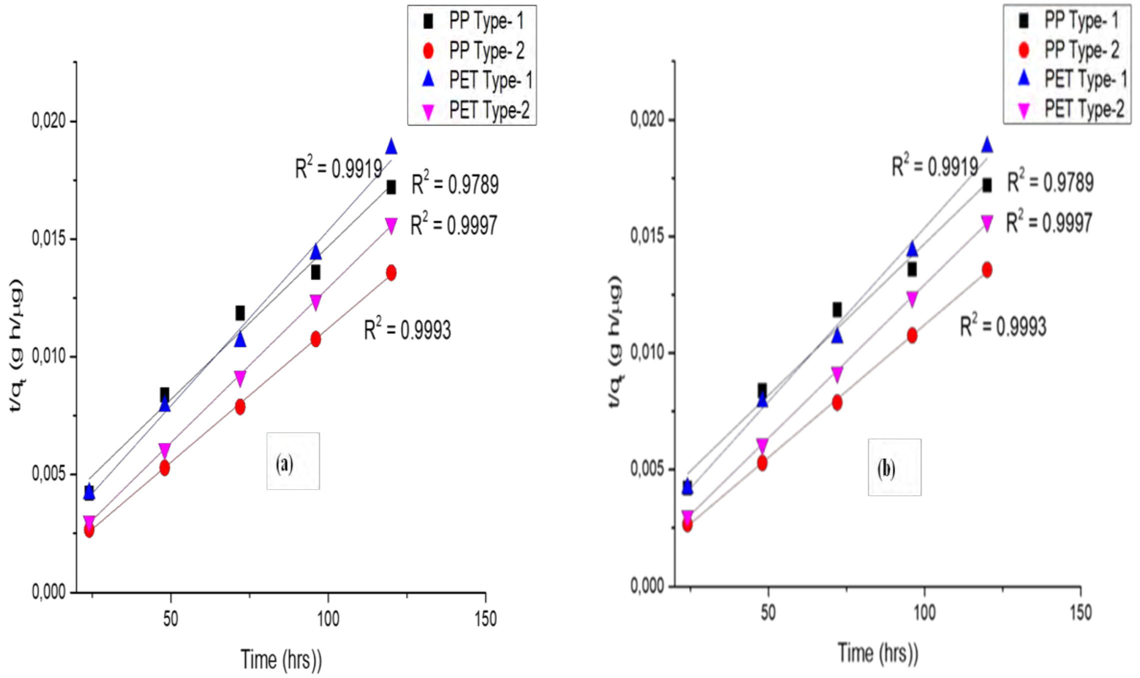


Figure 6.5: Linear plots for adsorption kinetics of (a)Sulfamethoxazole and (b) 17β-Estradiol on PET and PP microplastics particles of two size ranges, Type1 (2mm<-≤5mm), and Type2 (0.5mm<-≤2mm based on pseudo-second-order model.

Additionally, the intraparticle diffusion model was tested by fitting the data onto a Plot of q_t versus $t^{0.5}$ for intraparticle diffusion model (Figure6.6).

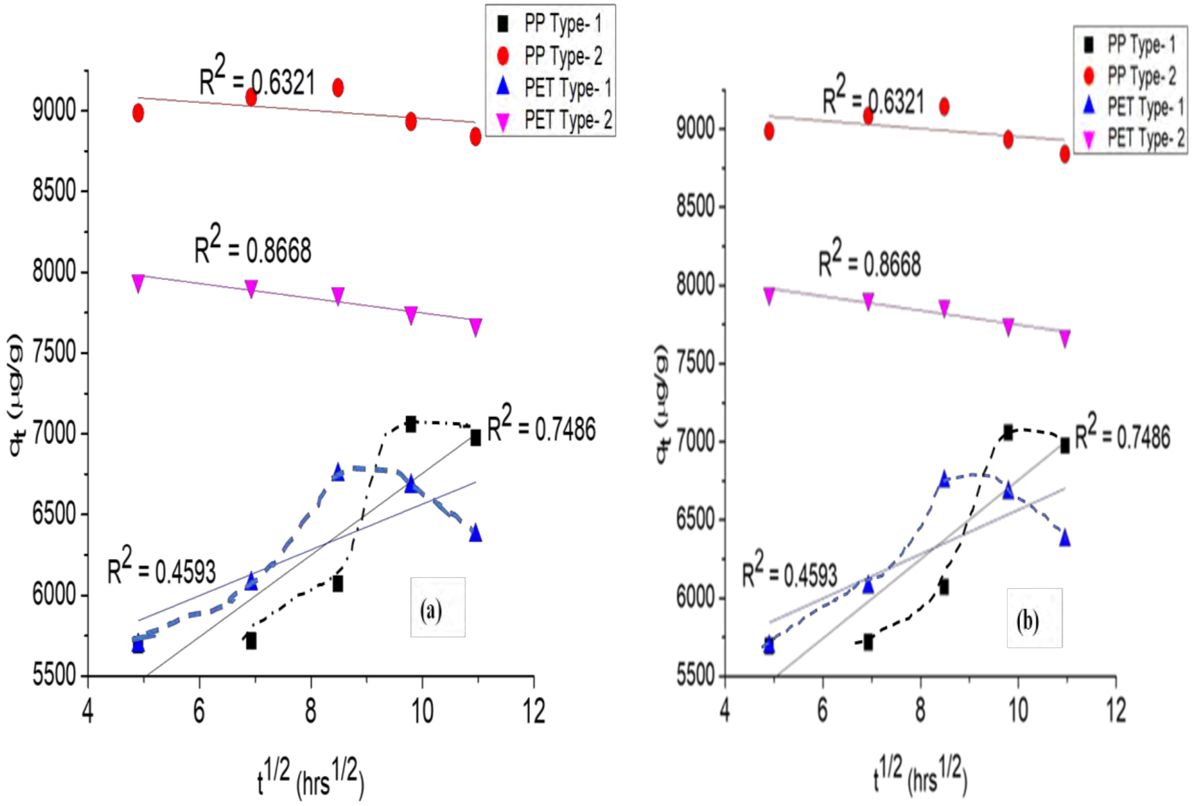


Figure 6.6: Linear plots for adsorption kinetics of (a)Sulfamethoxazole and (b) 17β-Estradiolon PET and PP microplastics particles of two size ranges, Type1 (2mm<-≤5mm), and Type2 (0.5mm<-≤2mm based on intraparticle diffusion model.

From the results recorded in Table 6.2 and Figures 6.3 to 6.5, the adsorption kinetics was better fitted by PSOM on the two considered size ranges for both PP and PET MPs than PFOM, judging from the high "**R^2**" values. For instance, sulfamethoxazole, PSOM (PP Type1 R^2 = 0.9789, PP Type 2 R^2 = 0.9993, PET Type1 R^2 = 0.9919, PET Type2 R^2 = 0.9997) compared with PFOM (PP Type1 R^2 = 0.4887, PP Type2 R^2 = 0.9944, PET Type1 R^2 = 0.0776, PET Type2 R^2 = 0.0702). A similar trend was observed with 17β-estradiol, probably indicating that besides the adsorption onto MP surface sites, mass transfer and intraparticle diffusion were involved in the adsorption process (Annadurai et al., 2008). A study by Wang and Wang, (2018b) also demonstrated that the adsorption kinetics of organic chemicals fitted well with the PSOM, with an R^2 value > 0.99 for three types of microplastics, including PE, polystyrene, and polyvinyl chloride. In contrast with studies for larger microplastics, >5 mm pellets in diameter, the sorption equilibriums of organic chemicals naphthalene and phenanthrene were achieved on PP at 21 and 28 days, and on PE at 63 and 105 days, respectively (Karapanagioti & Werner, 2019). These results indicate that the adsorption equilibrium time was affected by microplastic type, size range, and organic chemical type.

In the intraparticle diffusion model plots, qt versus t $^{0.5}$ a multi-linear graph is presented in Figure 6.6, implying that more than one step was required in the adsorption of both Sulfamethoxazole and 17β-Estradiol onto PP and PET microplastics. In this case, the adsorption process is generally believed to involve three steps, including transport of the sorbate to the external surface of the sorbent, the gradual adsorption by intra-particle diffusion, and achievement of equilibrium (Cheung et al., 2007).

6.5 Discussion

The adsorption of the two chemical pollutants, sulfamethoxazole and 17β-estradiol by the two polymer types of MP, PP and PET, increased over time until sorption equilibrium was reached after 80 hours, a state where all active sites on the adsorbate (MPs) are occupied (Figure 6.2). Beyond this point, the adsorption efficiency reduced, partly perhaps because the number of vacant active sites on the MP surface to be occupied by the chemical pollutants had been reduced(Wang & Wang, 2018b). Adsorption kinetics sheds light on the mechanism underlying the vector effect of MPs (Xu et al., 2018b, 2018a).

For pseudo-first-order kinetics models, the calculated q_e values are in order PP Type 2 > PP Type1 and PET Type 2 > PET Type 1 (Table 6.2). The differences in the quantity adsorbed can be explained based on size range differences and surface morphological differences. Compared with PET, the surface of PP was more wrinkled and less smooth from the morphology characterisation by SEM (Figure 3.3 and3.5). In PP, some more developed pore structures on the surface could have meant more adsorption sites for the pharmaceutical pollutants. This observation has been confirmed in other studies in the literature (Fan et al., 2021; Leng et al., 2023; Wu et al., 2020). Throughout the adsorption kinetics for the pseudo-first-order model, the estimated value q_e significantly deviated from the experimental data (Table 6.2). Furthermore, a detailed examination of the graphs for the pseudo-first-order model revealed that the linear regression correlation coefficient (R^2) was in the range of 0.1216–0.9944.

For the pseudo-second-order kinetics, the calculated qe values obtained were in an agreeable range with the experimentally measured q_e values (see Table 9). In addition, as is shown in the plots in Figure 6.1 of the fitted data, the linear correlation coefficient (R^2) for PP and PET polymer MPs is more significant than 0.9 for all the size ranges of the two polymer types. These findings show that the pseudo-second-order equation model well describes the sorption kinetics of Sulfamethoxazole and 17-Estradiol onto PP and PET MP Particles. These results imply that(i) the rate of adsorption of Sulfamethoxazole and 17β-Estradiolchemicals was proportional to the square of the number of free binding sites $(q_e - q_t)$;(ii) each of the Sulfamethoxazole and 17β-Estradiolmolecules is likely to be associated with two monovalent active sites on the MPs particle;(iii) chemisorption may be the rate-limiting step, that is, that the molecules of the pharmaceuticals stick to the MP adsorbent surface by forming chemical bonds and tend to find sites that maximise their coordination number with the surface. Previous research findings have also indicated that pseudo-second-order models are effective in a variety of cases for describing the binding of organic chemical pollutants to MP adsorbents(Wang & Wang, 2018a; Xu et al., 2018b; Karapanagioti & Werner, 2019; Atugoda et al., 2020; Leng et al., 2023)

The experimental data did not fit the intraparticle diffusion model, as evidenced by the non-linearity of the data trends and poor regression coefficients in Figure 6.6. The diffusivity processes are often slow and usually affected by multiple factors that could not have been examined, such as temperature, solution pH, and salinity of the solution, all of which required adjustments beyond this experiment's scope. A deeper examination of all the plots (dashed graph plots – Figure 6.6) reveals the same data feature trends (generally believed to involve three steps), which began with a curved trend and ended with a plateau. The first curved trend observed may be attributed to bulk diffusion, whereas the plateau could be attributed to sorption equilibrium. This observed data trend implies that the sorption of Sulfamethoxazole and 17β-Estradiol onto the MP particles occurred in a multi-stage process. The trends suggest that sorption typically comprises three steps: sorbent movement, progressive adsorption via intraparticle diffusion, and attainment of equilibrium. The initial curving data in the plots may refer to the transfer of Sulfamethoxazole and 17-Estradiol molecules from water to external surfaces and then into the internal pores of microplastics. At the same time, the ultimate plateau may represent final equilibrium. This transport mechanism has been observed in other studies cited in the literature (Lohmann, 2012; Wang & Wang, 2018a; Ma et al., 2019; Li et al., 2021).

Because the lines in all the plots in Figure 6.2 do not pass through the origin, it is possible that both film diffusion and intraparticle diffusion influenced the rate-limiting sorption step at the same time (Wang & Wang, 2018a). Given that the sorption process in the current study occurred under conditions of continuous agitation, a concentration gradient of Sulfamethoxazole and 17-Estradiol in the solution could not be avoided, implying that bulk transport of the chemicals from water to the external surfaces of the microplastics was not the rate-limiting step. The adsorption process is driven by physical adsorption and diffusive mass transfer between the solution and the MPs' surface matrix. In general, this model explains how the rate of chemisorption and external mass transfer on a heterogeneous substrate surface influences the total adsorption process in the context of microplastics acting as vectors for sulfamethoxazole and 17β-estradiol.

6.6 Conclusion

This chapter analysed the adsorption kinetics of sulfamethoxazole and 17β-estradiol onto different microplastics (PP and PET) types using three adsorption models. The kinetics study revealed that adsorption rates of sulfamethoxazole and 17β-estradiol onto microplastics were mainly controlled by intraparticle diffusion. However, the data fit well with the pseudo-second-order kinetics models based on the linear regression methods. It can be concluded that the concentration of organic pollutants in microplastics depends slightly on plastic size. PP demonstrated the highest adsorption affinity for both sulfamethoxazole and 17β-estradiol.

The quantitative effects of each polymer type could not easily be determined from the results of the variables gathered for this chapter. It should also be emphasised that the water type used in this study may also have caused adsorption through the salting-out effect (decreasing solubility) and polymer bridging (increasing adsorption capacity). Also, within the context of this chapter, the fitted data indicated that adsorption is most likely driven by compound repulsion from water rather than its affinity for MPs. Similar observations have been made in other studies (Zhang et al., 2019; Wu et al., 2020; Leng et al., 2023).

The results of this chapter are further evidence of the interaction mechanism and insights between MPs and organic pollutants that could facilitate technological development of organic pollutants' removal using polymer-based adsorbents, but more importantly, pave the way towards a better understanding of the behaviour of PP and PET MPs concerning their adsorption of sulfamethoxazole and 17β-estradiol, and the potential of both PP and PET to act as vectors of organic pollutants in water systems.

However, there is a need to investigate further the linear adsorption coefficient Kd, also called the partitioning coefficient, for the organic chemicals considered in this study and compare them with hydrophobic organic pollutants to understand clearly the extent to which microplastics may act as carriers for sulfamethoxazole and 17β-estradiol, which are hydrophilic organic pollutants.

CHAPTER 7: General Discussion, Conclusion and Recommendations

7.1 General Introduction

Freshwater contamination with microplastics is on the rise, driven by the extensive utilization of plastic products and the yearly surge in plastic production, as indicated by data from Plastics Europe in 2020. Coupled with their poor degradability in aquatic environments, as highlighted by Koelmans et al. (2019) and Rios Mendoza & Balcer (2019), microplastics are problematic, acting as vectors for pollutants (adsorbed toxic contaminants) in natural waters (de Sá et al., 2018; Cox et al., 2019). The fact that MP acts as a vector of chemical pollutants adds to the complexity of understanding the risks posed to the ecosystem and human health. Regarding the so-called vector effect, the role of seasonality, land use type, adsorption kinetics as well as MP properties has been suggested but has not received the necessary research attention in the literature, especially about pharmaceutically active compounds and other organic pollutants in river systems (Wang et al., 2018; Atugoda et al., 2021). This study, therefore, aimed to fill these research gaps. Overall, the study aimed to investigate the selected microplastic polymers of a particular size range as vectors of organic pollutants in urban rivers within the Eastern Cape of South Africa. This thesis intended to fill the knowledge gaps identified in the general introduction and literature review Chapter 1), which include: i) The effects of microplastic properties and spatial-temporal variability on adsorption of pharmaceutical pollutants and endocrine-disrupting compounds by microplastics in river ecosystems (Chapter 3 and chapter 4); ii)influence of physicochemical variables on the adsorption of pharmaceutical pollutants and endocrine-disrupting compounds by microplastics (Chapter 5), and iii) kinetic adsorption processes of pharmaceutical pollutants and endocrine-disrupting compounds on microplastics (Chapter 6). The Swartkops and Bloukrans Rivers were selected for the study due to the pronounced degradation in water quality as a result of chemical pollution stemming from various land-use activities, including wastewater treatment works (WWTWs), unregulated informal and urbanised human settlement activities, and inadequately managed agricultural practices. In the current chapter, a synthesis of the major findings, as discussed in the preceding chapters, about the original objectives, the implications of the results and approaches used, and recommendations for future research are provided.

7.1.1 Microplastic properties and spatial-temporal variability effects on the adsorption of pharmaceutical pollutants and endocrine-disrupting compounds onto microplastics

The adsorption capacity of MPs may be influenced by their physical and chemical characteristics, such as size, specific surface area, and morphology, among others (Ziccardi et al., 2016; Llorca et al., 2020). Spatial factors, especially those relating to anthropogenic activities, including land-use types such as WWTWs, industrial and human settlements, and agricultural activities, may influence the behaviour of MPs-pollutant interactions (Stanton et al., 2020; Xia et al., 2020). In Chapter 3 and Chapter 4, the effects of plastic properties and spatial-temporal variability of selected pharmaceutical pollutants and endocrine-disrupting compounds adsorption onto microplastics were investigated.

The concentration of the targeted pharmaceutical pollutants and endocrine-disrupting compounds adsorbed onto MPs followed a land-use practice gradient along the riverine ecosystem (Figure 3.5 and Figure 3.6; Figure 4.1 and Figure 4.2). As expected the concentration of sulfamethoxazole (SXM), ciprofloxacin (CIP), 17β-estradiol (E2) and 4-(2, 6-dimethyl-2-heptyl) Phenol ($C_{18}H_{24}O$) adsorbed significantly differed between sites (Figure 3.5 – Figure 3.10: Chapter 3 and Figure 4.1 – Figure 4.6: chapter 4), exhibiting organic chemical – specific correlation with sites and land-use. For example, the concentration of Sulfamethoxazole absorbed onto MPs was highest in agriculture and WWTWs impacted sites (B3 and B2-Blourkrans River-Chapter 3 and S3 and S1-Swartkops River- Chapter 4), explained by the possible application of this antibiotic in animal medicine and its introduction to the river water as a component of runoffs coupled with WWTWs being critical sources of sulphonamides in surface waters (Wanda et al., 2017; Vumazonke et al., 2020).

Concentration 17β-estradiol (E2) adsorbed, which is an indicator of the presence of hormones, was recorded highest from Municipal/informal- settlement-impacted sites (B1-Bloukrans River and S2- Swartkops River (Table S1 – Table S4, Appendix S) which was attributed to the observed liquid effluents from the domestic and industrial activities observed in the studied area that included chemical industries works, human settlements, hospital, where studies have indicated that human urine that is usually deposited daily is the most crucial source of estradiol (Farounbi & Ngqwala, 2020) (Figure 2.2 and Figure 2.3).

Among the targeted organic chemical pollutants, the concentration of ciprofloxacin adsorbed was highest in comparison to sulfamethoxazole (SXM), 17β-estradiol (E2) and 4-(2, 6-dimethyl-2-heptyl) Phenol ($C_{18}H_{24}O$) from MPs samples retrieved from the sites in the Bloukrans River (Table S1 and S2, Appendix S). This is not surprising given the observed high volume of liquid refuse waste discharged into the Bloukrans River channel from Grahamstown, which is densely populated and animals seen roaming through the streets uncontrolled, an implication of the everyday use of this antibiotic in the treatment of various bacterial infections in humans and livestock (Vumazonke et al., 2020). For MPs samples retrieved from sites along Swartkops River (Table S3 and S4, Appendix S), the concentration of 17β-estradiol (E2) adsorbed was high compared to the concentration of sulfamethoxazole (SXM) and ciprofloxacin (CIP), an indicator that Swartkops River is located in a catchment severally impacted by human and industrial liquid wastes (Odume et al., 2022).

The impact of land–use practices was further explained by the Tukey multiple comparisons (Tukey HSD post-hoc-test) analyses of the concentration adsorbed onto the MPs from the different sites (Table T1 and Table T2; Appendix T), and indeed. Statistically, the mean concentration adsorbed onto MPs at the different sites differed significantly. The results of this thesis indicated no significant differences ($P > 0.05$) in the concentration adsorbed onto MPs between the summer and autumn seasons (Figure 3.15 – Figure 3.18 and Figure 4.10 – 4.13). The following scientific explanations could explain the non-significant ($P > 0.05$) differences in the concentration adsorbed between the two seasons observed in the data analysis.

During summer, the rainy season for the study areas, more extreme flows exacerbate the MP-pollutant interactions as the chemical pollutants previously trapped in the sediments are re-suspended. However, this could favour desorption in addition to adsorption (Hurley et al., 2018; Talbot & Chang, 2022). Also, during autumn, which is a cold season in the study areas, the pollutants are stable, as they are not likely to break down because temperatures tend to be low (Harrower et al., 2021). Nevertheless, several studies have shown that there is always a higher concentration of chemical pollutants that could lead to higher adsorptivity in the high-flow season (summer) compared to the low-flow season (autumn) (He et al., 2013; F. Li et al., 2020; Harrower et al., 2021).

The results of this thesis confirmed that differences in microplastic particle sizes significantly influenced the adsorptivity of PP and PET MPs (Figures 3.19 – 3.21; and Figure 4.14 – Figure 4,.17), as the surface area will always increase with decreasing size of the MP (Ho & Tang, 2021). Both PP and PET Type2 MPs exhibited higher concentrations of sulfamethoxazole, ciprofloxacin, 17β-estradiol and 4-(2,6-Dimethyl-2-heptyl)phenol adsorbed than PP and PET Type1 MPs (Figure 3.5 – Figure 3.6 and Figure 4.1 – Figure 4.2). These results confirm the conclusion that MP particles with small sizes are associated with higher specific surface area (SSA), resulting in a more significant number of adsorption sites and greater concentrations of pollutants they can adsorb. It should also be emphasized that MPs of small sizes are less stable than larger particles (Atugoda et al., 2020, 2021; Ho & Tang, 2021). However, the effect of particle size on the adsorption capacity of MPs could result from the combined effects of particle size and agglomeration, which was not investigated in this study.

In conclusion, the results presented in Chapter 3 and Chapter 4 of this thesis suggests that the concentration of sulfamethoxazole (SXM), ciprofloxacin (CIP), 17β-estradiol (E2) and 4-(2, 6-dimethyl-2-heptyl) phenol ($C_{18}H_{24}O$) adsorbed onto PP and PET MPs were land-use type dependant, and influenced by specific MP size. The data provided more insights into land-use disturbance (spatial variability factors) effects on the vector role MPs for antibiotics and estrogen hormones in river water systems. They can be used for long-term monitoring of organic chemical pollution in water systems, plus compelling reasons for developing technologies to remove microplastics and associated pollutants from water systems.

7.1.2 Influence of water physicochemical variables on the adsorption of organic chemical pollutants onto microplastics

Adsorption of pollutants by MPs could be influenced by environmental water pH, electrical conductivity of the water, Temperature, seawater corrosion, or other water physicochemical quality variables through several mechanisms, including hydrophobic and electrostatic interactions (Fu et al., 2021; Joo et al., 2021). In Chapter 5, the Influence of different water physicochemical variables, including DO, electrical conductivity, Temperature, pH, turbidity, Total Alkalinity, Total hardness, TSS and TDS, among others, on pharmaceutical pollutants and endocrine-disrupting compounds adsorption onto microplastics, were investigated.

The results from this chapter indicated a significant relationship between sites with higher concentration adsorbed of sulfamethoxazole (SXM), ciprofloxacin (CIP), 17β-estradiol (E2) and 4-(2, 6-dimethyl-2-heptyl) phenol ($C_{18}H_{24}O$) and physicochemical variables of EC, Turbidity, Total Alkalinity, Total Hardness, TSS and TDS, based on the Kruskal-Wallis analyses. This indicates a relationship between the adsorption capacities of MPs for pollutants and water pollution levels that correspond to the land-use practices (Figures 5.1 and 5.2). At sites where the values of DO were highest, Site B4-Figure 5.1(B) and Site S4-Figure 5.2(B), the concentration adsorbed of the chemical pollutants onto MPs was least or, in some instances, not detected. Therefore, this indicates that water physicochemical variables are related to pollutant concentration levels, which in turn are related to the adsorptivity of pollutants onto MPs (Avazzadeh et al., 2023).

A clear indication of this relationship was observed from the regression analysis results and regression graphs for water physicochemical variables data and the concentration of sulfamethoxazole (SXM), ciprofloxacin (CIP), 17β-estradiol (E2) and 4-(2, 6-dimethyl-2-heptyl) phenol ($C_{18}H_{24}O$) adsorbed (Table 5.1 – Table 5.4 and Figure 5.5 – Figure 5.12). Generally, the higher concentration adsorbed of the chemical pollutants was strongly associated with physicochemical variables that indicate poor water quality criteria. Similarly, the least impacted study-control sites where the physico-chemical water variables indicated good quality water, especially DO, had low concentrations adsorbed onto the MPs. Therefore, the relationships between water physicochemical parameters and concentration of pollutants adsorbed onto MPs could be used to predict pollutants associated with specific land use types affecting the concentration patterns of organic chemical pollutants in the Bloukrans and Swartkops River ecosystems. Additionally, the relationship has indicated that monitoring organic chemical pollutants in the river systems should be done routinely, as most physicochemical parameters are unstable due to climatic fluctuations.

7.1.3 Kinetic adsorption processes between pharmaceutical pollutants and endocrine-disrupting compounds onto microplastics

Adsorption kinetic models are typically used to estimate the efficacy and rate-limiting steps of pollutant adsorption onto MPs and to investigate adsorption mechanisms that are key in developing technologies to remove microplastics and associated pollutants from water ecosystems (Cormier et al., 2022). The kinetics models considered in this study include pseudo-first-order, pseudo-second-order, and intraparticle diffusion models. The pseudo-first- and second models were used to evaluate the whole adsorption process, and intraparticle diffusion was added to describe the limiting steps of the adsorption process. In Chapter 6, I explored the kinetic adsorption processes of sulfamethoxazole and 17β-estradiol onto microplastics.

The pseudo-first-order model did not always provide a good fit for all the adsorption onto the MPs, as indicated by small R2 values (Figure 6.3 and Figure 6.4). However, the results data fitted the pseudo-second-order kinetic model indicated by $R^2 > 0.99$ values that were much higher than those of the pseudo-first-order model (Figure 6.5), and the difference between experimental and theoretical adsorption capacities (q_e) on MPs was small (Table 6.2). The implication is that the pseudo-second-order model appropriately represented the adsorption of Sulfamethoxazole and 17β-estradiol onto the MPs. Based on the plastic types deployed and the structure of the organic chemical pollutant targeted, adsorption mechanisms, including cation ligand, electrostatic, and hydrogen bonding interactions, were not likely involved. This implies that the whole adsorption mechanism is chemisorption that involves valence forces through sharing or exchanging electrons between an adsorbent (MPs) and adsorbate (targeted chemical pollutants) that involves π-π interactions (Cormier et al., 2022; Tseng et al., 2022).

The fitting curves of the intraparticle diffusion model did not contain the origin (curves did not pass through Ci = 0), indicating that intraparticle diffusion was not the only process governing the adsorption. This could imply that film diffusion (which is also a slow adsorption process) is involved in addition to intraparticle diffusion models. Chapter 6 results showed that the mechanism adsorption of sulfamethoxazole and 17β-estradiol onto PP and PET includes π-π interactions as a result of chemisorption and that multiple diffusion processes and multiple mechanisms are often involved in the whole adsorption process. These results highlighted further the evidence of the interaction mechanism. They provided insights into the interaction mechanisms between MPs and organic pollutants that could facilitate the technological development of organic pollutants' removal from river systems using polymer-based adsorbents, but more importantly, pave the way towards a better understanding of the complex interactions between MPs and water organic pollutants.

7.2 Implications and recommendations from the study

A deeper understanding of the MP vector role for organic chemical pollutants in rivers impacted by land use types, which can stimulate and facilitate effective mitigation strategies, such as reducing chemical pollutants inputs into water systems from the input source and the need to develop technologies to tackle MP contamination and ensure long-term sustainability of river ecosystems was a key motivation of this study. The results presented in this study demonstrate that the adsorption of chemical pollutants onto MPs influenced by MPs properties, land use practices and water physicochemical properties can be integrated into the assessment of the risk posed by MPs to aquatic organisms in river systems. To further improve the existing understanding of the risk posed by MPs, it is recommended that the vector role of MPs be further investigated using small-sized MPs of less than 20 microns.

Additionally, the findings contained in this thesis have clearly demonstrated a fundamental understanding of how MPs operate as a vector for chemical pollutants based on the investigated adsorption kinetics mechanism and environmental factors affecting the adsorption process. However, more investigation is needed to clarify the effect of microplastics on secondary environmental contamination, including various types of contaminants, as well as limited pilot-scale investigations, including multi-contaminant solutions. This could go hand in hand with spectroscopic investigations such as Extended X-ray absorption fine structure (EXAFS) and Raman to comprehend and explain the molecular interactions between the various chemical pollutants and MPs.

Also, future research could look at investigating the presence of the pollutants in the water inside the meshed MPs carrier bags, and on the water nearby to evaluate a potential cleaning effect of MPs on the water. But more importantly how physicochemical parameters can be used as real time monitoring system and how correlated they are to MPs pollution.

Overall, the results presented in this thesis can form components of the conceptual framework for incorporating the vector in assessing MP risk. A four-model framework is based on a perspective of connecting sources and drivers (Land use and MP properties) to dynamics distribution and impacts and, finally, to management response.

1. The sources (land-use practices) and drivers (MPs properties) - affect the input of microplastics and chemical pollutants into the river and the subsequent interaction (adsorption).
2. The dynamic (interaction/vector kinetic mechanisms) and distribution of the combined interaction could lead to combined pollution, which will be influenced by input sources (land use practices), MPs property of size, and water physicochemical parameters.
3. The combined interaction (vector effect) will, in turn, determine what is affected (aquatic organisms and or humans' health via the food chain)
4. Management or mitigation strategies could be formulated to respond to the demonstrated, perceived or potential impacts, and the management responses will, in turn, affect the sources and drivers to mitigate the microplastic vector risk.

7.3 Limitations and Future Studies

The findings of this PhD study have highlighted several gaps in the current MP's research in the context of freshwater ecosystems that warrant further investigation. Firstly, one of the potential drawbacks of this study was information on the most novel analytical method to extract pharmaceutical pollutants and endocrine-disrupting compounds adsorbed onto MP. Future research should be targeted towards the development and compilation of analytical extraction methodologies, including not only soaking in extractive solvents but other techniques like maceration, infusion percolation, soxhlet extraction and pressurized liquid extraction, especially for adsorption process involving MPs less than 20microns in the size range. Therefore, developing a standardized analytical methods database for extracting and quantifying chemical pollutants associated with MPs is recommended. Such research focus will stimulate more MP research studies, especially targeting the resulting synergistic effect due to the interaction between MPs and emerging contaminants such as pharmaceuticals in the context of MPs in South African freshwater ecosystems where more needs to be done.

Second, a significant limitation of this study is the slight seasonal gap difference that was considered (summer and autumn seasons are close) and the narrow MP size range (Two upper size ranges) of only two MP polymers. However, in the conditions of the aquatic environments, the interactions are usually with mixed MP polymers over different seasons that are pretty distant, providing for limited replication of pollutants gradient, which could lead to unconsidered intra-interactions and -season variability in the environment. Thus, the lack of seasonality in the concentration adsorbed observed in this thesis may reflect the relatively small sample size of MP polymers, considering the limited replication of the pollutants gradient and the closeness of the two seasons considered. Further studies into seasonal variability effects on the adsorption of organic chemical pollutants, covering a more significant number of size ranges, different polymers and seasons distant apart, are recommended.

Additionally, conducting studies over multiple years would allow exploration of inter-annual variation of concentration adsorbed and the effect of MP properties on the adsorption of chemical pollutants to longer-term climatic events, such as floods and low flows. In particular, additional sites in the two rivers studied and comparisons with other riverine systems in South Africa will enable further testing of some of the conclusions of this thesis across a larger spatial scale. Ultimately, this process will increase the depth of our understanding of the risks posed by microplastics to aquatic organisms and human health and assist in protecting these ecologically significant ecosystems by developing effective mitigation strategies and technologies to remove MP and associated pollutants from freshwater ecosystems.

Finally, the cost, time and novelty involved in MP deployment to the rivers studied, methods of extraction of the adsorbed pollutants and quantification of the adsorbed chemical pollutants limited the present study to the analysis of four organic pollutants and two MP polymers. Future work should seek to expand the number of targeted organic pollutants and different MP polymers, and this will become possible as the cost per sample run using Lc/Ms/Ms decreases. Such research will enable researchers to get clarity on the existing contradictory ‘school of thought’’, where, in some instances, the risk of the vector role of microplastics is downplayed since MPs represent <3% of the diet of aquatic organisms (Teuten et al., 2009; Avio et al., 2015).

7.4 Conclusion

The methodologies and data presented in this study contributed to the field of MP research in South Africa and generally across Africa by demonstrating the importance of integrating *In-situ* (actual field) experimentation with laboratory experimentation in assessing MP pollution of freshwater ecosystems. The study revealed the significant contribution of land use practices to the synergistic pollution of MPs and emerging contaminants such as pharmaceuticals and endocrine-disrupting chemicals in river ecosystems. Chapters 3 and 4) demonstrated the significant effect of spatial variability and MP property of sizes on the adsorption of selected pharmaceutical pollutants and endocrine disrupting compounds onto MPs in ambient environment ecosystems. Furthermore, the chapters presented statistical correlations of selected pharmaceutical pollutants and endocrine-disrupting compound concentrations adsorbed and land use practices. The chapters also present methods of quantifying organic pollutants associated with MPs using sensitive hyphenated LC/MS/MS instruments. The results of the relationship between water physicochemical variables associated with different land use practices along a riverine and the adsorption of pollutants onto MPs (Chapter 5) advance current understanding of the link relationship of physicochemical parameters to the occurrence of organic chemical pollutants in aquatic environments. Chapter 6 presents the kinetic mechanism involved in the interaction of selected organic chemical pollutants and MPs, which is essential in developing effective mitigation strategies and technologies to tackle MP contamination and associated organic chemical pollutants in river ecosystems. Despite these results, more MP research is required to understand the vector effect of MPs further compared to other sources in river ecosystems on the African continent.

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Appendices

Appendix P: The Fourier transform infrared monographs (FTIR) for the microplastics used in this study.

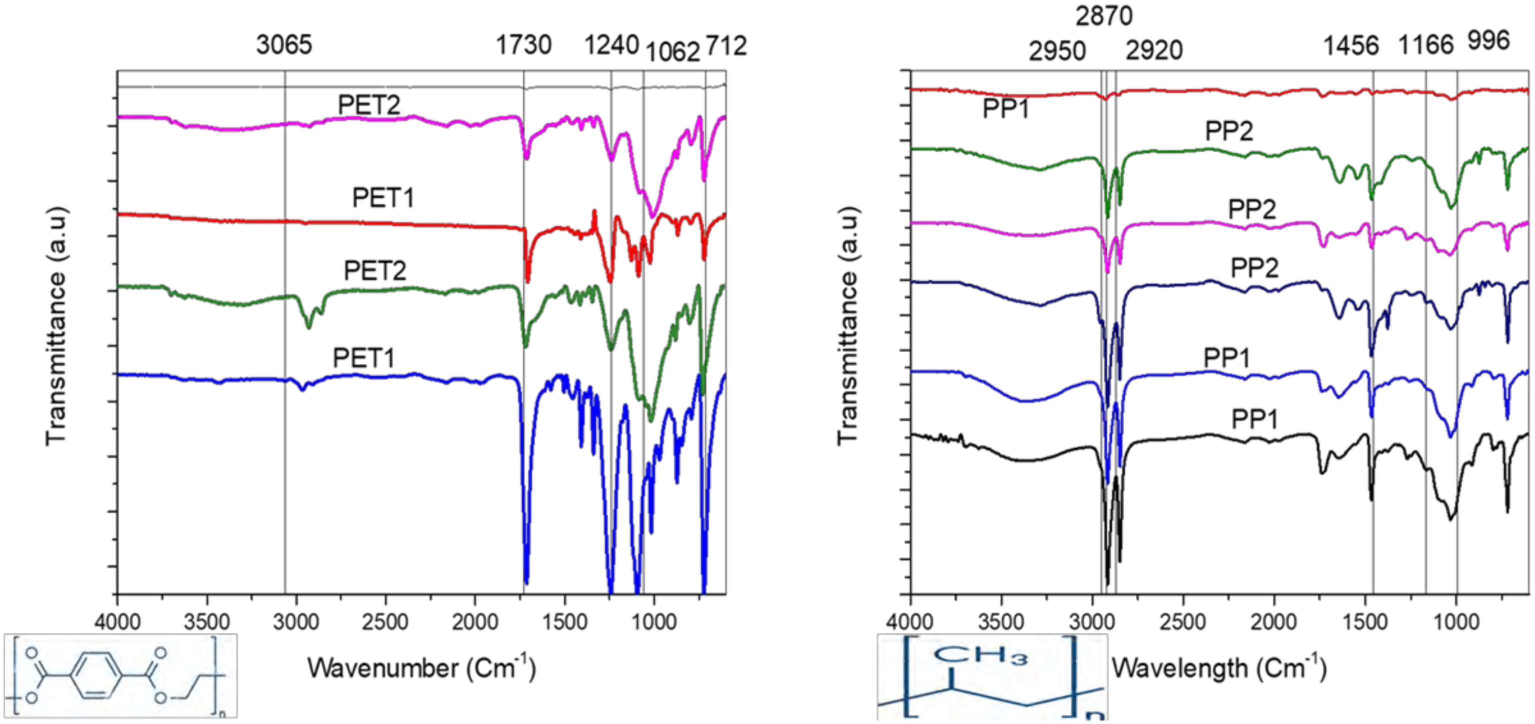


Figure P1: FTIR confirming PET and PP microplastics polymer particles used in this study. For PET1 (blue indicates prior deployment, green is after retrieval). PET2 (red indicates prior deployment, purple is after retrieval). PP1(black indicates prior deployment, blue and red is after retrieval) PP2 (navy blue indicates prior deployment and purple and green after retrieval)

Appendix Q**:** Water physico-chemical variables.

Table Q1: Physico-chemical water quality parameters for the for Bloukrans water samples

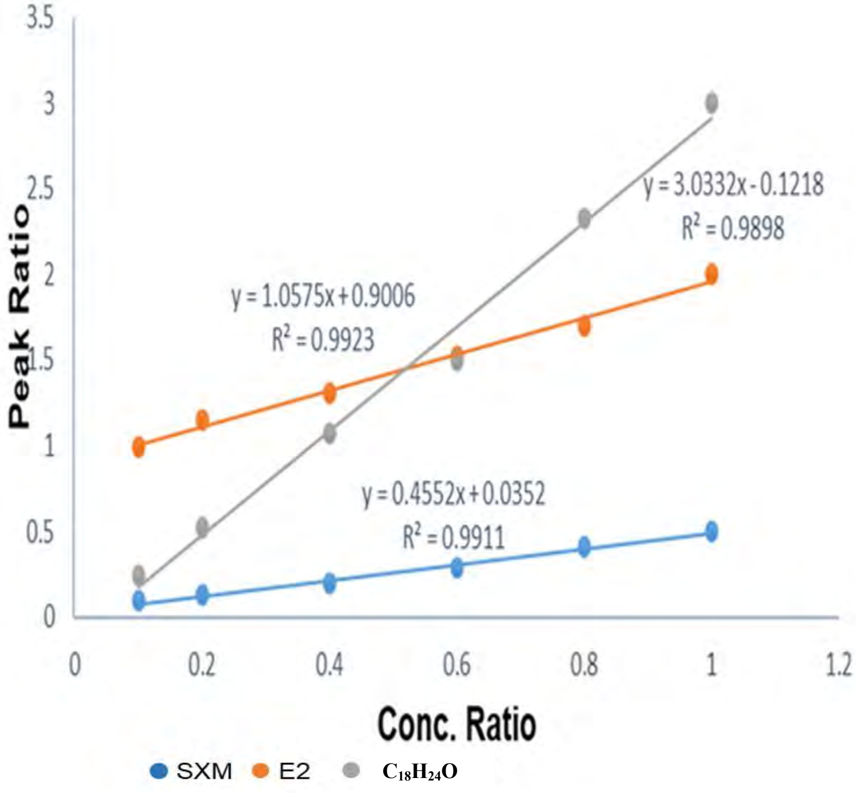
Days	Site	pH	Temp (^{o}C)	DO (mg/l)	Cond. (µs/cm)	Turbidity NTU	River width	Water depth	Water flow rate	TSS (mg/l)	TDS (mg/l)	Alkalinity (mg/l)	Hardness (mg/l)
27/1/22	B1	6.69	20	4.72	1247	12.64	3.1	22.33	52.67	13.33	3200	332.7	260
27/1/22	B2	6.7	23	2.35	1276	21.6	3.15	18.03	88.33	43	566.7	306	210
27/1/22	B3	6.61	25	2.91	1433	10.99	3.8	4.65	33	18.33	1600	398	285
27/1/22	B4	6.99	28	7.66	1919	3.46	1.77	23.6	36.67	14.33	1566.7	52.7	235
3/2/22	B1	10	24	3.59	1053	17.95	2.9	28.17	39.5	23	600	432.7	305
3/2/22	B2	9.55	23	3.07	1434	30.8	3.5	26.17	38	39	666.7	514	285
3/2/22	B3	9.59	23	4.99	1408	19.03	5.3	14.17	47.33	12.7	1200	62.7	245
3/2/22	B4	9.24	23	8.42	194.4	1.73	1.8	14	41	14.33	266.7	25.33	100
24/2/22	B1	7.58	25	0.36	1243	17.6	2.8	25.33	86.95	82.5	2000	670	620
24/2/22	B2	7.65	25	0.26	1519	32.7	1.5	32.5	3	46.5	900	419.5	265
24/2/22	B3	8.12	23	1.91	1533	5.49	3.3	7.5	18	38.5	950	317	355
24/2/22	B4	7.63	27	7.85	193.2	4.08	1.4	17.83	14	49.5	300	26	53.33
7/4/22	B1	7.41	19	1.95	3136	27.3	2.2	15	71	26	900	478	345
7/4/22	B2	7.22	19	0.43	2725	14.6	2.8	22	24.33	32.5	800	569	325
7/4/22	B3	7.2	20	0.59	1021	35.3	4.38	12.38	42.33	17.5	600	455	333
7/4/22	B4	8.05	18	8.35	205.8	2.4	1.55	15.83	12	15.5	200	17	101
14/4/22	B1	8.32	18	0.35	1543	16.97	2.2	13.33	48.67	17.5	1800	467	540
14/4/22	B2	8.19	19	1.19	1481	44.6	3.1	22.33	25.67	30.5	1000	428	530
14/4/22	B3	8.33	18	0.27	1627	13.21	4.75	9.17	44.67	23	700	453	210
14/4/22	B4	8	20	8.38	206.5	4.46	1.55	14.5	30.67	17.5	350	22	85
5/5/22	B1	8.29	14	2.39	1499	13.74	2.1	12.33	37.33	53.5	500	402	395
5/5/22	B2	8.28	15	0.69	1496	39.5	2.24	18	10.33	69	500	409	315
5/5/22	B3	8.34	14	0.87	1930	15.62	4.6	11.67	57.67	61.5	900	476	420
5/5/22	B4	7.49	15	8.21	192.1	5.98	1.65	10.33	27.67	20.75	300	10.5	75

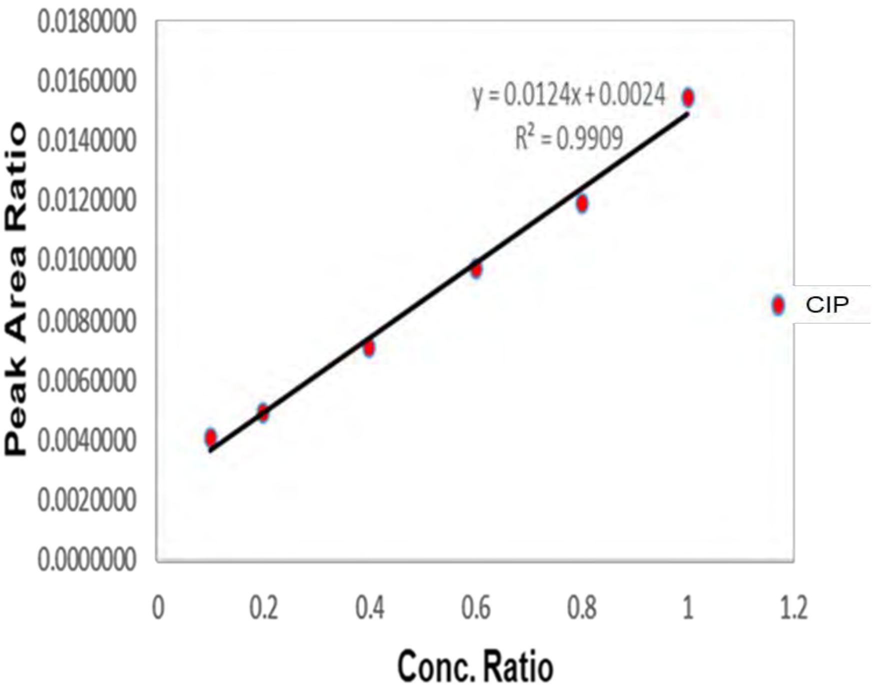
Table Q2: Physico-chemical water quality parameters for the for Swartkops water samples

Days	Site	pH	Temp (^{o}C)	DO (mg/l)	Cond. (µs/cm)	Turbidity NTU	River width (m)	Water depth (cm)	Water flow rate (m/s)	TSS (mg/l)	TDS (mg/l)	Alkalinity (mg/l)	Hardness (mg/l)
28/1/22	S1	6.89	19	1.95	1824	31.4	19	20.3	0.24	49	7600	321.5	321.51
28/1/22	S2	8.68	22	6.31	1952	14.14	7.65	19.80	0.27	50.33	733.33	276.00	276.00
28/1/22	S3	9.58	24	3.75	825	18.05	4.90	55.20	0.025	30	200	63.00	63.00
28/1/22	S4	10.23	24	8.31	260	3.56	6.4	30.33	0.3733	13.33	600	31.00	31.00
4/2/22	S1	8.8	25	2.88	2690	18.06	19.2	16.25	0.4867	110	1133.33	244.50	244.50
4/2/22	S2	6.21	27	8.97	2781	7.54	6.40	9.67	0.2567	36.67	1200	397.00	397.00
4/2/22	S3	7.2	27	4.23	461	6.99	5.00	60.67	0.07	15.33	400	49.50	49.50
4/2/22	S4	5.78	30	8.05	265	8.05	5.80	27.00	0.3800	7.67	333.33	18.333	18.3333
25/2/22	S1	7.86	17	2.39	2230	47.1	19.35	173.33	0.3933	106.55	700	277.00	277.00
25/2/22	S2	8.18	24	5.57	3180	13.40	6.80	14.33	0.3696	49.50	800	346.50	346.50
25/2/22	S3	7.22	25	3.65	400	12.44	5.00	60.50	0.0200	30.00	500	44.50	44.50
25/2/22	S4	7.31	25	8.38	244	2.54	5.80	24.00	0.200	13.00	600	26.00	26.00
8/4/22	S1	8.36	23	3.30	1592	15.58	19.00	18.00	0.3700	64.00	1500	363.00	363.00
8/4/22	S2	8.36	25	2.67	3330	6.98	8.40	18.50	0.1300	49.00	1600	326.00	326.00
8/4/22	S3	7.61	225	4.99	411	10.77	4.65	58.17	0.0350	55.50	1000	78.00	78.00
8/4/22	S4	7.71	26	7.77	254	2.52	5.35	17.83	0.2033	45.00	900	26.00	26.00
15/4/22	S1	8.13	18	3.48	2817	40.10	19.30	15.83	0.2533	61.00	1700	847.00	847.00
15/4/22	S2	8.29	18	4.22	3417	7.22	10.65	18.83	0.1867	24.00	2000	723.00	723.00
15/4/22	S3	7.55	19	5.11	416	12.50	5.10	45.00	0.0450	5.500	1100	138.00	138.00
15/4/22	S4	6.79	19	8.73	247	4.1	5.40	30.17	0.1800	12.00	500	27.00	27.00
6/5/22	S1	8.14	18	2.76	2615	19.09	19.10	16.83	0.4533	61.00	800	365.00	365.00
6/5/22	S2	8.28	19	1.83	3066	13.48	10.95	21.67	0.2000	74.50	1700	410	410.00
6/5/22	S3	7.16	20	4.54	392	12.86	4.75	54.67	0.0450	41.00	600	499.00	499.00
6/5/22	S4	7.80	16	8.39	249	7.51	5.15	29.50	0.1900	22.00	500	33.00	33.0

Appendix R: Calibration curves used to calculate the adsorbed concentration of the analytes on the MPs.

Sulfamethoxazole (SXM), Ciprofloxacin (CIP), 17β-Estradiol (E2) and 4-(2, 6-dimethyl-2-heptyl) Phenol ($C_{18}H_{24}O$). Peak Ratio is the ratio of peak height of standard analyte to the peak height of the respective internal standard. Concentration ratio (Conc. Ratio) concentration of the standard solution to the concentration of the respective internal standard solution used.





Appendix S: Mean ± standard deviation and range (in parenthesis) of the measured adsorbed concentration of the four selected pharmaceuticals and endocrine – disrupting compounds in the Bloukrans and Swartkops Rivers during the two study seasons (summer 2022 – autumn 2022)

Table S1: adsorbed concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol, 4-(2, 6-dimethyl-2-heptyl) Phenol on PET Type1 and PET Type2 Microplastic along Bloukrans River.

Site	**Season**	**MPs Size Types/ Concentration of Pharmaceutical Pollutant (ngL^{-1})**							
		PET Type1				**PET Type2**			
Site B1		**SXM**	**CIP**	**E2**	**$C_{15}H_{24}O$**	**SXM**	**CIP**	**E2**	**$C_{18}H_{24}O$**
	Summer	57.21101±42.3022 (9.2110-89.0521)	1203.628±239.3147 (1002.6408-1468.3612)	18.3856±6.5844 (13.0686-25.7507)	9.0764±6.1408 (3.7532-15.7948)	98.8963±79.3493 (11.2375-165.8182)	3829.641±1620.059 (2198.814-5438.712)	49.2182±17.9472 (35.2797-69.4687)	43.1305±42.5443 (17.8376-92.24905)
	Autumn	88.0354±64.9886 (17.4463-145.3845)	3182.428±1020.027 (2124.1518-4159.3314)	23.3597±10.7879 (16.7252-35.8073)	26.8457±19.96631 (10.6236-49.1443)	168.6046±24.3305 (150.869-196.342)	6769.847±4116.289 (2145.518±10033.6)	38.9917±12.1295 (27.5464-51.7057)	69.7775±18.5781 (62.1129-90.9616)
Site B2	**Summer**	36.8212±48.1237 (1.0259-91.5279)	1437.606±1595.449 (0-3154.1186)	18.0546±6.4029 (11.8931-24.6742)	8.8733±13.8142 (0-24.7895)	94.4620±33.4194 (60.5281-127.3543)	7232.4112±3839.882 (2839.701-9951.092)	32.3239±11.3668 (23.694-45.2032)	25.8657±23.7698 (0-46.7502)
	Autumn	80.3171±24.0671 (52.6654-96.5439)	1801.844±497.8326 (1281.5608-2273.6842)	10.7043±0.5024 (10.3768-11.2828)	17.2981±7.9703 (11.3791-26.3610)	137.5924±17.7011 (126.342-157.996)	4191.025±1117.524 (3515.036-5480.933)	25.8618±19.1704 (4.9994-42.7198)	55.9102±15.6012 (41.7367-72.6266)
Site B3	**Summer**	45.3732±49.9156 (0-98.8415)	1685.808±499.147 (0-2869.2702)	14.3236±2.2560 (12.2415-16.7204)	16.1022±16.9626 (5.6816-35.6751)	97.0160±86.9806 (0-168.0307)	5465.132±8781.155 (504.397-15603.96)	47.0218±23.9159 (30.39673-74.4308)	28.5129±15.9877 (10.1919-39.6391)
	Autumn	446.6334±374.7648 (182.8053-875.6076)	2813.038±1831.407 (1132.8843-4765.2582)	24.8066±9.4202 (13.9377-30.6157)	21.2932±17.8509 (9.2903-41.8068)	609.1765±431.4234 (201.0859-1060.654)	5864.837±1563.507 (4288.975-7415.689)	36.7808±7.3004 (30.1443-44.6006)	33.9926±24.7405 (11.8145-60.6762)
Site B4	**Summer**	0.5275±0.9136 (0-1.5824)	0.4747±0.8223 (0-1.4241)	0.7450±1.0882 (0-1.9939)	0.1926±0.1828 (0-0.3636)	0.6313±1.0935 (0-1.8939)	1.1737±1.0278 (0-1.9131)	2.8919±1.9553 (0.7134-4.4949)	1.7792±2.8367 (0-5.0506)
	Autumn	2.2608±3.0036 (0-5.6689)	1.4584±1.3664 (0-2.7091)	0.4831±0.5028 (0-1.0035)	0.2471±0.2650 (0-0.5271)	3.2808±5.5442 (0-9.6939)	0.8277±1.4337 (0-2.4832)	1.7041±0.5265 (1.1068-2.1008)	3.0493±3.2269 (0-6.4285)

Table S2: adsorbed concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol, 4-(2, 6-dimethyl-2-heptyl) Phenol on PP Type1 and PP Type2 Microplastics along Bloukrans River.

Site	**Season**	**MPs Size Types/ Concentration of Pharmaceutical Pollutant (ngL^{-1})**							
		PP Type1				**PP Type2**			
Site B1		**SXM**	**CIP**	**E2**	**C15H24O**	**SXM**	**CIP**	**E2**	**C18H24O**
	Summer	350.9417±207.9923 (251.994-589.936)	1870.731±1465.812 (1140.8451-3558.1913)	59.2758±25.0923 (37.7666-86.8424)	62.7282±35.5178 (25.042902-95.5844)	436.8646±173.2592 (328.3034-636.67726)	8073.286±2894.427 (8920.187-10449.796)	80.8983±17.828 (67.9464-73.5174)	93.2686±20.94853 (79.3503-117.36105)
	Autumn	856.8806±1301.544 (16.0867-2356.081)	8168.107±6545.06 (2860.9155-15481.431)	45.5565±1.3021 (44.6006-47.0397)	55.1623±38.0731 (16.4361-92.5468)	986.7923±1363±1363.146 (198.4743-2560.8167)	18689.41±12660.85 (5205.1439-20539.906)	82.3806±27.01683 (58.1303-111.5014)	211.1602±169.1862 (102.9091-406.1234)
Site B2	**Summer**	266.1329±109.2551 (180.5669-389.1998)	11119.6±12552.41 (3521.12676-4229.58169)	52.30183±16.1386 (49.4168-69.68835)	23.6832±28.6982 (0-55.5977)	340.0093±130.3684 (237.8434-486.8394)	30285.19±28783.78 (13252.6893-63518.3651)	78.3714±24.3838 (59.0302-105.7623)	68.5375±67.5992 (22.5151-146.1482)
	Autumn	1204.365±1314.816 (329.557-2716.373)	4170.732±3964.623 (0-7890.7178)	56.1141±20.2319 (39.1997-78.5269)	46.5598±44.7055 (11.2710-96.8326)	2316.272±1240.179 (984.4829-3438.0160)	8532.347±8941.035 (10.1868-17840.3756)	68.9693±24.7084 (45.5297-94.7762)	201.4551±62.4461 (130.5965-248.4519)
Site B3	**Summer**	171.538±44.0513 (123.745-210.514)	4526.125±4291.888 (1269.8882-9389.6713)	64.1594±9.1371 (56.9359-74.4308)	40.5277±42.7466 (11.8624-89.65978)	316.0354±68.7802 (242.3342-378.5146)	23058.48±15382.29 (5347.6476-33079.9199)	100.3635±29.6321 (80.2809-134.3959)	138.4706±80.6274 (110.5804-229.3403)
	Autumn	1937.146±2086.019 (233.916-1313.725)	7753.592±2942.183 (4694.8357-10563.3802)	48.7525±21.4921 (30.1444-72.2768)	40.2513±43.9781 (13.5008-91.0081)	3380.214±3224.89 (283.6747-6719.7124)	21326.16±16007.37 (10219.2963-39674.6677)	90.9184±38.2401 (48.3836-122.4524)	378.8924±224.233 (266.9569-232.664)
Site B4	**Summer**	12.9813±22.4843 (0-38.944)	0.7139±1.0565 (0-1.928)	1.9187±1.5386 (1.0084-3.6951)	1.9396±1.9667 (0.4316-4.1640)	22.9277±22.1637 (0-44.2388)	2.0881±0.8746 (1.1868±2.9333)	6.1466±0.3179 (1.9238±5.3348)	3.0867±2.6732 (0-4.6331)
	Autumn	13.0399±8.4757 (3.253-17.949)	2.7414±1.547 (1.5844-4.4978)	2.1331±1.0161 (1.1042-3.1360)	0.7381±0.8650 (0-1.6899)	13.8021±21.1513 (0-38.1532)	3.3459±3.1769 (0-6.3214)	3.5134±1.7172 (1.9238-5.3348)	4.0778±1.4383 (2.4324-5.0965)

Table S3: adsorbed concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol on PET Type2 and PET Type 1Microplastic along Swartkops River.

Site	**Season**	**MPs Size Types/ Concentration of Pharmaceutical Pollutant (ngL^{-1})**					
		PET Type2			**PET Type1**		
Site S1		**SXM**	**CIP**	**E2**	**SXM**	**CIP**	**E2**
	Summer	99.04162±42.42.5471 (66.4721-147.1803)	55.2396±25.9508 (25.3947-72.4871)	765.2759±65.2759 (683.8633-855.5542)	73.4956±38.1443 (33.7711-109.8341)	45.4197±4.2574 (40.5235-48.2495)	81.2188±46.9088 (49.3390-135.0823)
	Autumn	176.0181±237.6566 (25.0619-449.9648)	163.9624±140.4909 (45.8778±319.3330)	498.9038±120.2161 (360.1045-570.0301)	59.0460±29.1762 (25.5718-79.0784)	15.4381±9.1864 (7.6005-25.5471)	178.3661±175.1079 (59.4061-379.4412)
Site S2	**Summer**	121.5529±109.7802 (241.6443-26.3613)	108.2164±93.5196 (50.1812-216.1000)	473.1463±403.3504 (172.2812-931.4777)	59.0895±38.6772 (14.7180-85.6688)	53.6975±228.3296 (21.6152-75.2715)	59.2872±77.0225 (11.0921-148.1178)
	Autumn	128.9304±20.7927 (114.2277-143.6331)	264.876±110.6806 (141.4213-355.2286)	312.4877±297.2189 (112.3725-654.0085)	92.2214±61.4539 (34.3064-156.6889)	31.0227±16.1167 (12.4858-41.7167)	93.0304±78.5319 (53.2821-183.4901)
Site S3	**Summer**	95.1885±30.3079 (72.4879-129.6056)	121.3471±14.8021 (104.7351-141.8921)	116.2456±22321.0221 (12.2415-16.7204)	65.8571±28.5264 (32.9448-83.4721)	47.6334±51.1094 (17.8054-106.6483)	57.7397±48.4536 (12.2886-108.7211)
	Autumn	86.6515±48.2002 (35.8926-92.2594)	226.9621±163.4472 (95.4731-409.9583)	358.3556±350.8137 (131.6889-762.4413)	75.2102±50.8055 (17.2476-112.0309)	132.6525±100.8626 (54.3065-246.4559)	62.3289±72.1887 (10.5421-144.7892)
Site S4	**Summer**	ND	1.064e-4±8.8004e-5 (2.1e-5-1.968e-4)	2.0e-4	ND	3.9930e-4±5.369e-4	2.131e-4±1.315e4 (1.2e-5-3.06e-4)
	Autumn	ND	1.423e-3±1.032e-3 (ND-6.924e-4)	ND	1.5791e-4	3.2808±5.5442	1.35e-4±1.7688e-4 (1.0e-5-2.6e-4)

Table S4: adsorbed concentration of Sulfamethoxazole, Ciprofloxacin, 17β-Estradiol on PP Type2 and PP Type1 Microplastic along Swartkops River.

Site	Season	MPs Size Types/ Concentration of Pharmaceutical Pollutant (ngL^{-1})					
		PP Type2			PP Type1		
Site S1		**SXM**	**CIP**	**E2**	**SXM**	**CIP**	**E2**
	Summer	214.0419±135.1662 (81.2752-351.4861)	182.0338±147.6232 (113.3400-228.0317)	7028.2613±5797.9391 (3380.2805-13713.8621)	101.0469±21.9687 (101.0468-123.0157)	70.3114±74.7417 (24.3676-156.5542)	54.0228±23.3950 (27.3184-69.7395)
	Autumn	380.6985±431.3871 (120.6515-878.6573)	234.9993±10.4205 (224.7435-245.5772)	11732.5000±2308.6861 (10770.5055-14366.6197)	83.0815±44.3047 (34.1984-120.5898)	36.5205±10.0945 (26.6361±46.8129)	29.3831±1.5772 (28.0024-31.1020)
Site S2	**Summer**	344.2631±112.7335 (219.6750-439.3596)	193.6583±15.7459 (177.4630-208.9128)	4536.5151±4360.1791 (949.6311-9389.6718)	169.3613±63.36664 (90.0625-213.0854)	55.1876±28.4445 (23.6853-78.9883)	53.2831±13.3257 (38.5791-64.5622)
	Autumn	530.3466±16.6285 (118.6214-149.3772)	231.9773±114.3289 (113.6572-341.8459)	19613.2610±5600.179 (13581.4889-24647.8873)	77.6488±40.7638 (96.6531-105.4404)	33.4289±13.0550 (21.9811-47.6466)	44.86199±5.5754 (44.8619-54.9382)
Site S3	**Summer**	1762.1339±3076.321 (101.6373-417.3912)	152.8162±11.9775 (142.8767-166.1144)	4913.3441±4649.5921 (278.4175-9577.4647)	160.0227±81.5299 (79.0784-241.6443)	33.9281±11.6536 (21.1346-43.9374)	45.5754±40.0386 (19.5598-91.6814)
	Autumn	465.1583±63.1812 (20.4826-109.8341)	123.1972±18.6971 (106.9487-143.6328)	6603.8841±5812.2281 (1986.2766-13130.5391)	49.3309±51.2090 (11.6546-107.6373)	44.2548±21.7183 (27.2085-68.7075)	22.9989±5.5269 (16.7206-27.1304)
Site S4	**Summer**	ND	ND	3.7e-4	2.0e-6	3.559e-4±1.842e- (1.803e-4-3.4e-4)	4.7621±3.278e-4 (4.438e-4-8.190e-3)
	Autumn	ND	5.471e-3	5.631e-3	ND	1.8884±2.089e-e	2.135e-3±2.731e-3 (1.194e-3-5.213e-3)

Appendix T: Tukey multiple comparisons (Tukey HSD post-hoc-test).

Table T1: Tukey multiple comparisons (Tukey HSD post-hoc-test) of the adsorbed concentration of the targeted analytes between different study sites (land-use types) and deployment periods along Bloukrans River.

Diff (Sites/Days)	**Sulfamethoxazole (*P* value)**	**Ciprofloxacin (*P* value)**	**17β-Estradiol (*P* value)**	**4-(2,6-dimethyl-2-heptyl) Phenol (*P* value)**
B2-B1	0.01999	0.017858	0.015919	0.84579
B3-B1	0.04199	0.018205	0.99816	0.22329
B4-B1	0.002756	0.017234	0.03214	0.008748
B3-B2	0.999948	0.019998	0.68592	0.019936
B4-B2	0.0030845	0.02657	0.19579	0.023337
B4-B3	0.002892	0.02905	0.0406	0.04127
14-7	0.02129	0.94101	0.96123	0.40358
35-7	0.01614	0.37354	0.91335	0.009623
35-14	0.04988	0.01547	0.03728	0.122243

Table T2: Tukey multiple comparisons (Tukey HSD post-hoc-test) of the adsorbed concentration of the targeted analytes between different study sites (Land use types) and deployment periods along Swartkops River.

Diff (Sites/Days)	**Sulfamethoxazole (*P* value)**	**Ciprofloxacin (*P* value)**	**17β-Estradiol (*P* value)**
S2-S1	0.85355	0.84685	0.22321
S3-S1	0.72448	0.46279	0.011073
S4-S1	0.05076	0.01944	2.022e-4
S3-S2	0.99457	0.9059	0.45149
S4-S2	0.008745	0.0.0412	0.01829
S4-S3	0.003504	0.009623	0.031793
14-7	0.96881	0.12842	0.84579
35-7	0.009116	0.00464	0.006521
35-14	0.10562	0.21415	0.2327

Appendix V:Calculated concentration adsorbed of Sulfamethoxazole and 17β-Estradiol onto PET and PP particle of two size ranges

Table V1: Kinetics experimental data results

Time (hrs)	24	48	72	96	120
Mixture of PP and PET (alone)	ND	ND	ND	ND	ND
Mixture of SXM (alone)	998.6679±63.0032	997.8296±60.0353	997.6609±61.3874	997.6605±46.2010	997.6602±74.1031
Mixture of E2 (alone)	999.31705±22.2141	999.00184±36.3400	998.17551±36.0013	998.1752±27.4013	998.1750±335.1930
Mixture of PP1 and SXM	429.38872±61.3840	420.2784±55.1180	378.25059±56.0061	269.80499±43.3110	271.11137±41.0010
SXM - adsorbed	**569.2792**	**577.5512**	**619.41031**	**727.8551**	**726.5488**
PP1 and E2	228.4759±35.2014	216.2346±30.1080	189.0010±26.0134	199,40485±18.0874	199.5258±10.0208
E2 - Sorbed	**770.8412**	**782.7672**	**809.1745**	**798.77035**	**798.6492**
Mixture PP2 and SXM	100.0426±30.4302	80.2691±27.7080	64.67023±22.4032	76.7335±15.1002	76.7701±21.0042
SXM - adsorbed	**898.6253**	**917.5605**	**932.9907**	**920.9270**	**920.8901**
Mixture of PP2 and E2(17β)	48.6442±26.1293	43.9367±23.9301	39.0548±29.0412	39.7556±23.0026	39.7248±13.0113
E2- adsorbed	**950.6729**	**955.0651**	**959.1207**	**958.4474**	**958.4502**
Mixture of PET1 and SXM	429.5254±17.0358	384.2965±16.0073	309.1969±18.6801	309,4210±12.1081	334,0101±11.05153
SXM - adsorbed	**569.1425**	**613.5331**	**688.4640**	**688.2395**	**663.6501**
Mixture of PET1 and E2	666.2368±39.0093	594.3937±27.7010	221.7022±14.7182	236.7351±17.0703	236.7086±15.0207
E2 - adsorbed	**333.0803**	**404.6082**	**776.4733**	**761.4401**	**761.4664**
Mixture of PET2 and SXM	204.0237±35.2671	198.6061±26.1807	194.8479±17.0267	198.8929±21.1023	198.1205±18.0061
SXM - adsorbed	**794.6442**	**799.2235**	**802.8130**	**798.7106**	**799.5397**
Mixture of PET2 and E2	375.87952±14.3401	272.18055±13.7201	98,2592±14.3502	108.1081±11.0022	104.0547±17.0623
E2 -adsorbed	**623.4375**	**726.8213**	**899.9163**	**890.0671**	**894.1203**